Research Results in Pharmacology

Research Results in Pharmacology 7(2): 31–37
UDC: 615.214.22
DOI 10.3897/rrpharmacology.7.67499

Research Article

Screening of anxiolytic properties and analysis of structure-activity relationship of new derivatives of 6-(4-methoxy)-7H-[1,2,4]triazolo[3,4-a][2,3] benzodiazepine under the code RD

Maria O. Skripka[1], Alexander A. Spasov[1,2], Dmitriy V. Maltsev[2], Mikhail V. Miroshnikov[1], Dmitriy S. Yakovlev[2], Kira T. Sultanova[2], Maxim A. Kochergin[1], Lyudmila N. Divaeva[3]

1 *Volgograd State Medical University, Department of Pharmacology and Bioinformatics, 1 Pavshikh Bortsov, Volgograd 400131, Russia*

2 *Volgograd Medical Research Center, 1 Pavshikh Bortsov Sq., Volgograd 400131, Russia*

3 *Research Institute of Physical and Organic Chemistry, Southern Federal University, 194/2 Stachki Ave., Rostov-on-Don 344090, Russia*

Corresponding author: Maria O. Skripka (rete.mirabile.renis@gmail.com)

Academic editor: Mikhail Korokin ♦ **Received** 18 March 2021 ♦ **Accepted** 17 May 2021 ♦ **Published** 15 June 2021

Citation: Skripka MO, Spasov AA, Maltsev DV, Miroshnikov MV, Yakovlev DS, Sultanova KT, Kochergin MA, Divaeva LN (2021) Screening of anxiolytic properties and analysis of structure-activity relationship of new derivatives of 6-(4-methoxy)-7H-[1,2,4] triazolo[3,4-a][2,3]benzodiazepine under the code RD. Research Results in Pharmacology 7(2): 31–37. https://doi.org/10.3897/rrpharmacology.7.67499

Abstract

Introduction: Searching for new compounds with anti-anxiety activity resulting from the combination of privileged scaffolds is a promising direction in medicinal chemistry and in the development of new drugs. Anxiolytic potential and cytotoxic properties of previously synthesized molecules, containing fragments of 2,3-benzodiazepine and 1,2,4-triazole – 6-(4-methoxyphenyl)-7H-[1,2,4]triazolo[3,4-A][2,3]benzodiazepines under the generic code RD were studied.

Materials and methods: Screening for anxiolytic activity was performed on elevated plus maze (EPM) and open field (OF) test models. Structural and functional analysis of the anti-anxiety activity of the studied substances was carried out. A degree of muscle relaxant effect of the substances was assessed in the tests Grid, Wire, and Rotarod. A cytotoxicity study of RD compounds was carried out using an MTT assay on human hepatocellular carcinoma cells HepG2.

Results and discussion: For a number of novel triazolo[3,4-a][2,3]benzodiazepine derivatives, a prominent anxiolytic activity was manifested in terms of EPM test. The results of OF test were consistent with the obtained data and confirmed the presence of the sought activity in the leading compounds. There was no significant effect on muscle tone for the compounds under study. It was observed that RD compounds possessed no cytotoxic properties and were safe for further studies in vivo.

Conclusion: Among the new derivatives of 6-(4-methoxyphenyl)-7H-[1,2,4]triazolo[3,4-a][2,3]benzodiazepine under the code RD, substances (RD-4, 12, 13) with a high anxiolytic activity comparable to diazepam and tofisopam were found. The most promising compound is RD-4 due to its pronounced anxiolytic and low cytotoxic properties.

Keywords

triazolo[3,4-A][2,3]benzodiazepines, combined structures, HepG2, anxiolytic, screening, open field, elevated plus maze, muscle relaxation.

Introduction

Anxiety disorders, such as panic attacks/agoraphobia, generalized anxiety disorder, and social phobia are the most common mental disorders (Bandelow 2020). Anxiety-depressive diseases are among the main causes of disability in the world and contribute significantly to the global burden of diseases (World Health Organization, 2020). According to the data obtained by Wang et al. (2020), 53.8 of respondents in China rated the psychological impact of the COVID-19 outbreak as moderate or severe, 16.5% reported moderate to severe depression symptoms, and 28.8% reported moderate to severe anxiety symptoms (Sher 2020). During the pandemic, the risk of developing pathological anxiety (Ornell et al. 2020), depression (Shigemura et al. 2020), sleep disturbances (Xiao et al. 2020), and suicidal behavior (Lai et al. 2020) increased significantly. For the treatment of these socially significant diseases, psycho- and pharmacotherapy are used in various combinations. Despite high efficiency and widespread clinical use of classical benzodiazepine derivatives (diazepam, lorazepam), these drugs are not the drugs of choice for the treatment of anxiety disorders at the present stage due to their powerful addictive potential (Balon and Starcevic 2020). The known side effects of benzodiazepine drugs also include unwanted sedation and muscle relaxation, which limit the use of this class of drugs in patients whose profession is associated with increased concentration of attention (Platt et al. 2016). As a result of the development of pharmacotherapy, "daytime" sedatives were created, devoid of these side effects (fabomotizol, phenibut, mebicar, etc.). However, the effectiveness of these drugs is not expressed in all patients with phobic disorders, and the onset of the anxiolytic action is often delayed and is noted only a few weeks after starting the drug (Uyanaev and Fisenko 2006). Daytime sedatives – the products of benzodiazepine scaffold optimization, such as tofisopam, – are characterized by a pronounced anxiolytic effect, but also by the rapid development of tolerance to their action, as well as an increase in aggressiveness and psychomotor agitation. Currently, there is an active search for new compounds with a short latency period and mitigated side effects, as well as more pronounced anxiolytic properties (Miroshnikov et al. 2020).

