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SYNTHETIC ANTICONVULSANTS, ANTIHYPOXICS, AND LIVER MONOOXYGENASE SYSTEM INDUCERS BASED ON AMIDES AND UREA.

VI*. SYNTHESIS AND SEARCH FOR LIVER MONOOXYGENASE SYSTEM INDUCERS AMONG COMPOUNDS CONTAINING THE BENZHYDRYL GROUP

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The idea of the pharmacological utilization of compounds which induce liver cytochrome P-450-dependent monooxygenase system enzymes by dosage with hydrophobic xenobiotics was proposed by A. Conney [10] more than 20 years ago. Later studies [4] supported this concept in clinical practice, using zixorin (3-trifluoromethyl-α-ethylbenhydrol; Gideon Richter, Hungary), which was recommended for the treatment of liver and bile tract disease.

The benzhydryl group is a key pharmacological fragment for many biologically active compounds with a wide spectrum of pharmacological activities [1, 8, 9].

There has thus far been no systematic analysis of the liver monooxygenase enzyme system-inducing activity among compounds containing the benzhydryl group.

The aim of the present work was to synthesize different benzhydryl compounds, to screen them for enzyme-inducing activity, and to identify potential classes of organic compounds as inducers of the liver monooxygenase system. We synthesized a series of compounds (I-XII) containing the benzhydryl group, and studied their effects on the liver monooxygenase system using the hexobarbital sleep test in experimental animals. The yields and physicochemical properties of compounds I-XII are shown in Table 1, and their effects on the liver monooxygenase system are shown in Table 2.

Ph_2CHR,
I—XII

where R=H (I), OH (II), NH_2 (III), NH_2·HCl (IV), NHCHO (V), $NHCOCF_3$ (VI), $NHCONH_2$ (VII), NHCONHAC (VIII), $NHCONHCONH_2$ (IX), $NHCONHCHPh_2$ (X), $N{=}CHSO_3Na$ (XI), N=CHCH=CHPh (XII).

Diphenylmethane I and benzhydrol II were obtained in pure form by standard methods [3]. Benzhydrylformamide V was synthesized by our modification of the Leickhardt reaction [2];

*See [2] for Communication V.

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TABLE 1. Properties of Benzhydryl Derivatives I-XII

Compound	Yield %	Melting temperature, °C	Atomic formula
I	91	25—26	$C_{13}H_{12}$
II	92	68—69	$C_{13}H_{12}O$
III	90	Boiling point 178°C at 23 mm Hg	$C_{13}H_{13}N$
IV	74	274—275	$C_{13}H_{14}ClN$
V	94	132—134	$C_{14}H_{13}NO$
VI	79	158—159	$C_{15}H_{12}F_3NO$
VII	78	143—144	$C_{14}H_{14}N_2O$
VIII	81	151—152	$C_{16}H_{16}N_2O_2$
IX	68	169—170	$C_{15}H_{15}N_3O_2$
X	78	269—271	$C_{27}H_{24}N_2O$
XI	62	149—251	$C_{14}H_{12}NO_3$
XII	51	116—118	$C_{22}H_{19}N$

TABLE 2. Effects of Compounds I-XII in Equimolar Doses on the Duration of Hexobarbital Sleep in Mice (mean of 6-12 experiments) (M ± m)

Compound	Duration of hexobarbital sleep, min		% of control
	experimental	control	
I	79,0±3,21	79,0±2,70	100
II	80,0±2,72	79,0±2,70	100
III	49,0±3,45*	79,0±2,70	62
IV	47,4±2,94*	79,0±2,7	60
V	19,8±2,51*	79,0±2,7	25
VI	47,5±3,62*	79,0±2,7	60
VII	23,1±2,54*	70,0±2,2	33
VIII	24,5±3,12*	70,0±2,2	35
IX	43,4±2,96*	70,0±2,2	62
X	69,5±3,22	70,0±2,2	100
XI	66,5±2,75	70,0±2,2	95
XII	52,5±2,17*	70,0±2,2	75

*Differences with control significant ($p \leq 0.05$).

hydrolysis of V yielded benzhydrylamine III, and treatment of this with gaseous hydrogen chloride converted it into the chlorhydrate IV. Azomethynes XI and XII were prepared by condensation of benzhydrylamine III with the appropriate aldehydes. Benzhydryltrifluoroacetylamide VI was synthesized by acetylation of amine III with trifluoroacetic anhydride in benzene. Benzhydrylurea was synthesized by a method published by us [9], and compounds VIII, IX, and X were obtained from benzhydrylurea VII: (a) by acetylation with acetic anhydride for compound VIII; (b) by carbamolylation with isocyanic acid for compound IX; (c) by thermal disproportionation at 210°C for 1,3-dibenzhydrylurea X [1].

Shortening of hexobarbital sleep in experimental animals demonstrates induction of the liver monooxygenase system responsible for metabolism of xenobiotics entering the body [5]. In addition, hexobarbital is metabolized by PB-cytochrome P-450, which is induced by phenobarbital, with increases the rate of hexobarbital metabolism in PB-microsomes to a level significantly greater than that in MX-induced microsomes [6]. Thus, hexobarbital sleep provides a fairly specific in vivo screening test for potential PB-type inducers [7].

