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Anxiolytic Properties of Derivatives of 1-Methoxycarbonylmethyl-3-Arylamino-7-Bromo-5-Phenyl-1,2-Dihydro-3*H*-1,4-Benzodiazepin-2-one

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TSPO receptors are peripheral benzodiazepine receptors (PBDRs). Unlike central benzodiazepine receptors (CBDRs), PBDRs (TSPO receptors) are widely presented in organs and tissues of animals and humans; these receptors are involved significantly in the regulation of many physiological processes in the norm and pathologies. Elucidation of the molecular bases of interactions between the TSPO receptors and their ligands is an important task of modern pharmacochemistry. We studied the anxiolytic properties of some derivatives of 1-methoxycarbonylmethyl-3-arylamino-7-bromo-5-phenyl-1, 2-dihydro-3H-1,4-benzodiazepin-2-one (compounds 1-7), which demonstrate considerable affinity for TSPO and CBDRs. The anxiolytic activity was estimated in experiments on rats in the "Conflict situation" test, while the intensity of motor activity was estimated in the standard "Open field" test. All tested compounds demonstrated a rather high selectivity in binding with TSPO receptors; compounds 1–4 manifested significant anxiolytic properties. Compound 2 demonstrated the maximum anxiolytic activity; after binding with TSPO receptors, $K_{i(TSPO)}$ = 19, while $K_{i(CBDRs)}$ > 10000 nM. All studied compounds were characterized by low toxicity; their LD_{50} exceeded 500 mg/kg.

Keywords: benzodiazepine, affinity, TSPO receptors, anxiolytic activity.

INTRODUCTION

In recent years, peripheral benzodiazepine receptors (PBDRs) are considered promising therapeutical targets in the treatment of some pathologies, such as epilepsy, Alzheimer's disease, cerebral ischemia, and inflammatory processes [1]. It has been demonstrated that numerous PBDRs are present in many tissues and organs [2-8]. They fulfill various functions (depending on the tissue where they are expressed) and, therefore, are polymodal receptors. It has been found that some PBDR ligands (e.g., emapunil, XBD-173) are effective anxiolytics, whose side effects are less expressed than those of traditional benzodiazepine preparations.

We studied the anxiolytic efficiency of seven derivatives of 1-methoxycarbonylmethyl-3--arylamino-7-bromo-5-phenyl-1,2-dihydro-3*H*-1, 4-benzodiazepin-2-one (Fig. 1; preparations 1–7) synthesized in our laboratory, as well as their toxicity and effects on motor activity. In preparations 1–4, the functional group in the benzene ring R^1 was not replaced (hydrogen remained), while this hydrogen was replaced by chlorine in preparations 5–7. In the benzene ring R^2 of preparation 1, hydrogen was not replaced, while in preparations 2-4 and 5-7, hydrogen in this ring was replaced by a nitro-group ($-NO_2$) in positions 2, 3, or 4, respectively.

METHODS

Experiments were carried out on mongrel albino male rats weighing 180–200 g. The animals were kept under standard vivarium conditions with free access to food and water. The anxiolytic activity of the tested preparations was estimated according to the results of a standard "Conflict situation" test [8]. General motor activity was estimated in the "Open field" test [8], while acute toxicity was estimated using the Litchfield–Wilcoxon method [9]. As a standard for comparison, we used diazepam [10] (UA/8579/01/01; No. 274 from 05.04.13), tablets, 0.05 g each (UA/8579/01/02 No. 274 from 05.04.13). All compounds used and the reference preparation (diazepam) were administered *per os*

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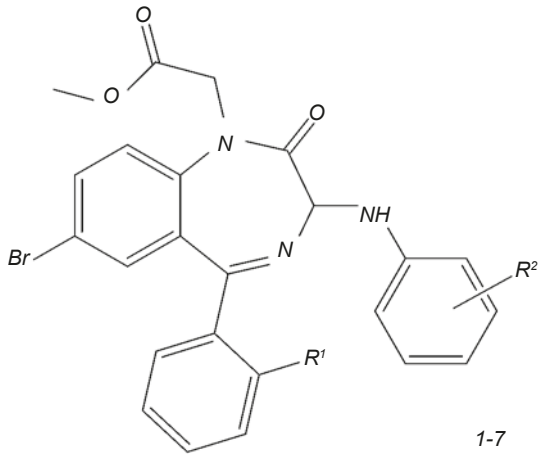
as a suspension in Tween-80 in a dose of 5 mg/kg 30 min prior to the beginning of the study.

