DOI 10.1007/s10517-018-4006-0

PHARMACOLOGY AND TOXICOLOGY

Nootropic Activity of a Novel (-)-Cytisine Derivative (3a*R*,4*S*,8*S*,12*R*, 12a*S*,12b*R*)-10-Methyl-2-Phenyloctahydro-1*H*-4,12a-Etheno-8,12-Methanopyrrolo[3',4':3,4]Pyrido[1,2-*a*][1,5]Diazocine-1,3,5(4*H*)-Trione

N. S. Makara[1], T. A. Sapozhnikova[1], R. Yu. Khisamutdinova[1], I. P. Tsypysheva[1], S. S. Borisevich[1], A. V. Kovalskaya[1], P. R. Petrova[1], C L. Khursan[1], and F. S. Zarudii[1,2]

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 164, No. 10, pp. 427-432, October, 2017
Original article submitted March 13, 2017

We performed screening of nootropic properties of 10 new derivatives of quinolizidine alkaloid (-)-cytisine. Compounds with β-endo stereochemistry were more active than α-endo-isomers. Under stress conditions (3a*R*,4*S*,8*S*,12*R*,12a*S*,12b*R*)-10-methyl-2-phenyloctahydro-1*H*-4,12a-etheno-8,12-methanopyrrolo[3',4':3,4]pyrido[1,2-*a*] [1,5]diazocine-1,3,5(4*H*)-trione enhanced memory and had a positive effect on cognitive functions of rats. According to molecular docking data, the nootropic activity of the compound can be associated with its affinity for the glutamate-binding subunits GluK1 and GluR2 of the kainate and AMPA receptor, respectively.

Key Words: *(-)-cytisine; nootropic activity; mnestic activity; molecular docking*

Age-related neurodegenerative diseases, such as Alzheimer and Parkinson disease, vascular dementia and other cognitive disorders of various genesis, typical of the population of developed countries with high life expectancy, are an acute medical and social problem [2]. Drug therapy of neurodegenerative disorders usually represents symptomatic treatment and is often associated with complications and side effects [1]. Complexity of pathogenesis of most neurological diseases necessitates the search for new nootropic compounds with low toxicity and wide therapeutic effect and requires the development and application of drugs that affect different stages of the pathological process [11]. Compounds of natural origin, especially plant alkaloids, are of particular interest in this respect [9]. Our previous studies demonstrated that some derivatives of the quinolizidine alkaloid (-)-cytisine **1**, well-known nicotinic acetylcholine receptor ligand, exhibit pronounced nootropic activity [3].

We studied the effect of new (-)-cytisine derivatives **2a,b-6a,b** (Fig. 1) on learning, memory, and cognitive capacities of laboratory animals under stress conditions and evaluated the ability of the leader compound to interact with active sites of ionotropic glutamate receptor subunits: GluR2 (AMPA receptor), GluN2A (NMDA receptor) and GluK1 (kainate receptor) involved in learning and memory formation [5].

[1]Laboratory of Bioorganic Chemistry and Catalysis, Ufa Institute of Chemistry, Russian Academy of Sciences; [2]Department of Pharmacology No. 1, Bashkir State Medical University, Ministry of Health of the Russian Federation, Ufa, Russia. ***Address for correspondence:*** newpharm@anrb.ru. N. S. Makara

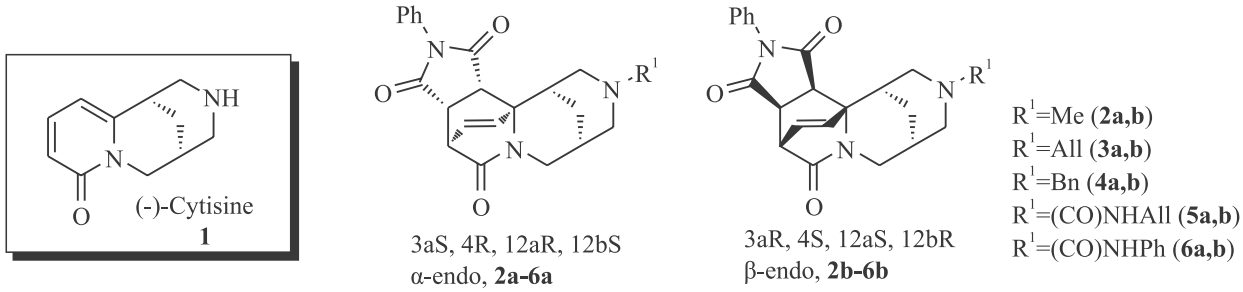

Fig. 1. (-)-Cytisine **1** and its derivatives **2a,b-6a,b**.

