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Preparative synthesis and pharmacological activity of Albicar racemate and enantiomers

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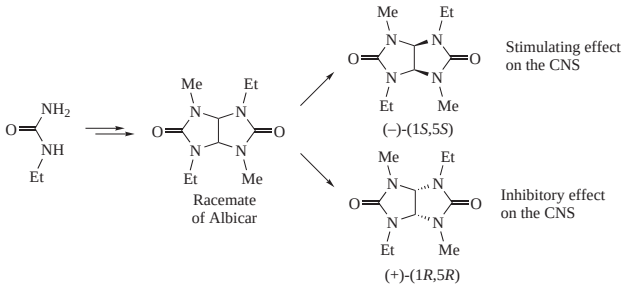
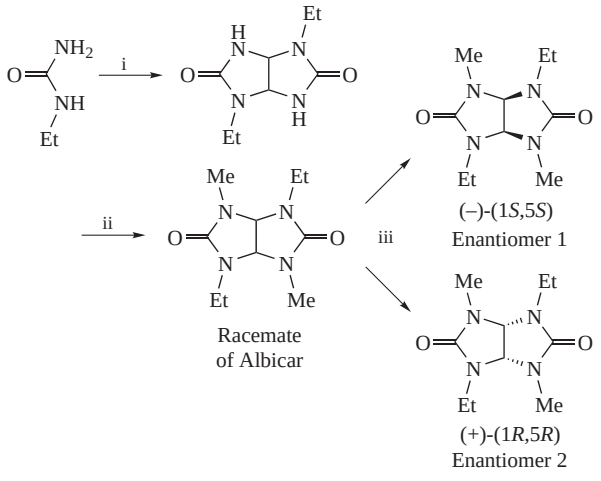
Racemic Albicar (2,6-diethyl-4,8-dimethylglycoluril) has been synthesized and resolved into enantiomers on a preparative scale by chiral HPLC. A comparison of the pharmacological effects of these compounds has been performed for the first time. It has been demonstrated that the (–)-(1*S*,5*S*)-enantiomer exerts a stimulating effect on the central nervous system due to activation of the serotonergic system, since it potentiates the effects of 5-hydroxytryptophan (the serotonin precursor), while the antagonist of serotonin receptors blocks its activity; the (+)-(1*R*,5*R*)-enantiomer evinces an inhibitory effect.



Glycolurils represent a class of pharmacologically active compounds.[1] One of them, 2,4,6,8-tetramethylglycoluril (Mebicar, Mebix or Adaptol), is used in Russia for the treatment of neuroses.[2] Yet another compound, 2,6-diethyl-4,8-dimethylglycoluril (Albicar), has passed preclinical trials and has been recommended for clinical trials as a tranquillizer and an agent for the treatment of vegetative neuroses.[1(c)] Unlike achiral Mebicar, Albicar 'gull wings'-shaped molecule[2(b)] is chiral since its C^1 and C^5 atoms are asymmetric. The pharmacologic tests of Albicar were performed using a racemate. However, in general only one enantiomer is useful in racemic pharmaceutical agents (see, *e.g.*, ref. 3). Moreover, of two enantiomeric diastereomers of glycolurils obtained from (*R*)- and (*S*)-*N*-carbamoylmethionine and 4,5-dihydroxyimidazolidin-2-one only one manifests neurotropic activity.[1(g),(j)]



Scheme 1 *Reagents and conditions*: i, glyoxal, H_2O, pH 1 (HCl), reflux, 1 h;[4(a)] ii, Me_2SO_4, KOH (aq.), 70–75 °C, 3 h, then HCl, 20 °C, extraction (CH_2Cl_2), column chromatography (silica, CH_2Cl_2) and crystallization from Et_2O;[2(a)] iii, chiral HPLC on Chiralcel OD.

In search for an agent with optimum properties based on Albicar, in this study we synthesized an Albicar racemate and for the first time accomplished its separation into enantiomers. A comparative study of the pharmacological properties of the racemate and enantiomers was performed.

