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# Screening benzimidazole derivatives for atypical antipsychotic activity

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The development of innovative antipsychotic drugs is one of the key tasks of modern pharmacology. Due to their unique chemical properties, benzimidazole derivatives demonstrate diverse neuropsychotropic effects, highlighting their high potential as antipsychotic agents. Bioinformatics methods enable optimization of the process of identifying compounds with high affinity for target receptors.

**The aim.** To identify and evaluate benzimidazole derivatives with atypical antipsychotic activity using QSAR analysis and pharmacophore modeling, followed by *in vivo* experimental testing in preclinical models of psychotic disorders.

**Materials and methods.** QSAR models were constructed based on data from 2615 compounds from the ChEMBL database. Pharmacophore modeling was performed based on the structure of the 5-HT2A receptor (PDB ID: 6A94). The antipsychotic activity of the most promising compound was assessed *in vivo* using tests with apomorphine in rats and mice.

**Results.** Machine learning models were developed and tested to predict the antipsychotic activity of benzimidazole derivatives. The Neural Networks (MAE=0.019) and Random Forest (MAE=0.020) algorithms demonstrated the highest prediction performance. Pharmacophore modeling of interaction with the 5-HT2A receptor identified a promising compound for further testing. Compound RU-31 demonstrated significant reduction ($p$ <0.05) in climbing behavior in mice ($ED_{80}$=10.16 mg/kg intraperitoneally) and high efficacy when administered with low presynaptic doses of apomorphine (yawning frequency decreased by 49,3% compared to control, $p$ <0.05).

**Conclusions.** Compound RU-31 showed activity in the climbing test and in the test with low presynaptic doses of apomorphine, suggesting potential atypical antipsychotic effects. Benzimidazole derivative RU-31 is a promising candidate for further investigation in the development of novel atypical antipsychotics.

**Keywords:** QSAR; pharmacophore modeling; benzimidazole derivatives; antipsychotic activity

**Abbreviations:** MAE — mean absolute error; MLR — multiple linear regression; NN — neural networks; PLSR — partial least squares regression; QSAR — quantitative structure-activity relationship; SVR — support vector regression; RF — random forest.

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# Поиск веществ с атипичной антипсихотической активностью среди производных бензимидазола

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Разработка инновационных антипсихотических препаратов является одной из ключевых задач современной фармакологии. Производные бензимидазола, благодаря их уникальным химическим свойствам, демонстрируют широкий спектр нейропсихотропных эффектов и представляют высокий потенциал в качестве антипсихотических агентов. Применение методов биоинформатики позволяет оптимизировать процесс идентификации соединений с высокой аффинностью к целевым рецепторам.

**Цель.** Провести поиск и оценку производных бензимидазола с атипичной антипсихотической активностью, используя методы QSAR-анализа и фармакофорного моделирования с последующей экспериментальной проверкой *in vivo* на доклинических моделях психотических расстройств.

**Материалы и методы.** Были построены QSAR-модели на основе данных о 2615 соединениях из базы данных CheMBL. Фармакофорное моделирование проводилось на основе структуры 5-HT2A рецептора (PDB ID: 6A94). Антипсихотическая активность наиболее перспективного соединения была оценена *in vivo* в тестах с апоморфином на крысах и мышах.

**Результаты.** В ходе исследования были разработаны и протестированы модели машинного обучения для предсказания антипсихотической активности производных бензимидазола. Наилучшие результаты показали нейронные сети (MAE=0,019) и метод случайного леса (MAE=0,020), которые показали высокую точность в прогнозировании активности. Фармакофорное моделирование взаимодействия с 5-HT2A рецептором позволило выделить перспективное соединение для дальнейшего тестирования. Соединение РУ-31 продемонстрировало значительное ($p$ <0,05) снижение вертикализации у мышей ($ЭД^{80}$=10,16 мг/кг внутрибрюшинно), а также высокую эффективность при введении малых пресинаптических доз апоморфина (число зеваний снизилось на 49,3% по сравнению с контролем; $p$ <0,05).