In previous studies, the products of the combination of a diazepine scaffold with benzimidazole moiety were studied – 2,3,4,5-tetrahydro[1,3]diazepino[1,2-a]benzimidazole hydrochlorides under the general code DAB (Spasov et al. 2018). For various representatives of this series of compounds, a wide range of neurotropic effects have been noted – anxiolytic, antidepressant, anticonvulsant, hypnotic, and some others (Miroshnikov et al. 2020, Spasov et al. 2020a); however, no adverse drug reactions of the benzodiazepine group were noted for them. Further search for effective anxiolytics among the combinations of the benzodiazepine core structure with the hit structures of various chemical classes seems promising: in the present study, the products of the combination of 1,2,4-triazole and 2,3-benzodiazepine, derivatives of 6-(4-methoxyphenyl)-7H-[1,2,4]triazolo[3,4-A][2,3] benzodiazepine under the general laboratory code RD were investigated.

Materials and methods

Experimental animals

For the study, 360 white outbred two-month-old male mice weighing 20±2 g were used, obtained from Rappolovo Nursery, Leningrad region. The animals were kept in a vivarium with a natural light regimen on a standard diet of laboratory animals, with access to food and water *ad libitum*. The keeping of the animals corresponded to the rules of Good Laboratory Practice during preclinical research in the Russian Federation (GOST 351.000.3-96 and 51000.4-96), the Order of the Ministry of Health of the Russian Federation dated August 23, 2010 No. 708n "*On the Approval of the Rules of Laboratory Practice*". The experimental procedures on the animals were carried out in accordance with the Local Ethics Committee of Volgograd State Medical University, Volgograd, Russia (Protocol No. IRB 00005839 IORG 0004900 (OHRP)). The mice were randomly assigned to 60 experimental groups – 12 groups for each test (n = 6).

Cell cultures

Human hepatocellular carcinoma cells, HepG2 (CLS Cell Lines Service), were used for assessment of the cytotoxic properties of the compounds. Cells were cultured in Gibco F-12 medium containing 10% fetal calf serum (Gibco), 1% penicillin-streptomycin (Gibco), 1% essential amino acids (NEAA, Sigma-Aldrich), and 2 mM sodium pyruvate (Sigma-Aldrich) in a CO_2 incubator at 37 °C in an atmosphere of 5% CO_2.

Drugs and treatment

Synthesis of compounds – derivatives of 6-(4-methoxyphenyl)-7H-[1,2,4]triazolo[3,4-a][2,3]benzodiazepine under the code RD-2, 13 (Khabarov et al. 2009), RD-3, 4, 5, 10, 11, 12, 14 (Kharaneko et al. 2013) – was carried out according to the methods described in these works. Comparison drugs were ones with a known anxiolytic effect, structural analogues of the studied substances – diazepam [7-chloro-1-methyl-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one] (RelaniumTM, Polfa, Poland, 1 mg/kg) and tofisopam [1-(3,4-dimethoxy-phenyl)-4-methyl-5-ethyl-7,8-dimethoxy-5H-2,3-benzodiazepine] (GrandaxinTM, EGIS CJSC Pharmaceutical Plant, Russia, 2 mg/kg (Skripka et al. 2018).

For the evaluation of cytotoxicity properties, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich) and dimethyl sulfoxide (DMSO; Helicon; used as a solubilizer) were used. The substanc-

es were tested over a concentration range from 0.001 to 10 μM. Doxorubicin (Sigma-Aldrich) was used at equal concentrations as a positive control drug.

Experimental design

At the first stage of the study, the safety of the new compounds was assessed using an MTT assay on human hepatocellular carcinoma cells HepG2. Further, to study the anxiolytic activity of the compounds under the code RD, the methods of Elevated plus maze (EPM) and Open field (OF) were applied; to assess the development of muscle relaxation under the influence of these substances, a battery of tests was used: Rotarod, Grid, and Wire. The studied substances were injected into mice with an atraumatic metal probe intragastrically 30 minutes prior to a test, at doses equimolar to diazepam (Taran et al. 2017). The animals of the control group were injected with a solvent (distilled water) in a volume of 0.1 ml per 10 g of animal's weight.