RESULTS AND DISCUSSION

Examination of the interaction between compounds 1–7 and receptors using a technique of radioreceptor binding showed that PBDRs are molecular targets for these agents [7] (Table 1). Among the studied substances, we found compounds possessing the highest affinity for PBDRs. These data are in good correlation with the values of anxiolytic activity of these compounds. Among compounds 1–7, compound 2 demonstrated maximum anxiolytic activity. Under its action, the number of punishable water consumptions by animals in the "Conflict situation" test was close to the corresponding index for diazepam (comparison preparation).

Compounds 1–4 were characterized by rather high anxiolytic activity; in the above-mentioned test, the respective indices significantly exceeded the mean value in the control group of animals. Incorporation of a chlorine atom into the *ortho*-position 5 of the phenyl substituent (compounds 5-7) led to a decrease in the number of punishable water consumptions, which was especially significant for compounds 5 and 7. In other words, these compounds demonstrated rather clearly expressed anxiogenic effects (Table 1).

The estimate of the intensity of general motor activity in the "Open field" test showed that compound 5 and diazepam (comparison agent) did not reduce significantly this index, as compared with the control. From this aspect, some sedative effects were typical of compounds 2–4; however, the respective shifts were relatively moderate (Table 1).



F i g. 1. Structural formula of 1-methoxycarbonylmethyl--3-arylamino-7-bromo-5-phenyl-1,2-dihydro-3*H*-1,4-benzodiazepin-2-one. Structural elements, to which functional groups were added, are shown (see the text).

All tested compounds belong to low-toxicity ones; in estimations of acute toxicity, their LD_{50} in all cases exceeded 500 mg/kg.

All stages of the study were carried out according to the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (86/609/EEC, 1986, Strasbourg). In the

T a b l e 1. Affinity for Peripheral Benzodiazepine Receptors (PBDRs) and Anxiolytic Properties of Derivatives of 1-Methoxycarbonylmethyl-3-arylamino-7-bromo-5-phenyl-1,2-dihydro-3*h*-1,4-benzodiazepin-2-one Used in a Dose of 5 mg/kg

Compounds	Functional groups in		Anxiolytic activity in the Conflict situation test (number of punishable water consumptions)	K_i, nM (with respect to PBDRs)	General motor activity in the Open field test (total number of motor acts)
	R^1	R^2			
Control	–	–	9.0 ± 1.1	–	35.3 ± 180.0
1	H	H	69.0 ± 3.7*	740.0 ± 180.0	25.8 ± 3.0
2	H	2-NO_2	109.5 ± 8.3*	19.1 ± 3.0	13.0 ± 41.0
3	H	3-NO_2	40.6 ± 3.9*	360.0 ± 41.0	17.0 ± 10.1
4	H	4-NO_2	38.7 ± 2.6*	87.3 ± 10.1	19.0 ± 7.7
5	Cl	2-NO_2	3.4 ± 1.2*	32.2 ± 7.7	40.0 ± 121.3
6	Cl	3-NO_2	8.2 ± 3.4	398.6 ± 121.3	24.5 ± 121.9
7	Cl	4-NO_2	4.9 ± 2.2	430.9 ± 121.9	25.2
Diazepam	–	–	120.0 ± 4.9*	40 ± 9.2	32.5

Footnote. * Differences from the control group are significant with $P \leq 0.05$.

course of performance of experiments, care of experimental animals and their ethical treatment were performed according to the techniques approved by the Ethics Committee of the State Pharmacological Center of the Ministry of Public Health of Ukraine (protocol No. 20 from September 20, 2005).

The authors of this study, S. A. Andronati, T. L. Karaseva, and A. V. Zamkovaya, confirm that, in the course of performance of the experiments, they had no conflict of interest pertinent to commercial or financial relations and relations with organizations or persons somehow or other related to the study, as well as to interaction within the research group.

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