**Заключение.** Соединение РУ-31 проявило активность в тестах вертикализации и при использовании малых пресинаптических доз апоморфина, что может указывать на его атипичное антипсихотическое действие. Производное бензимидазола РУ-31 является перспективным кандидатом для дальнейшего изучения в рамках разработки новых атипичных антипсихотиков.

**Ключевые слова:** QSAR; фармакофорное моделирование; производные бензимидазола; антипсихотическая активность

**Список сокращений:** MAE — средняя абсолютная ошибка; MLR — множественная линейная регрессия; NN — нейронные сети; PLSR — регрессия частичных наименьших квадратов; QSAR — количественное соотношение структура-свойство; SVR — метод опорных векторов; RF — случайный лес.

## INTRODUCTION

The search for novel compounds with antipsychotic activity is a key priority in modern pharmacology, particularly in the context of developing drugs for Schizophrenia and other psychotic disorders [1]. Traditional antipsychotic drugs, despite their widespread use, are often associated with serious side effects, including extrapyramidal disorders, metabolic disturbances, and neuroleptic malignant syndrome [1]. These limitations motivate the search for alternative molecules with improved safety and efficacy profiles. Of particular interest is the study of benzimidazole

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derivatives, which, due to their structure, exhibit a broad spectrum of biological activity and may be promising candidates for the creation of new antipsychotics [2].

Benzimidazole derivatives are known for their diverse pharmacological properties, including anticonvulsant [3–5], antidepressant [6], anxiolytic [7], and other neuropsychotropic activity [8, 9]. However, their potential in treating neuropsychiatric diseases, particularly their antipsychotic effects, remains underexplored. Over recent years, study in this area has gained new momentum thanks to the development of molecular modeling and machine learning methods, which can significantly accelerate the process of identifying and evaluating promising compounds. The use of modern tools, such as QSAR analysis and pharmacophore modeling, opens up new possibilities for predicting the activity of substances at the early preclinical stage of development [10]. These methods not only reduce the time required to find new compounds with high affinity for target receptors but also significantly lower the costs associated with expensive biological tests.

The application of machine learning in QSAR analysis allows for the prediction of the biological activity of new chemical compounds based on data from the structure and activity of already known substances. One of the most critical factors for success in this area is the selection of the most appropriate methods capable of identifying the key structural features of compounds responsible for their activity. In this work we use a series of machine learning algorithms, including both linear (multiple linear regression [MLR], partial least squares regression [PLSR]) and non-linear methods (support vector regression [SVR], random forest [RF], neural networks [NN]). This approach will allow for a comparative analysis of the effectiveness of different algorithms and help identify the most suitable ones for this task [11].

The 5-$HT_{2A}$ receptor, whose crystal structure is available in the RCSB PDB database[1], was chosen as a model for pharmacophore analysis. 5-$HT_{2A}$ receptors play a key role in the mechanisms of action of atypical antipsychotics. This makes them a priority target in the development of new drugs for psychotic disorders [12]. These receptors, located predominantly in the cerebral cortex, are involved in regulating dopaminergic and glutamatergic transmission [13], which is directly related to the development of symptoms of Schizophrenia and other psychoses [14, 15]. 5-$HT_{2A}$ receptor antagonists have demonstrated the ability to reduce both positive and negative symptoms of mental disorders while minimizing extrapyramidal side effects, which differentiates them from typical antipsychotics [16].

To assess antipsychotic activity, several apomorphine-based models were used, including hyperactivity, stereotypy, and aggressive behavior in rats, as well as climbing behavior in mice[2]. These models are widely used in pharmacological studies to evaluate both typical and atypical neuroleptics [17]. The use of such models allows not only for the evaluation of the effectiveness of new compounds but also for the identification of their potential side effects, which is an important stage in the development process of new antipsychotics.

