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INVESTIGATION OF THE ACTION OF 1,3-DIPHENYLPYRAZOLECARBOXYLIC ACIDS ON THE LIVER CYTOCHROME p-450 SYSTEM

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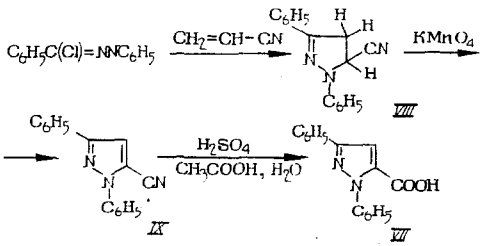
Intensive investigations of the cytochrome P-450 system within the framework of molecular pharmacology have been associated chiefly with problems of resistance, habituation to drugs, potentation and prolongation of their action, as well as certain other pharmacological manifestations determined by the state of the cytochrome P-450-dependent enzyme systems.

The targeted synthesis of relatively nontoxic inducers of liver cytochrome P-450, exhibiting minimal side effects and suitable for use in clinical practice, is dictated by the necessity of correcting the activity of monooxygenase systems under pathological conditions, when there is a change in the pharmacological and toxic effects of drugs as a result of a disruption of processes of their biotransformation [1, 2, 5, 6].

In search of new cytochrome P-450 inducers, we synthesized derivatives of pyrazolecarboxylic acids (I-VI) and studied their influence on the content of cytochrome P-450 and b_5, as well as on the rate of demethylation of aminopyrnee and hydroxylation of aniline in liver microsomes. The association between the effects on the monooxygenase system and the structure of I-VI was analyzed.

Compounds II-VI were synthesized by the reaction of the chlorides of 1,3-diphenylpyrazole-4-carboxylic acids with the corresponding amines. To obtain the amide I, 1,3-diphenylpyrazole-5-carboxylic acid (VII) was synthesized according to the scheme:



1,3-Diphenyl-4-R^1-5-R^2-pyrazoles (I-VI)
R^1=H (I), CONHCH$_2$CH$_2$CH(CH$_3$)$_2$ (II), CON(CH$_2$CH$_2$)$_2$NCH$_2$C$_6$H$_5$·HCl (III), CONH(CH$_2$)$_3$·Morpholino·HCl (IV) CO·Morpholino (V), CONH$_2$ (VI);
R^2=H (III, IV, VI), CH$_3$ (II, V), CON(n-C$_4$H$_9$)$_2$

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TABLE 1. Influenmce of Derivatives of 1,3-Diphenylpyrazolecarboxylic Acids on the Liver Cytochrome P-450 System

Compound	Cytochrome, nmoles per mg protein		Rate of demethylation of amidopyrin	Rate of hydroxylation of aniline
	P-450	b_5	nmoles/min per mg protein	
Control	0,53±0,02	0,46±0,03	8,72±0,62	0,93±0,02
I	0,55±0,05	0,46±0,03	11,89±2,30	1,00±0,03
II	0,61±0,03	0,49±0,01	12,74±0,34***	1,05±0,02**
III	0,80±0,11**	0,59±0,04*	18,04±2,78**	1,42±0,03***
IV	0,57±0,07	0,50±0,03	10,57±0,59*	1,00±0,12
V	0,96±0,07***	0,61±0,04**	18,63±2,80**	1,49±0,22**
VI	0,53±0,03	0,46±0,04	9,45±2,44	1,05±0,30

CTP 5

Note. Average values of 3 to 4 experiments are presented; 5 animals were used in each experiment. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

EXPERIMENTAL (CHEMICAL)

The synthesis of compounds II, III, V, and VI was presented earlier [3]. 1,3-Diphenylpyrazole-4-N(3-morpholinopropyl)carboxamide hydrochloride (IV) was produced analogously by the reaction of 0.01 mole of the chloride of 3,5-diphenylpyrazole-4-carboxylic acid in 30 ml of absolute benzene with a solution of 1.44 g (0.01 mole) of 3-morpholinopropylamine in 10 ml of absolute benzene at a bath temperature of 4-5°C. On the following day the precipitate was filtered off, washed on the filter with absolute benzene, and recrystallized from butanol. We obtained 3.35 g (78.5%) IV, mp 187.5-188.5°C. $C_{23}H_{26}N_4O_2 \cdot HCl$, the data of elementary analysis satisfy the calculated values.