Cytotoxicity assessment

Evaluation of cytotoxic properties of a new series of compounds was carried out according to the technique described earlier (Yakovlev et al. 2020, Maltsev et al. 2021). The cell suspension was seeded into a 96-well plate, and incubated for 24 h to allow the cells to adhere to the bottom of the plate. Thereafter, the compound under study or the positive control drug, doxorubicin, was added to the wells in the concentration range from 0.001 to 10 μM. The control wells were filled with an equivalent volume of solvent. The exposure to the compounds was carried out within 48 h. The medium was subsequently removed, and DMSO was added to dissolve the formazan crystals that were formed. The absorbance was measured using a CLARIOstar microplate reader (BMG Labtech) at 555 nm (reference λ = 650 nm), reflecting the quantitative assessment of the MTT reagent converted into formazan by mitochondrial and cytoplasmic reductases.

Behavioral tests

Elevated plus maze

The EPM technique is based on the rodents' natural preference for dark burrows, as well as on the fear of being in open areas and falling from a height (Kraeuter et al. 2019). The animals were placed in the center of the unit and, for 5 min, the following were recorded: the time spent in the open arm (s), the number of entrances into the open arm, the total number of transitions between the arms, as well as the detailing of the transitions between the dark and light arms.

Open field test and spontaneous locomotor activity assessment

The OF test was used to assess the behavioral patterns of the animals. Within 5 minutes of the test, the number of transitions between the quadrants (horizontal locomotor activity), the number of rearings (vertical locomotor activity), the number of holes examined (exploratory activity), and the number of entries to the center of the unit were recorded. In addition to the behavioral activity of the animals, the OF technique also made it possible to assess the nonspecific muscle relaxant capacity of new compounds (Maltsev et al. 2020). A battery of muscle relaxation tests – Rotarod, Grid, and Wire – were described previously (Spasov et al. 2020b).

Statistical analysis

For cytotoxicity study, statistical data processing was carried out using MARS Data Analysis Software, Microsoft Office Excel 16 and GraphPad Prism v.8.0 and using nonlinear regression analysis methods. For behavioral experiments, the results were statistically processed by means of GraphPad Prism 8.0 software, using the Kruskal-Wallis test and post-processing with the Dunn's test. The data are presented as mean ± standard error of mean (M±SEM). The $p \leq 0.05$ values were considered statistically significant.

Results and discussion

During the cytotoxicity study of compounds RD-2, 3, 4, 5, 10, 11, 12, 13, 14, the absorption values were obtained at 555 nm (reference λ = 650 nm), reflecting the quantitative assessment of the MTT reagent (bromide 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium, Sigma-Aldrich) converted into formazan (dimethyl sulfoxide, Helicon, was used as a solubilizer) by mitochondrial and cytoplasmic reductases. For compounds RD-10, 11, 12, 13, 14, the LC_{50} value (the concentration that suppresses the vital activity of cells by 50% relative to the intact control) lies within the range 0.12–9.96 nM. For compounds RD-2, 3, 4, 5, the LC_{50} value is more than 10 μM and is outside the maximum investigated concentrations. Thus, at the maximum studied concentration of 10 μM, cell survival for compound RD-2 was – 74.3±2.19%; for RD-3 – 80.7±0.67%; for RD-4 – 79.0±3.06%; and for RD-5 – 69.3±4.67%. For doxorubicin, this indicator was 25.2±2.12%. The obtained data indicate a low level of cytotoxicity of the studied compounds in comparison with the reference cytostatic agent doxorubicin (LC_{50} = 5.7 μM), which makes it possible to view these substances as promising for further study of their anxiolytic properties.

At the second stage of the study, the anxiolytic activity of new benzodiazepine derivatives in the EPM test was screened. In the row {[6-(4-methoxyphenyl)-7H-[1,2,4]triazolo[3,4-a][2,3]benzodiazepin-3-yl]methyl}amine (RD-14) – {2-[6-(4-methoxyphenyl)-7H-[1,2,4]triazolo[3,4-a][2,3]benzodiazepin-3-yl]ethyl}amine (RD-3) – 3-[6-(4-methoxyphenyl)-7H-[1,2,4]triazolo[3,4-a][2,3]benzodiazepin-3-yl]propyl}amine (RD-11), the level of anti-anxiety activity was visibly reduced. Thereby, under the influence of RD-11, the mice spent in the open arm of

EPM, on average, 12% less time than in the RD-3 group, and 30% less time compared to the RD-14 group. A similar relationship was noted in terms of the number of enterances into the open arm. Thus, lengthening the side chain by increasing the number of methyl groups preceding the amino group negatively affects the anti-anxiety effect of the new compounds (Table 1).