**THE AIM** of this study is to develop and implement a comprehensive approach to the search and evaluation of benzimidazole derivatives with atypical antipsychotic activity, using molecular modeling and machine learning methods. Within this aim, the study involves conducting a QSAR analysis to identify structural features that determine antipsychotic activity, building a pharmacophore model based on interaction with the 5-$HT_{2A}$ receptor, and experimentally evaluating the identified compounds *in vivo* using preclinical models of psychotic disorders.

## MATERIALS AND METHODS

### Study design

The study design included several sequential stages. In the first stage, QSAR models were built to evaluate a series of benzimidazole derivatives, with the aim of identifying compounds with high affinity for the 5-$HT_{2A}$ receptor, based on data from compounds with established activity. Next, a pharmacophore analysis was conducted based on the 5-$HT_{2A}$ receptor complex with zotepine. This analysis helped identify compounds that best matched the pharmacophore model and select candidates for further testing. In the final stage, the identified lead compound underwent experimental verification of its antipsychotic activity in a series of tests with apomorphine.

### QSAR analysis

A dataset was prepared for the study, including 2 615 compounds with known biological activity ($IC_{50}$), obtained from the ChEMBL database and various literature sources. The validation dataset was formed from compounds obtained from the database

[1] RCSB Protein Data Bank (RCSB PDB). Available from: https://www.rcsb.org/

[2] Ostrovskaya RU, Raevskiy KS, Voronina TA, Garibova TL, Kovalev GI, Kudrin VS, Narkevich VB, Klodt PM. [Methodological recommendations for the study of neuroleptic activity of drugs]. In: [Guidelines for preclinical studies of medicinal products]. Part 1. Moscow: Grif i K; 2012. P. 252–255. Russian

"Benzimidazole derivatives with neurotropic and psychotropic action"[3]. Duplicates were removed, and compounds with missing activity data were filtered out. All biological activity data were converted to micromolar units of $IC_{50}$.

The chemical structures of the compounds were converted into a machine-readable format (SMILES) using the RDKit package. To calculate molecular descriptors (physicochemical properties, topological indices, geometric properties, electronic characteristics, etc.), the PaDEL-Descriptor Software was used (1875 descriptors for each substance, including 1444 1D, 2D descriptors and 431 3D descriptors). Data processing included removing descriptors with zero variance and high correlation.

The dataset was split into training and test sets in an 80 / 20 ratio. For data, stratified splitting was applied to preserve the activity distribution across the sets. For regression tasks, several machine learning algorithms were selected and tested, including multiple linear regression (MLR), partial least squares regression (PLSR), support vector regression (SVR), random forest (RF), and neural networks (NN). The models were trained on the training dataset. To optimize model parameters and prevent overfitting, 5-fold cross-validation was used.

The performance of the models was evaluated on the validation set by calculating metrics such as the coefficient of determination ($R^2$) and mean absolute error (MAE) averaged across the 5 folds. Statistical evaluation of performance was carried out by comparing the parameters of the constructed models with those of a baseline regression model. In the final stage, predictive activity estimates ($IC_{50}$) were obtained for the most promising substances in the test set.

### Pharmacophore analysis

Modeling pharmacophores based on the structure of a protein-ligand complex is widely used in the drug development process. The created pharmacophore model helps to understand the critical structural features of the target protein's active site with the ligand, which are necessary for pharmacological activity. In this work, an energy-optimized pharmacophore (e-pharmacophore) was created using the Schrodinger Phase tool, based on a ligand-protein complex. The crystal structure of the $5\text{-}HT_{2A}$ receptor (PDB ID: 6A94) from the Protein Data Bank (RCSB PDB), co-crystallized with zotepine, was chosen to create the pharmacophore model. The Protein Preparation Wizard tool was used for preliminary protein processing, which allows for assigning bond orders, removing water, building missing side chains, and minimizing the protein structure. The pharmacophore hypothesis, obtained using the Phase module, includes features: a hydrogen bond donor (D), an aromatic ring (R), a hydrophobic group (H), and a negative ion (N). Ligands from the database of benzimidazole derivatives with neurotropic activity were tested for compliance with the generated model and evaluated using fitness score, align score, vector score, volume score, and the number of matched features.