1,3-Diphenyl-5-cyano-2-pyrazoline (VIII). To a suspension of 46 g (0.2 mole) α-chlorobenzal phenylhydrazone in 400 ml of absolute benzene we added 13.8 g (0.26 mole) acrylonitrile, and while mixing after 20 min 100 ml of triethylamine was added. The reaction mixture heated up spontaneously, the suspension dissolved, and a precipitate formed from the solution during subsequent mixing (total reaction time 10 h); it was filtered off, washed with absolute benzene, dried, tirturated with 300 ml of water, filtered off, washed with 200 ml of water, and dried. We obtained 23.16 g VIII, mp. 138.5-140°C (according to [13], mp. 138-140°C). The benzene mother liquor was evaporated to three fourths of its volume, the precipitate formed was filtered off, washed with absolute benzene, dried, and an additional 13.18 g of VIII was obtained. Total yield 36.34 g (73.5%).

1,3-Diphenyl-5-cyanopyrazol (X). To a solution of 24.73 g (0.1 mole) of the pyrazoline VIII in 300 ml of absolute acetone, 20.6 g of $KMnO_4$ was added in small portions in several sessions with mixing after 20 min, maintaining the temperature in the reaction mass around 20°C by cooling with water. Mixing was continued for another 1 h, 300 ml of alchol was added, the precipitate was filtered off, washed with acetone and alcohol (50 ml each). The filtrate was evaporated, and the residue crystallized from 250 ml of isopropanol. We obtained 12.7 g (51.8%) IX, mp 136-137°C (according to [13], mp 133-135°C).

1,3-Diphenylpyrazol-5-carboxylic Acid (VII). We boiled 6.9 g of the nitrile IX in a mixture of 140 ml of glacial CH_3COOH, 28 ml conc. sulfuric acid, and 28 ml of water for 10 h, poured the solution out into 200 ml of water, and left at 5°C for 4 h. The precipitate formed was filtered off, washed from the filter with water to a neutral reaction according to Congo, then suspended in 150 ml of water, 50 ml of a 10% sodium hydroxide solution was added, the solution was filtered off and acidified with 50 ml of a 10% solution of hydrochloric acid. The mixture was allowed to stand for 16 h at 5°C. The residue was filtered off, washed with water, and dried at 100-120°C for 5 h. We obtained 6.04 g (81.2%) VII, mp 228.5-229.5°C with decomposition (according to [13], mp 225-227°C, decomposition).

1,3-Diphenylpyrazole-5-(n-dibutyl)carboxamide (I). To a solution of 0.025 mole of the acid chloride corresponding to VII in 70 ml of absolute benzene, a soluiton of 6.54 g (9.05 mole) n-butylamine in 15 ml of absolute benzene was added with cooling (bath temperature 0°C) and mixing after 5 min (temperature of the reaction mass about 8°C), mixed for 30 min and left overnight at room temperature. Then it was treated successively with

water, a 5% sodium hydroxide solution, and again with water to a neutral pH. The benzene extract was dried over anhydrous magnesium sulfate and evaporated under vacuum. The residue was triturated with hexane, the crystallized precipitate was filtered off, recrystallized from a mixture of alcohol and water (5:1), and 6.34 g (66.8%) I was obtained, mp 72.5-73.5°C (frompetroleum ether), $C_{24}H_{29}N_3O$; the data of elementary analysis satisfied the calculated values.

EXPERIMENTAL (PHARMACOLOGICAL)

The work was conducted on $F_1(CBA \times C_{57}Bl_6)$ male mice weighing 20-22 g, obtained from the Stolbovaya nursery of the Academy of Medical Sciences of the USSR and kept on the standard vivarium diet. The compounds studied were administered to the animals intraperitoneally in the form of a suspension in 0.5% starch solution in a dose of 50 mg/kg once a day for three days.