The introduction of a propionic acid residue (RD-5) or a phenyl substituent (RD-10) into position 3 of the triazole ring did not result in the manifestation of anxiolytic properties of the substances: their effect corresponded to the control values. At the same time, the presence of 2-furyl (RD-4) in the same position led to a significant increase in the sought effect, both in time and in the number of entrances into open arms, at a level comparable to diazepam at a dose of 1 mg/kg ($p \leq 0.05$). The most active compounds of the studied series were 3-(2-furil)-6-(4- methoxyphenyl)-7H-[1,2,4]triazolo[3,4-a][2,3] benzodiazepine (RD-4), 3-(1,3-dimethyl-1H-pyrazol-5-yl)-6-(4-methoxyphenyl)-7H-[1,2,4]triazolo[3,4-a] [2,3]benzodiazepine (RD-12) and 6-(4-methoxyphe-

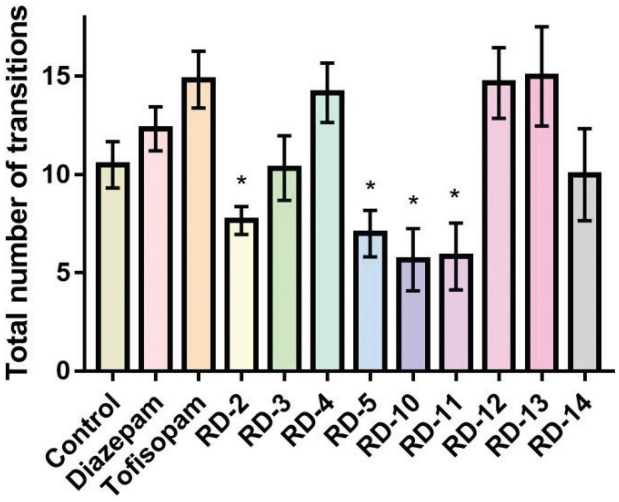
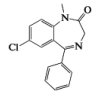
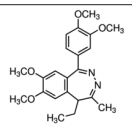
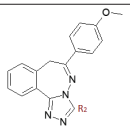


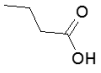
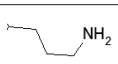
Figure 1. Influence of Diazepam (1 mg/kg), Tofisopam (2 mg/kg) and RD Compounds[#] on Locomotor Activity of Mice in Elevated Plus Maze test (M±SEM). **Note:** * – differences are significant compared to control ($p \leq 0.05$, Kruskal-Wallis test, Dunn's post hoc test); [#] – doses are equimolar to diazepam at a dose of 1 mg/kg.

Table 1. Anxiolytic Effect of Benzodiazepine Derivatives in Elevated Plus Maze test (M± SEM)

Compound	Chemical structure	Time spent in open arms (s)	Number of entries to open arms
Control	–	36.8±10.04	2.8±0.60
Diazepam (1 mg/kg)		94.8±15.24*	3.2±0.68
Tofisopam (2 mg/kg)		102.2±3.62*	5.3±0.49*



7H-[1,2,4]triazolo[3,4-a][2,3]benzodiazepine with R_1 = 6-(4-methoxyphenyl)

Compound	R_2	Time spent in open arms (s)	Number of entries to open arms
RD-2#		56.17±4.91*	2.0±0.37
RD-3#		61.0±16.34	2.7±0.56
RD-4#		97.0±7.22*	5.8±0.65*
RD-5#		44.8±6.86	1.7±0.33
RD-10#		34.0±7.42	1.7±0.42
RD-11#		54.5±18.8	2.2±0.70
RD-12#		113.2±19.68*	5.7±0.99*
RD-13#	$-CH_3$	106.3±26.96*	5.8±1.58*
RD-14#		76.7±7.53*	3.5±0.92

Note: * – differences are significant compared to control ($p \leq 0.05$, Kruskal-Wallis test, Dunn's post hoc test); [#] – doses are equimolar to diazepam at a dose of 1 mg/kg.