### *In vivo* investigation of antipsychotic activity

#### Test compounds

In animal experiments, the derivative 1-(2-diethylaminoethyl)-2-(4-methoxyphenyl)-imidazo[1,2-a]benzimidazole — compound RU-31 (Research Institute of Physical and Organic Chemistry, Southern Federal University, Russia); clozapine (Organica, Russia); haloperidol (Gedeon Richter, Hungary); and apomorphine (Sigma, USA) were used.

#### Ethics approval

Animal experiments were conducted in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, the principles of Good Laboratory Practice (GLP) (GOST 33044-2014, 2021), and the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. The study was approved by the Local Ethics Committee of the Volgograd State Medical University (Registration number IRB00005839 IORG0004900, Minutes No. 2024/221 dated April 3, 2024).

#### Animals

The experiments were performed on male outbred white rats weighing 260–280 g and male outbred white mice weighing 18–25 g. The animals were housed under standard vivarium conditions with a 12-hour light cycle, a temperature range of 22 ± 2 °C, and *ad libitum* access to food and water.

#### Apomorphine-induced climbing behavior

Mice were divided into four groups of 8 animals each. The experimental groups received intraperitoneal injections of haloperidol, clozapine, compound RU-31 in increasing doses, or saline solution (10 mL/kg, vehicle group). The substances were administered 20 minutes before a subcutaneous injection of apomorphine

[3] Kalitin KY, Mukha OY, Spasov AA, et al. [Benzimidazole derivatives with neurotropic and psychotropic action] [Database]. Certificate of State Registration of a Database No. 2023624590. Russian Federation. Applied Nov 18, 2023; Registered Dec 12, 2023. Copyright holder: Volgograd State Medical University. Russian

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(5 mg/kg). Ten minutes after the apomorphine injection, the animals were placed in cylindrical chambers (height 14 cm, diameter 12 cm, made of 2 mm thick wire rod, with 1 cm spacing between rods) for observation of stereotypical behavior. The intensity of climbing was assessed on a four-point scale: 0 points — no paws on the wire mesh; 1 point — one paw on the mesh; 2 points — two paws on the mesh; 3 points — three paws on the mesh; 4 points — four paws on the mesh. The assessment was performed every 2 min for 10 s over a one-hour period. At the end of the experiment, the total score for each animal was calculated.

Dose-response curves were constructed, from which the $ED_{80}$ values for each compound were interpolated. The analysis was performed using non-linear regression with a variable slope model and calculation of the coefficients of determination ($R^2$). The obtained $ED_{80}$ values were used in further experiments.

### Apomorphine-induced hyperactivity in the Open field test

Rats were divided into four groups of 8 animals each. Thirty minutes before the apomorphine injection, the following were administered intraperitoneally: saline (10 mL/kg, vehicle), haloperidol (1 mg/kg), clozapine (7.5 mg/kg), or compound RU-31 (10 mg/kg). Apomorphine was administered subcutaneously (5 mg/kg) 3 minbefore testing. Horizontal motor activity was recorded in the Open field apparatus for 5 min after the apomorphine injection.

### Effect on stereotyped behavior induced by apomorphine

Rats were divided into four groups of 8 animals each. Thirty minutes before the apomorphine injection, the following were administered intraperitoneally: saline (10 mL/kg, vehicle group), haloperidol (1 mg/kg), clozapine (7.5 mg/kg), or compound RU-31 (10 mg/kg). Apomorphine was administered subcutaneously (1 mg/kg). Stereotyped behavior was assessed every 15 min for 2 ho on a three-point scale (1 — weak, 2 — moderate, 3 — intense stereotypy).