The functional state of the cytochrome P-450 system was determined in the liver microsomal fraction, which was isolated by differential centrifugation. The content of cytochrome P-450 and b_5 was determined according to the method of [14] on an Aminco spectrophotometer. The amount of microsomal protein was determined according to a modified Lowry method [12]. To estimate the metabolism of drugs in vitro, the rate of N-demethylation of amidopyrine and hydroxylation of aniline was determined in the microsomal fraction [4].

The results were treated statistically using the Student t-criterion.

RESULTS AND DISCUSSION

In the series of comounds (I-VI), according to the quantitative estimation, there should be a decrease in lipophilicity of the radicals in positions 4 and 5 of the pyrazole ring. As is well known, for the alkyl groups of aliphatic compounds C_nH_{2n-1}, a larger value of n corresponds to greater solubility in nonpolar solvents. On the basis of this, we should consider that compound I will be more lipophilic than II. Aromatic containing other elements, in particular, oxygen and nitrogen, are known to have greater affinity for water molecules than hydrocarbon fragments, and in cases when they are bound to hydrocarbon chains, they correspondingly increase the hydrophilicity of the entire molecule [7]. Hence it is quite significant that the lipophilicity decreases in the series II > IV > V. The amide group R^1 in comopund VI, as is generally known, has pronounced hydrophobic properties and low lipophilicity.

Microsomal oxidation in the heterocyclic system of pyrazole occurs at the C(4) position. Thus, although 3,5-dimethylpyrazole has two metabolically vulnerable methyl groups, the main metabolite is 4-hydroxy-3,5-dimethylpyrazole, which agrees with the high reactivity of C(4) in the pyrazole molecule [11]. There is no information in the literature with respect to N-oxidation. The reactivity at C(4) under conditions of microsomal oxidation, moreover, may depend on the three-dimensional structure of this center of the substituent, for example, in the C(5) position, and may also be the group bonded precisely to C(4), and it may also depend on the overall and local lipophilicity of the investigated compound, which will influence the specifics of the binding of the low-molecular-weight ligand to the macromolecular structure of the monooxygenase system. Taking into into account, we performed a directed modification of structures in the pyrazole series precisely at the C(4) position.

Let us examine the structures of the compounds studied from the standpoint of the possibility of their microsomal oxidation. For compounds I and VI, C(4) in the heterocycle is readily accessible to oxidation. In compounds III and V, such heterocycles as piperazine and morpholine are in direct proximity to C(4), and they hinder the access to the metabolizable position in the pyrazole molecule to an even greater degree.

Earlier [8-10] it was suggested the xenobiotics of the phenobarbital type exert their inducing effects through the original moelcule, rather than products of metabolism in the microsomes. The blocking of the site in the molecule that undergoes oxidation in the active site of cytochrome P-450 provides the xenobiotic with pronounced inducting properties.

The results of an investigation studying the effects of chemical compounds on the liver cytochrome P-450 system are presented in Table 1. Both compound VI with a hydrophilic radical in position C(4) and compound I, which carries its own lipophilic substituent, did not exhibit any pronounced effect on the liver cytochrome P-450 sytem. Compounds II and IV, less lipophilic in comparison with I, caused a small increase in the cytochrome P-450 content

(by 7-15%) and the enzyme activity (by 10-45%). The most pronounced properties exhibited b compounds III and V, in which access to the C(4) position is sterically hindered to the greatest degree. Thus, compound III increases the content of cytochrome P-450 by 50.9%, changing the rate of N-demethylation of amidopyrine and the hydroxylation of aniline by 106.9 and 52.7%, respectively. The creation of supplementary steric hindrance by the introduction of amethyl group into position C(5) of the heterocycle, not subjected to primary hydroxylation (compound V), is responsible for an 80% increase in the cytochrome P-450 content.

Thus, targeted chemical modification of the pyrazole molecule has yielded new inducers of the cytochrome P-450-dependent monooxgenase system.

Earlier it was shown that the ability of barbiturates to induce drug metabolism is inversely dependent on their solubility in lipids [15]. However, the authors also did not exclude an influence of the nature of the chemical groups in the molecule.

A comparison of the qualitatively estimated lipophilicity of the investigated compounds permits us to conclude that although a definite degree of lipophilicity is a necessary determinant for manifestation of the properties of inducerse, the factor of blocking of the metabolizable site in the substrate molecule is more important in determining the inducing activity of the xenobiotic.

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