Racemic Albicar was synthesized in two stages (Scheme 1)[†] by the reaction of ethylurea with glyoxal followed by N-methylation of intermediate 2,6-diethylglycoluril.[2(a),4(a)]

Though we have previously synthesized and characterized Albicar enantiomers,[2(a)] that method cannot be used to produce these compounds in amounts sufficient for biological trials (50 mg of each). Therefore, we were the first to use HPLC for the preparative separation of the racemate into the enantiomers. Various columns, eluents and separation conditions were tested. Attempts to separate the racemate on Chiralpak AD and Chiralcel OJ columns (Daicel) using propan-2-ol–hexane system in various ratios were unsuccessful. The best results were achieved on using two sequentially connected commercial columns Chiralcel OD.[‡] Taking into account the published data,[2(a)] the configurations of asymmetric bridging carbon atoms were assigned as 1*S*,5*S* for enantiomer 1 (retention time 11 min) and 1*R*,5*R* for enantiomer 2 (retention time 28 min).

[†] *2,6-Diethyl-2,4,6,8-tetraazabicyclo[3.3.0]octane-3,7-dione (2,6-diethylglycoluril)*. Yield 60%, mp 286–288 °C (lit.,[4(a)] mp 286–288 °C). ^{1}H NMR (DMSO-d_6) δ: 1.02 (t, 6H, Me, ^3J 7.2 Hz), 2.94–3.06 (AMX_3, 2H, CH_2N, ^2J 14.2 Hz, ^3J 7.1 Hz), 3.15–3.27 (AMX_3, 2H, CH_2N, ^2J 14.2 Hz, ^3J 7.1 Hz), 5.22 (s, 2H, CH), 7.48 (s, 2H, 2NH).

2,6-Diethyl-4,8-dimethyl-2,4,6,8-tetraazabicyclo[3.3.0]octane-3,7-dione (racemic Albicar). Yield 72%, mp 108–110 °C (lit.,[4(a)] mp 108–110 °C). ^{1}H NMR (DMSO-d_6) δ: 1.08 (t, 6H, Me, ^3J 7.1 Hz), 2.81 (s, 6H, Me), 3.16 (dq, 2H, CH_2N, ^2J 14.2 Hz, ^3J 7.1 Hz), 3.36 (dq, 2H, CH_2N, ^2J 14.2 Hz, ^3J 7.1 Hz), 5.18 (s, 2H, CH) [cf. ref. 4(*b*)].

To study the pharmacological effect of the compounds on the central nervous system (CNS), we used internationally recognized methods, namely, the 'open field' and the 'elevated plus maze' tests.[5,§]

At the first stage, we studied the behavioural reactions of mice after administration of racemic Albicar in doses from 50 to 300 mg kg^{-1} in the 'open field' integral test and in an effective dose of 150 mg kg^{-1} in the 'elevated plus maze' test (Figures S1 and S2, Online Supplementary Materials). This 'dose–effect' relationship for Albicar was found to be similar to those of many anxiolytics and antidepressants: small doses show some stimulating effect, while higher doses cause CNS depression. The transition to CNS depression by Albicar occurs at doses around 150 mg kg^{-1}. It is expressed as a *ca*. threefold decrease in the motional and exploratory activity. Furthermore, upon administering 75 mg kg^{-1} Albicar, the motional activity of mice did not obey a normal distribution. Namely, the mice were distinctly divided into two populations: the behaviour of one half did not change, whereas the locomotion of the other half decreased abruptly. This effect may be due to the existing modes of behaviour (defeatists/tyrants), *i.e*., the agent has a sedative effect on some mice and normalizes the behaviour of others.[6] In the 'elevated plus maze' test, administering the racemate did not result in changes in the behavioural reactions of mice in comparison with the reference group.

At the second stage, a comparative *in vivo* study of the racemate and enantiomers 1 and 2 in the 'open field' test was performed (Figure 1). The Albicar racemate was administered in 150 mg kg^{-1} dose, while enantiomers 1 and 2 (EN1 and EN2) in 75 mg kg^{-1} dose. The first group of mice was placed into 'open field' 30 min after administering the compounds, the second one in 60 min, and the third one in 90 min. The data were compared with those for three reference groups to which physiological saline was administered abdominally 30, 60 and 90 min before the test. It was found that Albicar racemate did not affect the motional and exploratory activity of laboratory animals by the 30th minute of the experiment, but it acted as an inhibitor on both parameters by the 60th minute. Enantiomer 1 stimulated the motional and exploratory activity by the 30th minute of the experiment and inhibited both parameters by the 60th minute. Enantiomer 2 showed an inhibitive effect on the motional and exploratory activity throughout the entire experiment. Apparently, the lack of effect of Albicar racemate on the motional and exploratory activity by the 30th minute of the experiment is due to the mutually exclusive effects of the two enantiomers, while both enantiomers act in the same way by the 60th minute.