### Effect on the effects of low (presynaptic) doses of apomorphine

Animals were divided into four groups of 10 individuals each. Thirty minutes before the apomorphine injection, rats received intraperitoneal injections of: saline (10 mL/kg), haloperidol (1 mg/kg), clozapine (7.5 mg/kg), or compound RU-31 (10 mg/kg). Apomorphine was administered subcutaneously (0.1 mg/kg). The number of yawning movements for each animal was recorded over 60 min.

### Effect of substances on aggressive behavior

The same pairs of animals were used throughout the study, with pairs always selected from adjacent cages and receiving the same therapy. To induce aggressive behavior, rats were administered apomorphine (1 mg/kg, s.c.) daily for 15 days. Aggressive behavior was assessed on days 1, 3, 6, 9 and 12 of the experiment. The following were observed: (1) latency period (time to the first attack or first aggressive posture) and (2) intensity of aggressive behavior using a four-point scale: 0 — no aggression; 1 — weak aggression without vocalization; 2 — intense aggression with vocalization but without biting; 3 — continuous attacks or attempts to bite. The test was stopped upon reaching the maximum level of aggression to avoid injury. Animals not showing aggressive behavior by day 15 were excluded from further study. On day 15, the study of the effect of the test compounds on apomorphine-induced aggression began. Haloperidol (1 mg/kg, $n$ = 8), clozapine (7.5 mg/kg, $n$ = 8), compound RU-31 (10 mg/kg, $n$ = 8), or saline solution (10 mL/kg, $n$ = 8) were administered intraperitoneally 30 min before the apomorphine injection (1 mg/kg, s.c.). Immediately after the apomorphine injection, pairs of rats were placed in a testing cage, and aggressive behavior was recorded for 15 min.

### Statistical analysis

GraphPad Prism 10.1 with an academic license (Dotmatics, USA) was used for data processing. After checking for normality of distribution using the Shapiro–Wilk test, group comparisons were performed using one-way ANOVA with Dunnett's *post hoc* test for normally distributed data, and the Kruskal–Wallis test with Dunn's post hoc test for non-normally distributed data. Differences between groups were considered statistically significant at $p < 0.05$.

## RESULTS

### QSAR analysis

Table 1 presents the performance evaluation results of various machine learning models on the test and validation datasets. For each model, the mean absolute error (MAE), coefficient of determination ($R^2$), and *p-value*, reflecting the statistical significance of the metrics compared to a baseline regression model, were calculated. All considered models showed statistically significant results ($p < 0.05$), confirming their applicability to this task.

**Table 1 – Performance evaluation of models on the test and validation datasets**

| Model | | MAE | $R^2$ | *p*-value |
|---|---|---|---|---|
| MLR | Test | 0.01645 | 0.87 | < 0.05 |
| | Validation | 0.02323 | 0.76 | < 0.05 |
| PLSR | Test | 0.01593 | 0.89 | < 0.05 |
| | Validation | 0.02188 | 0.80 | < 0.05 |
| SVR | Test | 0.01471 | 0.91 | < 0.05 |
| | Validation | 0.01974 | 0.83 | < 0.05 |
| RF | Test | 0.01453 | 0.91 | < 0.05 |
| | Validation | 0.01961 | 0.84 | < 0.05 |
| NN | Test | 0.01405 | 0.92 | < 0.05 |
| | Validation | 0.01920 | 0.85 | < 0.05 |

Note: the *p*-value reflects the statistical significance of the model's metrics compared to a baseline regression model.