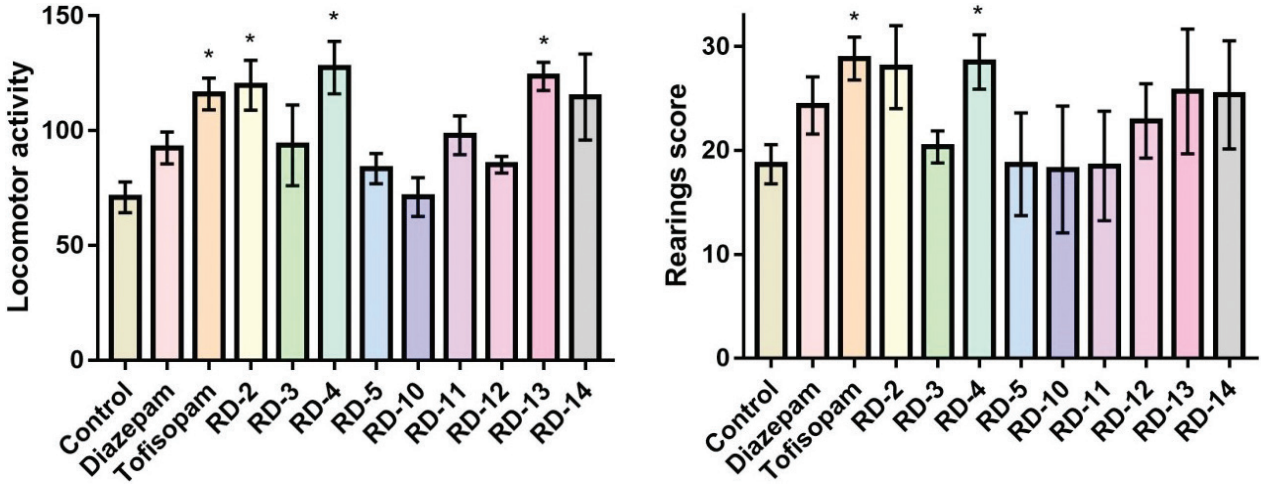


Figure 2. Influence of Diazepam, Tofisopam and RD compounds[#] on Locomotor Activity of Mice in Open Field test (M±SEM). **Note:** * – differences are significant compared to control ($p \leq 0.05$, Kruskal-Wallis test, Dunn's post hoc test); [#] – doses are equimolar to diazepam at a dose of 1 mg/kg.

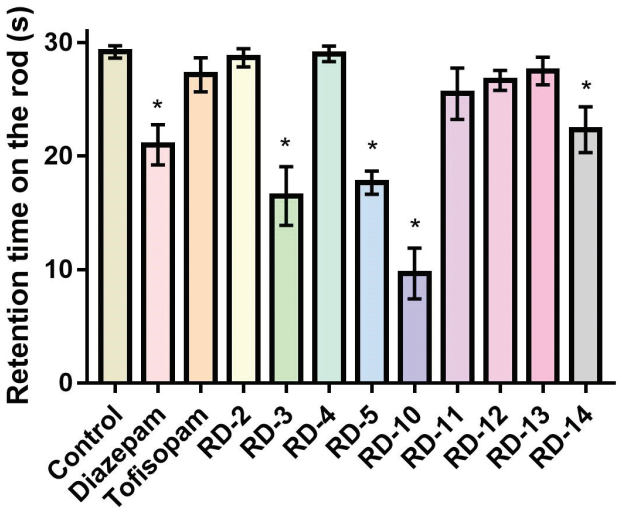


Figure 3. Muscle Relaxant Properties of Diazepam, Tofisopam and RD compounds[#] in Rotarod test (M±SEM). **Note:** * – differences are significant compared to control ($p \leq 0.05$, Kruskal-Wallis test, Dunn's post hoc test); [#] – doses are equimolar to diazepam at a dose of 1 mg/kg.

nyl)-3-methyl-7H-[1,2,4]triazolo[3,4-a][2,3]benzodiazepine (RD-13). It was noted that the introduction of the pyrrole cycle at position 3 (RD-12) had practically no effect on the value of the anti-anxiety effect of the substances. However, lengthening the side chain of RD-13 by only one methyl group (RD-2) led to a decrease in the desired activity by almost 2–3 times ($p \leq 0.05$). It can be noted that the synthesized RD compounds are structurally closer to tofisopam than to diazepam, and the core structure of 2,3-benzodiazepine leads to the manifestation of a slightly higher anti-phobic effect than 1,4-benzodiazepine. The total number of the mice's transitions in the EPM test was not significantly reduced in comparison with controls in any experimental group, which may indicate the absence of a significant muscle relaxant effect of the RD compounds. For the leading compounds RD-4, 12, 13, the number of transitions between the light and dark arms of the EPM was at the level of tofisopam ($p \leq 0.05$), which confirms the presence of an anti-anxiety effect in them.

At the third stage, the behavioral activity of the animals was assessed in OF test. The profile of the locomotor activity of the animals under the influence of the experimental compounds was not significantly changed, which is consistent with the results of EPM test (Fig. 2). Under the influence of RD compounds, the profile of locomotor activity is more similar to such of tofisopam than to that of diazepam, which can be explained by a high degree of resemblance between molecules of tofisopam and the compounds under study.