Experimental pharmacological data (see Table 2) showed that compounds I and II, which lack a nitrogen atom, had no effect on the duration of hexobarbital sleep. The presence of a nitrogen atom in the exocyclic methyne carbon atom (compounds III-XII) produced a significant shortening in hexobarbital sleep, except for compounds X, XI, and XII. The absence of enzyme-inducing effects in 1,3-dibenzhydrylureas is presumably caused largely by their steric bulk, which may significantly limit the ability of compound X to penetrate to a possible receptor. Compounds with azomethyne bonds, i.e. XI and XII, also had low activity.

The benzhydrylamine chlorhydrate IV and its base III significantly shortened the duration of hexobarbital sleep, to virtually the same extents. Stepwise carbamolyation of benzhydrylamine III led to alterations in the duration of hexobarbital sleep, VII producing a greater reduction than III = IX. Primary carbamolyation of compound III, i.e. to form a carbamide pharmacophore group (compound VII) was almost twice as effective in increasing enzyme-inducing activity compared with the initial benzhydrylamine III. Subsequent carbamolyation of benzhydrylurea VII, i.e. formation of a Biuret functional group (compound IX), resulted in a significant decrease in activity in comparison to that of benzhydrylurea VII. Comparative analysis of reductions in hexobarbital sleep in compounds III, VII, and IX showed that the carbamide pharmacophore group on the benzhydryl framework was preferred over the amino and Biuret groups (compounds III and IX respectively). This conclusion was also supported by analysis of N-benzhydryl-N-acetylurea VIII, which, despite the additional presence of the acetyl group in benzhydrylurea VII, had high enzyme-inducing activity, i.e. at the same level as that of the carbamide-containing precursor VII itself.

Trifluoroacetylation of benzhydrylamine (compound VI) had virtually no effect on the enzyme-inducing properties of the initial amine III (see Table 2). However, formylation of benzhydrylamine I (compound V) significantly reduces hexobarbital sleep in experimental animals. Benzhydrylformamide V is one of the probable products of hydrolytic (enzymatic) splitting of benzhydrylurea VII, and their common high enzyme-inducing activity is probably associated with the formation of an active fragment in the body.

These studies lead us to suggest that the classes of compounds with the greatest potential for further study of their liver monooxygenase system-inducing properties are the benzhydrylformamides (of which V is a representative) and the benzhydrylureas (e.g. VII). More detailed studies of their enzyme-inducing effects will be the subject of our next report.

CHEMICAL METHODS

Diphenylmethane I and benzhydrol II were synthesized and extracted as described in [3]. Benhydrylformamide V, benzhydrylamine III, the chlorhydrate of benzhydrylamine IV, benzhydryl-Biuret IX, and 1,3-dibenzhydrylurea X were prepared and purified as described in [2]. Benzhydrylurea was synthesized as described in [9].

N-(Trifluoroacetyl)-N-(benzhydryl)amine (VI). Benzhydrylamine III (0.01 mole) solution in 10 ml of anhydrous benzene was thoroughly stirred for 10 min while 0.012 mole of freshly-prepared $(CF_3CO)_2O$ in 5 ml of anhydrous benzene was added. The reaction mix was kept for 30 min and the solvent was evaporated; the resulting precipitate was recrystallized from hexane.

N-Benzhydryl-N-acetylurea (VIII). A mixture of 4 g of benzhydrylurea VII, 20 ml of Ac_2O, and 0.2 ml of sulfuric acid was heated to 60°C and stirred for 3 h. The reaction mix was then cooled to 20°C, and 100 ml of water was added with stirring. The resulting precipitate was collected by filtration, washed to pH 7.0, and recrystallized from ethanol this yielded the desired product.

Sodium methylenesulfonate benzhydrylamine (XI). Benzhydrylamine III (15 g) and 9 ml of water were heated to 70°C with stirring, and a previously prepared solution of sodium formaldehyde bisulfite was added over 1 h, and the reaction mix was kept for a further 2 h. The resulting precipitate was recrystallized from ethanol. Schiff base XII was synthesized and purified by a similar method.

Yields and properties of benzhydryl compounds I-XII are presented in Table 1. Elemental analyses agreed with calculated values.

BIOLOGICAL METHODS

Studies were carried out using 250 white male mongrel mice (18-22 g), in groups of 6-12 animals. Compounds were given p.o. in equimolar doses, i.e. I (77.0 mg/kg), II (84 mg/kg), III (84 mg/kg), IV (100 mg/kg), V (93 mg/kg), VI (120 mg/kg), VII (100 mg/kg), VIII (120 mg/kg), IX (120 mg/kg), X (170 mg/kg), XI (120 mg/kg), and XII (130 mg/kg), once daily for three days, as suspensions in 1% starch paste. Control animals received equal volumes of starch paste.

Hexobarbital was given i.p. at a dose of 80 mg/kg 24 h after the last dose of compounds I-XII. The duration of hexobarbital sleep was measured using the absence of the turning-over reflex. Results were analyzed using Student's t test (T. F. Lakin, 1980). Biological results are presented in Table 2.

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