**Table 2 – Activity prediction for compounds**

| Model | $IC_{50}$ RU-31 (μM) | $IC_{50}$ RU-30 (μM) | $IC_{50}$ RU-204 (μM) |
|---|---|---|---|
| MLR | 0.066 | 0.074 | 0.167 |
| PLSR | 0.067 | 0.081 | 0.165 |
| SVR | 0.064 | 0.079 | 0.164 |
| RF | 0.062 | 0.076 | 0.167 |
| NN | 0.063 | 0.074 | 0.164 |
| Experiment[4] | 0.044 | 0.069 | 0.15 |

**Table 3 – Codes of the most promising substances and their chemical structures**

| Compound | IUPAC | SMILES |
|---|---|---|
| RU-30 | 2-(2-(4-ethoxyphenyl)-9*H*-benzo[d]imidazo[1,2-a] imidazol-9-yl)-*N*,*N*-diethylethan-1-amine | CCOC1=CC=C(C=C1)C4=C[N]3C2=C(C=CC=C2)[N] (CCN(CC)CC)C3=N4 |
| RU-31 | *N*,*N*-diethyl-2-(2-(4-methoxyphenyl)-9*H*-benzo[d] imidazo[1,2-a]imidazol-9-yl)ethan-1-amine | CCN(CC)CC[N]3C1=C(C=CC=C1)[N]4C=C(C2=CC=C(C=C2) OC)N=C34 |
| RU-204 | *N*,*N*-diethyl-2-(2-(thiophen-2-yl)-9*H*-benzo[d] imidazo[1,2-a]imidazol-9-yl)ethan-1-amine | CCN(CC)CC[N]3C1=CC=CC=C1[N]4C=C(C2=CC=CS2) N=C34 |

**Table 4 – Screening results of benzimidazole derivatives for compliance with the pharmacophore hypothesis**

| Compound | Matched ligand sites | Align Score | Vector Score | Volume Score | Fitness Score |
|---|---|---|---|---|---|
| RU-31 | 3 | 0.795 | 0.828 | 0.231 | 1.013 |
| RU-30 | 3 | 0.879 | 0.838 | 0.225 | 0.989 |
| RU-204 | 2 | 0.313 | 0 | 0 | 0.208 |

**Table 5 – Effect of haloperidol (1 mg/kg), clozapine (7.5 mg/kg), and compound RU-31 (10 mg/kg) on yawning behavior in rats induced by low doses of apomorphine (0.1 mg/kg)**

| Group | Number of yawns | *p*-value |
|---|---|---|
| Vehicle | 24.3 ± 3.4 | – |
| Haloperidol | 4.5 ± 1.3 | < 0.05 |
| Clozapine | 8.324 ± 2.2 | < 0.05 |
| Compound RU-31 | 12.324 ± 6.8 | < 0.05 |

Note: data are presented as mean ± SEM.

[4] Yakovlev DS. [Condensed azoles as a new class of serotonin receptor ligands] [dissertation for the degree of Doctor of Medical Sciences]. Volgograd; 2016. 98 p. Russian

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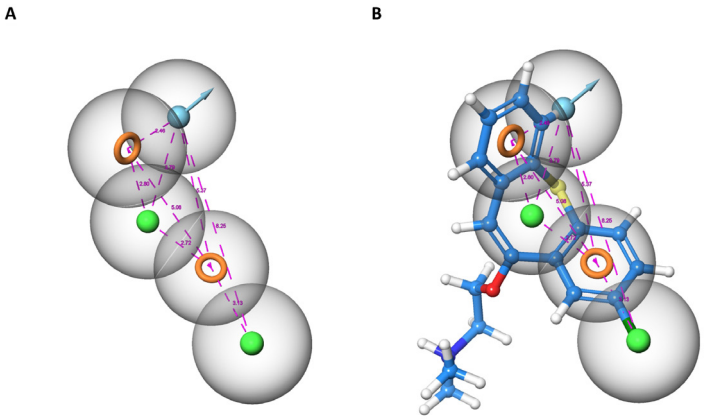


**Figure 1 – Pharmacophore model for 5-HT2A receptor antagonism, based on the receptor-ligand complex structure, with five features: one hydrogen bond donor (blue sphere), two hydrophobic sites (green spheres), and two aromatic rings (orange rings) [A] and the distance between zotepine's pharmacophore features in angstroms [B]**