The level of exploratory (search) activity of the animals was the highest when compounds RD-4, 11, 14 were administered to the animals. For compounds RD-12 and 13, the search activity was higher than in the control GROUP; however, it did not reach the values of the comparison groups. The number of entrances to the center of OF for the leading compounds RD-4, 12, 13 does not differ significantly from the values of the diazepam group, being at its level ($p \leq 0.05$). The results of Grid and Wire tests are at the level of the control values for the entire range of the RD compounds, and in Rotarod test only the RD-2, 4, 11, 12, 13 values do not deviate from the control values (Fig. 3).

The obtained data are consistent with the previous studies (Spasov et al. 2018) on the combination of benzodiazepine and benzimidazole scaffolds, which also resulted in obtaining the compounds – diazepino[1,2-a]benzimidazoles – with a pronounced neurotropic activity due to the native diazepine fragment, and mitigated undesirable effects due to the presence of the benzimidazole group. According to the data, the acute toxicity of diazepam when administered orally to the mice is 48 mg/kg (Vinogradova et al. 1983). For 1,2,4-triazole derivatives, the LD_{50} is at the level of 1250–2000 mg/kg, which makes it possible to classify this class of compounds as low-toxic (Ovsyannikova et al. 2017). 1,2,4-triazoles are also characterized by antimicrobial, antifungal, anti-inflammatory, analge-

sic, antidepressant, antiplatelet, and antioxidant activities (Klyon et al. 2008), which suggests the pleiotropic action of this heterocycle, as well as the presence of psychotropic properties in triazole derivatives. Thus, by combining the diazepine and triazole fragments, it is possible to obtain novel, less toxic drugs with a pronounced neurotropic activity and reduced adverse reactions relative to classical benzodiazepines, which is confirmed by the results of the conducted study.

Conclusion

Thus, the search for compounds with anxiolytic activity among derivatives of 6-(4-methoxyphenyl)-7H-[1,2,4]triazolo[3,4-a][2,3]benzodiazepine (RD) on the elevated plus maze model made it possible to identify the compounds with a pronounced anti-anxiety activity. The leading compounds were 3-(2-furyl)-6-(4-methoxyphenyl)-7H-[1,2,4]triazolo[3,4-a][2,3]benzodiazepine (RD-4), 3-(1,3-dimethyl-1H-pyrazol-5-yl)-6-(4-methoxyphenyl)-7H-[1,2,4]triazolo[3,4-a][2,3] benzodiazepine (RD-12), and 6-(4-methoxyphenyl)-3-methyl-7H-[1,2,4]triazolo[3,4-a][2,3]benzodiazepine (RD-13). Elongation the side chain at position 3 of the main molecular fragment led to a decrease in the anti-anxiety effect of the compounds. The introduction of the pyrrole ring in the same position did not affect the magnitude of the desired effect, and the 2-furyl fragment increased a level of the antifhobic action of the compounds. Unlike diazepam, the RD compounds are not characterized by muscle relaxant and sedative effects. The most promising compound is RD-4 due to its pronounced anxiolytic and low cytotoxic properties.

Acknowledgments

The reported study was funded by The Russian Foundation for Basic Research, Project № 20-015-00164.

Conflict of interest

The authors declare no conflict of interests.