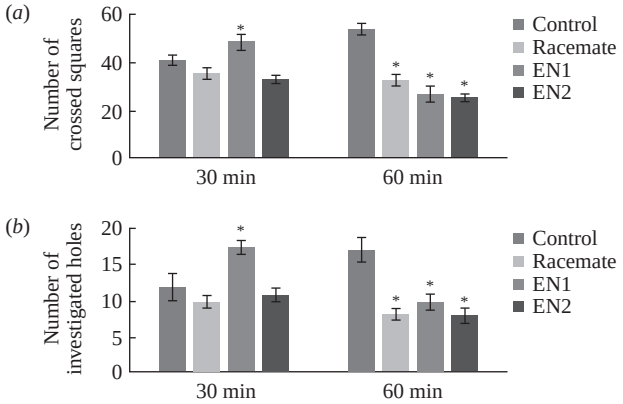


Figure 1 Variation of parameters in the 'open field' test after administering Albicar racemate and enantiomers (EN1 and EN2).

Comparison of the behavioural reactions of mice after administering racemic Albicar and its enantiomers against the effects of Yohimbine and Ketanserin was carried out in order to study the assumed mechanism of action of enantiomers 1 and 2. It is known that the action of Mebicar (Mebix, Adaptol) is related to its ability to affect the serotonergic system by enhancing the action of tryptophane, which is a serotonin precursor.[7] To identify the presence of a serotonergic component and an adrenergic component intertwined with the former in the mechanism of Albicar action, we performed experiments to estimate the effects of Albicar racemate and enantiomers on the action of Yohimbine and Ketanserin in the 'open field' test and on the action of 5-hydroxytryptophan the direct serotonin precursor, which can pass the hematoencephalic barrier, unlike the mediator itself.[8]

In our experiments, enantiomer 1 removes the inhibitive effect of Yohimbine on the motional and exploratory activity of laboratory animals on the 30th minute of the experiment, *i.e*., at the peak of its stimulating action (Figure 2). Racemic Albicar and enantiomer 2 throughout the experiment and enantiomer 1 on the 60th minute of the experiment demonstrate an inhibitive effect on the behavior of animals. Ketanserin which does not affect the psycho-

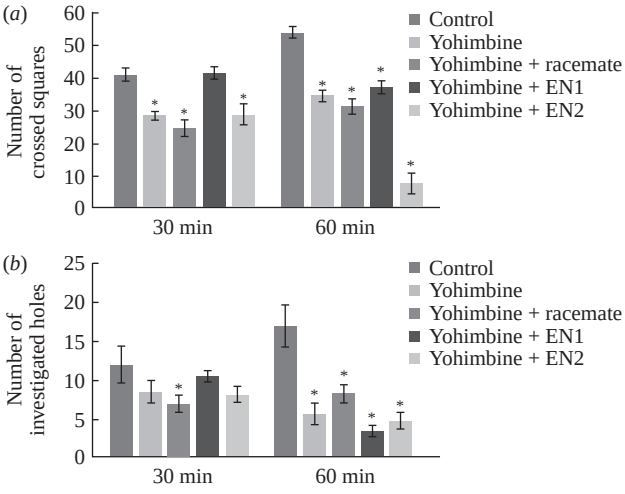


Figure 2 Changes in the 'open field' test results after administering Albicar racemate and its enantiomers in the presence of Yohimbine.

‡ *Preparative resolution of racemic Albicar*. The racemate (100 mg) was dissolved in propan-2-ol (1 ml), and this solution (20 μl) was injected into a Rheodyne 7725 loop sampling valve (100 μl) of chromatographic system comprising Waters 501 pump and RIDK 102 chromatographic detector (Laboratorni pristroje Praha). The micropreparative separation was carried out on two sequentially connected commercial Chiralcel OD 4.5×250 mm columns, particle size 10 μm (Daicel, US Department, Chiral Technologies, Inc., West Chester, PA, USA). Propan-2-ol–hexane (1:1, v/v) was used as the mobile phase (flow rate 1.5 ml min^{-1}). The retention times of Albicar enantiomers were 11 min (enantiomer 1) and 28 min (enantiomer 2) with full separation of the peaks to the baseline.