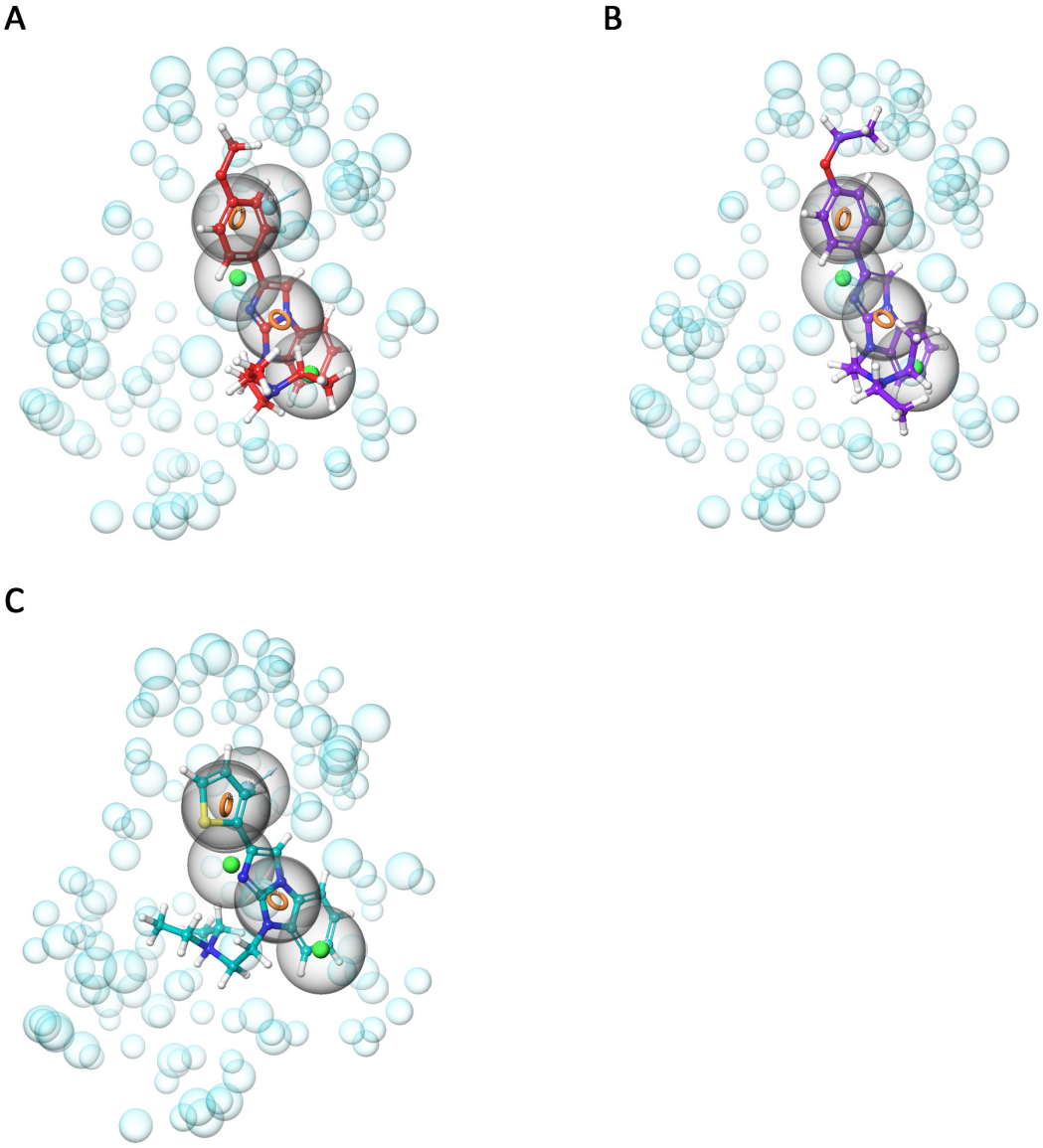


**Figure 2 – Alignment of the DHHRR pharmacophore hypothesis with benzimidazole derivatives RU-31 [A], RU-30 [B], and RU-204 [C].**