References

- Bandelow B (2020) Current and novel psychopharmacological drugs for anxiety disorders. Advances in Experimental Medicine and Biology 1191: 347–365. https://doi.org/10.1007/978-981-32-9705-0_19 [PubMed]
- Balon R, Starcevic V (2020) Role of benzodiazepines in anxiety disorders. Advances in Experimental Medicine and Biology 1191: 367–388. https://doi.org/10.1007/978-981-32-9705-0_20 [PubMed]
- Khabarov KM, Kharaneko OI, Bogza SL (2009) 2,3-Benzodiazepine-1-thione in the synthesis of substituted and hetero-annelated 2,3-benzodiazepines. Chemistry of Heterocyclic Compounds 45: 468–474. https://doi.org/10.1007/s10593-009-0280-0
- Kharaneko OI, Popov VYu, Bogza SL (2013) 4-Aryl-1-hydrazino-5H-2,3-benzodiazepine and 1-aryl-4-hydrazino-5H-2,3-benzodiazepine in the synthesis of condensed [1,2]diazepines. Chemistry of Heterocyclic Compounds 2: 343–350. https://doi.org/10.1007/s10593-013-1249-6
- Klyon EE, Khaliullin FA, Spasov AA, Makarova NN, Bagautdinova LF, Naumenko LV (2008) Synthesis and rheological activity of new 1,2,4-triazole derivatives. Pharmaceutical Chemistry Journal 42(9): 510–512. https://doi.org/10.1007/s11094-009-0171-9 [in Russian]
- Kraeuter AK, Guest PC, Sarnyai Z (2019) The elevated plus maze test for measuring anxiety-like behavior in rodents. Methods in Molecular Biology 1916: 69–74. https://doi.org/10.1007/978-1-4939-8994-2_4 [PubMed]
- Lai J, Ma S, Wang Y (2020) Factors associated with mental health outcomes among health care workers exposed to Coronavirus Disease 2019. JAMA Network Open 3(3): e203976. https://doi.org/10.1001/jamanetworkopen.2020.3976 [PubMed] [PMC]
- Maltsev DV, Spasov AA, Miroshnikov MV, Skripka MO, Divaeva LN (2020) Influence of diazepino[1,2-a]benzimidazole derivative (DAB-19) on behavioral aspects of animals. Research Results in Pharmacology 6(3): 9–14. https://doi.org/10.3897/rrpharmacology.6.55142
- Maltsev DV, Spasov AA, Yakovlev DS, Vassiliev PM, Skripka MO, Miroshnikov MV, Sultanova KT, Kochetkov AN, Divaeva LN, Kuzmenko TA, Morkovnik AS (2021) Searching for new anxiolytic agents among derivatives of 11-dialkylaminoethyl-2,3,4,5-tetrahydrodiazepino[1,2-a]benzimidazole. European Journal of Pharmaceutical Sciences 161: 105781. https://doi.org/10.1016/j.ejps.2021.105792 [PubMed]
- Miroshnikov MV, Maltsev DV, Spasov AA, Taran AS, Skripka MO, Surkova EO, Gontareva AV, Divaeva LN, Morkovnik AS (2020) The anxiolytic activity of a new derivative of diazepinobenzimidazole (DAB-19). Russian Journal of Experimental and Clinical Pharmacology [Eksperimental'naya i Klinicheskaya Farmakologiya] 83(10): 3–8. https://doi.org/10.30906/0869-2092-2020-83-10-3-8 [in Russian]
- Ornell F, Schuch JB, Sordi AO (2020) "Pandemic fear" and COVID-19: mental health burden and strategies. Brazilian Journal of Psychiatry 42(3): 232–235. https://doi.org/10.1590/1516-4446-2020-0008 [PubMed] [PMC]
- Ovsyannikova LN, Lalaev BYu, Yakovlev IP, Anisimova NA, Kirillova EN, Ksenofontova GV (2017) Biological activity of new derivatives of triazoles. Pharmacy [Farmaciya] 66(3): 47–50. [in Russian]
- Platt LM, Whitburn AI, Platt-Koch AG, Koch RL (2016) Nonpharmacological alternatives to benzodiazepine drugs for the treatment of anxiety in outpatient populations: a literature review. Journal of Phychosocial Nursing and Mental Health Services 54(8): 35–42. https://doi.org/10.3928/02793695-20160725-07 [PubMed]
- Sher L (2020) COVID-19, anxiety, sleep disturbances and suicide. Sleep Medicine 70: 124–124. https://doi.org/10.1016/j.sleep.2020.04.019 [PubMed]
- Shigemura J, Ursano RJ, Morganstein JC (2020) Public responses to the novel 2019 coronavirus (2019-nCoV) in Japan: mental health consequences and target populations. Psychiatry and Clinical Neurosciences 74(4): 281–282. https://doi.org/10.1111/pcn.12988 [PubMed] [PMC]
- Skripka MO, Gontareva AV, Zolotova EA, Miroshnikov MV, Nurmagomedova BR (2018) Neuropsychotropic activity of new diazepinobenzimidazole derivatives under the codes QLR-9 and QLR-10. Youth for practical health care: Proceedings of the XII International

Scientific and Practical Conference of Students and Young Medical Scientists, Tver, 909–913. [in Russian]