*(–)-(1*S*,5*S*)-2,6-Diethyl-4,8-dimethyl-2,4,6,8-tetraazabicyclo[3.3.0]-octane-3,7-dione (enantiomer 1)*. Mp 138–139 °C (lit.,[2(a)] mp 138–139 °C). $[\alpha]_D^{20}$ = –41.5° (*c* 1.6, H_2O).

*(+)-(1*R*,5*R*)-2,6-Diethyl-4,8-dimethyl-2,4,6,8-tetraazabicyclo[3.3.0]-octane-3,7-dione (enantiomer 2)*. Mp 138–139 °C (lit.,[4(a)] mp 138–139 °C). $[\alpha]_D^{20}$ = +41.7° (*c* 1.6, H_2O).

§ All experiments involving animals were performed in accordance with the guidelines set forth by the European Communities Council Directive of November 24, 1986 (86/609/EEC) and the protocol of experiments approved by the Animal Care and Use Committee of IPAC RAS. Mongrel albino male mice (1.5–2 months old, 20–25 g) were purchased from the Laboratory Animal Breeding Facility (Branch of M. M. Shemyakin–Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Puschino, Moscow Region) and were allowed to acclimate to their environment in the vivarium for at least 5 days before experiments. Animals were kept in sawdust-lined plastic cages in a well-ventilated room at 20–22 °C in a 12 h light/dark cycle, 40−70% relative humidity, and given *ad libitum* access to food and water.

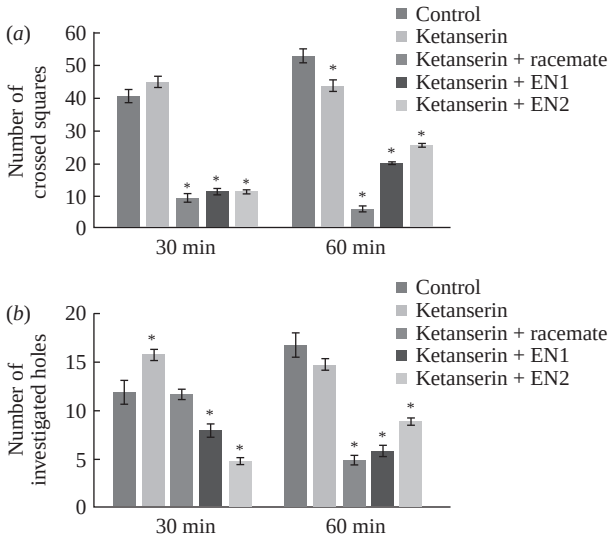


Figure 3 Changes in the results of the 'open field' test after administering Albicar racemate and its enantiomers in the presence of Ketanserin.

pharmacological characteristics of animals blocks the stimulating properties of enantiomer 1 by the 30th minute and enhances the inhibitory properties of both enantiomers and Albicar racemate throughout the entire experiment (Figure 3).

In the tests with 5-hydroxytryptophan, Albicar racemate and enantiomer 2 did not reliably change the intensity of hyperkinesis caused by 5-hydroxytryptophan. However, enantiomer 1 reliably potentiated the action of the dose of 300 mg kg^{-1} 5-hydroxytryptophan: the number of head shakings increased to 50.00±5.33 in comparison with 27.80±2.40 in the reference group of animals (Figure S3).

In conclusion, we have performed a two-stage synthesis of Albicar racemate. To obtain enantiomers 1 and 2 in 50 mg amounts, a chiral HPLC method for racemate separation has been used for the first time. An *in vivo* study of Albicar racemate showed the effective dose to be 150 mg kg^{-1}, which was then used in the comparative analysis of the effects of Albicar racemate and enantiomers (the dose of the latter was half that, *i.e.*, 75 mg kg^{-1}). The lack of an effect of Albicar racemate on the motional and exploratory activity by the 30th minute of the experiment was found to be due to the competing mutually exclusive effects of the two enantiomers (enantiomer 1 has a stimulating effect on the CNS while enantiomer 2 has an inhibitive effect), whereas both enantiomers act in an inhibitive way at the 60th minute. Comparison of the behavioural reactions of mice after administering racemic Albicar and enantiomers with background action of Yohimbine and Ketanserin has shown that the action of enantiomer 1 is most likely due to the activation of the serotoninergic system, since it potentiates the effects of 5-hydroxytryptophan, a serotonin precursor, while the antagonist of serotonin receptors blocks its activity. The mechanism of action of enantiomer 2 requires further studies.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2018.05.030.

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