MATERIALS AND METHODS

Synthesis of derivatives **2a,b-6a,b** has been described previously [13]. All compounds were isolated individually and completely characterized, their physicochemical constants corresponded to those described previously [13].

In vivo experiments were performed on Wistar rats (n=152) of both sexes weighing 170-200 g. Acute toxicity was determined in outbred male mice (n=42) weighing 18-20 g. The animals were kept under standard vivarium conditions on a standard diet according to the European Convention for the Protection of Vertebrate Animals used for Experiments or Other Scientific Purposes (Strasbourg, 1986).

Specific nootropic activity of compounds **2a-6a** and **2b-6b** was assessed in the conditioned passive avoidance response (CPAR) as described elsewhere [4]. CPAR performance was tested 24 h after learning: latency of the first entry, number of entries into the dark compartment and total time spent in the dark compartment were recorded. Mnestic activity of the compounds was evaluated by the ratio of the difference between the times spent in the dark compartment by animals of the control and experimental group to the time spent in the dark compartment by control animals; the results were expressed in percentage. The effective dose of the leader compound was determined in the CPAR in rats.

Nootropic activity of compounds under stress conditions was studied in extrapolation escape test (EET) using corresponding experimental facility (Open Science). The latency of diving under the edge of the cylinder was recorded for 2 min [4].

The test compounds and the initial alkaloid (-)-cytisine were administered in a screening dose of 50 μmol/kg [7], the reference drug Piracetam (Oblpharm) was administered in a dose of 400 mg/kg. Control animals received equivalent volume of distilled water. Compounds were administered orally 1 h prior to the experiment.

Acute toxicity (LD_{50}) and mean effective dose (ED_{50}) were determined by the Litchfield—Wilcoxon method. The data were processed statistically by Student's t test using Statistica 7.0 software (StatSoft, Inc.). The differences were significant at $p<0.05$.

Preparation of the receptor and ligand structure and molecular docking procedure were performed using LeadIT 2.2.0 software. All calculations were carried out on the cluster computer of the Ufa Institute of Chemistry. Biological targets included active binding sites of native ligands (endogenous agonists, competitive and allosteric antagonists) located in the following subunits of ionotropic glutamate receptors: GluR2 AMPA receptor (PDB codes 1FTM, 1FTL), GluN2A NMDA receptor (PDB code 5U8C), and GluK1 kainate receptor (PDB codes 5MFQ and 5M2V) [6]. Geometric parameters of biological structures were downloaded from Protein Data Bank [6]. The binding site regions were determined by surrounding of the native ligands of the ionotropic glutamate receptors with amino acid residues within a radius of 8 Å. Redocking correctly reproduces the geometric parameters of the ligand and the way of binding to functional amino acids of the receptors determined by crystallography [6]. The root-mean-square deviation (RMSD) value did not exceed 0.8 Å. The algorithm of incremental construction was used throughout the step for molecular docking: the ligand structure is first fragmented, the rigid core of the molecule is placed in the active site, and then is completed to the original structure. In this case, there can be a lot of docking solutions, and the choice of optimal solution is based on minimum binding energy ΔG_{FlexX} (kJ/mol) and minimum RMSD value. Thus, selected position is assessed by the energy of ligand affinity for the binding site ΔG_{HYDE} (kJ/mol), taking into account the influence of the solvent (water), as well as by the ligand efficiency (LE):$LE=\Delta G_{HYDE}/N$, where N is the number of non-hydrogen atoms [12].

RESULTS

According to the obtained data, practically all compounds **2b-6b** of the β-endo range improved the first stage of CPAR: latency increased, the number of en-