- Spasov AA, Divaeva LN, Maltsev DV, Kuzmenko TA, Morkovnik AS, Miroshnikov MV, Taran AS, Zolotova EA (2018) Anxiolytic potential of a new series of diazepinobenzimidazole derivatives. Journal of Volgograd State Medical University [Vestnik VolgGMU] 3(67): 19–23. https://doi.org/10.19163/1994-9480-2018-3(67)-19-23 [in Russian]
- Spasov AA, Maltsev DV, Miroshnikov MV, Taran AS, Nurmagomedova BR, Skripka MO, Kuzmenko TA, Morkovnik AS, Divaeva LN (2020a) Antidepressant activity and potential mechanisms of action of the diazepinobenzimidazole derivative DAB-19. Russian Journal of Experimental and Clinical Pharmacology [Eksperimental'naya i Klinicheskaya Farmakologiya] 83(4): 31–36. https://doi.org/10.30906/0869-2092-2020-83-4-31-36 [in Russian]
- Spasov AA, Zhukovskaya ON, Maltsev DV, Miroshnikov MV, Skripka MO, Sultanova KT, Morkovnik AS (2020b) Anxiolytic activity of 11H-2,3,4,5-tetrahydro [1,3] diazepino [1,2-a] benzimidazole and 2-mercaptobenzimidazole derivatives. Russian Journal of Bioorganic Chemistry [Bioorganicheskaya Himiya] 46(1): 92–100. https://doi.org/10.31857/S0132342320010145 [in Russian]
- Uyanaev AA, Fisenko VP (2006) Studies of long-term noopept and afobazol treatment in rats with learned helplessness neurosis. Bulletin of Experimental Biology and Medicine [Biulleten' Eksperimental'noi Biologii i Meditsiny] 142(2): 202–204. https://doi.org/10.1007/s10517-006-0327-5 [PubMed]
- Taran AS, Maltsev DV, Yakovlev DS, Karavaeva TV, Tkachenko YuO, Divaeva LN, Morkovnik AS, Kuzmenko TA (2017) Study of anxiolytic activity in a series of new diazepinobenzimidazole derivatives using the elevated plus maze installation. Volgograd Scientific Medical Journal [Volgogradskiy Nauchno-Meditsinskiy Zhurnal] 1(53): 24–26. [in Russian]
- Vinogradova VI, Yunusov MS, Rezhepov Z, Sadritdinov FF (1983) Synthesis and sedative properties of dI-tetrahydropalmatine. Pharmaceutical Chemistry Journal 17: 30–31.
- Wang C, Pan R, Wan X (2020) Immediate psychological responses and associated factors during the initial stage of the 2019 Coronavirus Disease (COVID-19) epidemic among the general population in China. International Journal of Environmental Research and Public Health 17(5): E1729. https://doi.org/10.3390/ijerph17051729 [PubMed]
- Xiao H, Zhang Y, Kong D (2020) Social capital and sleep quality in individuals who self-Isolated for 14 days during the Coronavirus Disease 2019 (COVID-19) outbreak in January 2020 in China. Medical Science Monitor 26: e923921. https://doi.org/10.12659/MSM.923921 [PubMed]
- Yakovlev DS, Sultanova KT, Zolotova EA, Gasainieva AG, Spasov AA (2020) Optimization of MTT assay for evaluation of cytotoxicity of new chemical compounds on MCF-7 cell line. Volgograd Scientific Medical Journal [Volgogradskiy Nauchno-Meditsinskiy Zhurnal] 1: 58–61. https://doi.org/10.24412/1995-7225-2020-1-58-61 [in Russian]

Author contributions

- **Maria O. Skripka**, PhD student of the Department of Pharmacology and Bioinformatics, Volgograd State Medical University, Volgograd, Russia, e-mail: rete.mirabile.renis@gmail.com, **ORCID ID** http://orcid.org/0000-0002-4173-7143. The author took part in conducting experimental work, analysing the material, writing and editing the text of the article.

- **Alexander A. Spasov**, Doctor Habil. of Medical Sciences, Full Professor, Academician of the Russian Academy of Sciences, Head of the Department of Pharmacology and Bioinformatics, Volgograd State Medical University, Volgograd, Russia, e-mail: aspasov@mail.ru, **ORCID ID** http://orcid.org/0000-0002-7185-4826. The author consulted on the research idea, concept and design of the study.

- **Dmitriy V. Maltsev**, PhD, Lecturer of the Department of Pharmacology and Bioinformatics, Volgograd State Medical University, Volgograd, Russia, e-mail: maltsevdmitriy@rambler.ru, **ORCID ID** http://orcid.org/0000-0002-2005-6621. The author defined the idea of research and developed a research plan.

- **Mikhail V. Miroshnikov**, PhD, Assistant professor of the Department of Pharmacology and Bioinformatics, Volgograd State Medical University, Volgograd, Russia, e-mail: mihailmiroshnikov@mail.ru, **ORCID ID** http://orcid.org/0000-0002-9828-3242. The author took part in conducting experimental work and analysing the material**.**

- **Dmitriy S. Yakovlev,** Doctor Habil. of Medical Sciences, Professor of the Department of Pharmacology and Bioinformatics, Volgograd State Medical University, Volgograd, Russia, e-mail: dypharm@list.ru, **ORCID ID** http://orcid.org/0000-0001-8980-6016. The author consulted on the research idea**.**

- **Kira T. Sultanova,** PhD**,** Assistant professor of the Department of Pharmacology and Bioinformatics, PhD student, Volgograd State Medical University, Volgograd, Russia, e-mail: sultanova.pharma@gmail.com, **ORCID ID** http://orcid.org/0000-0002-9846-8335. The author took part in conducting experimental work.

- **Maxim A. Kochergin**, student of Volgograd State Medical University, Volgograd, Russia, e-mail: maximcko@mail.ru. The author took part in conducting experimental work.

- **Lyudmila N. Divaeva,** PhD, Researcher in the Laboratory of Organic Synthesis. Research Institute of Physical and Organic Chemistry, Southern Federal University, Rostov-on-Don, Russia, e-mail: divaevaln@mail.ru, **ORCID ID** http://orcid.org/0000-0002-7275-0797. The author took part in synthesis of the substances.