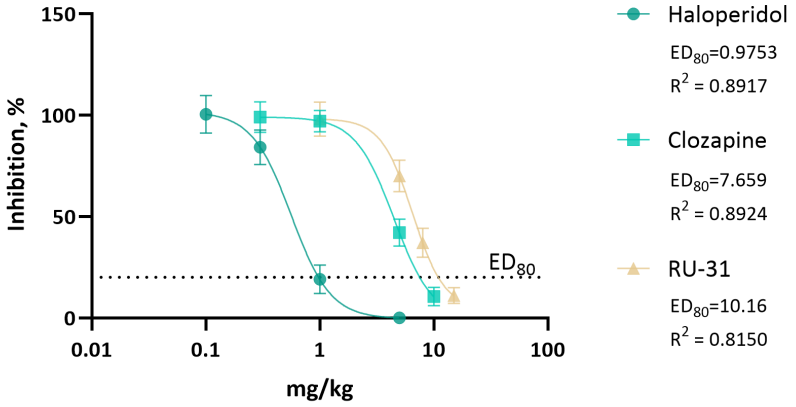


**Figure 3 – Dose-response curves for increasing doses (i.p.) of haloperidol, clozapine, and compound RU-31 in the climbing test in mice with stereotypic disorder induced by a single injection of apomorphine (5 mg/kg).**

Note: data are presented as mean ± SEM.

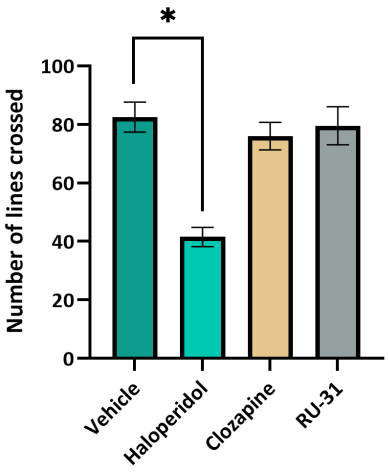


**Figure 4 – Effect of haloperidol (1 mg/kg), clozapine (7.5 mg/kg), and compound RU-31 (10 mg/kg) on hyperactivity in rats induced by apomorphine (5 mg/kg).**

Note: data are presented as mean ± SEM. Differences are statistically significant relative to the vehicle group: * — $p < 0.05$.

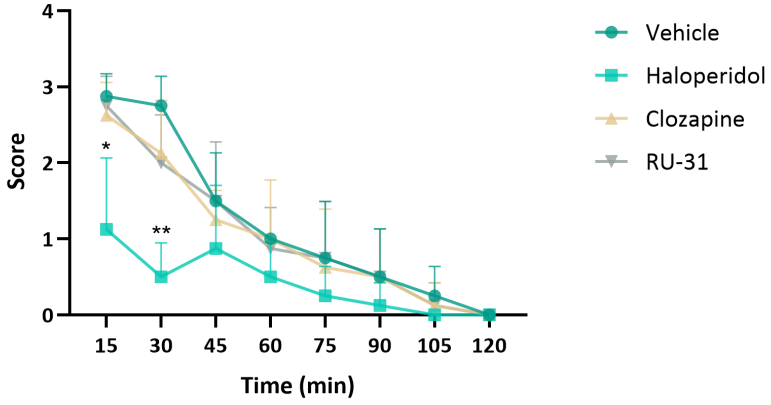


**Figure 5 – Effect of haloperidol (1 mg/kg), clozapine (7.5 mg/kg), and compound RU-31 (10 mg/kg) on stereotyped behavior in rats induced by apomorphine (1 mg/kg).**

Note: data are presented as mean ± 95% CI. Differences are statistically significant relative to the vehicle group: * — $p < 0.05$; ** — $p < 0.01$.

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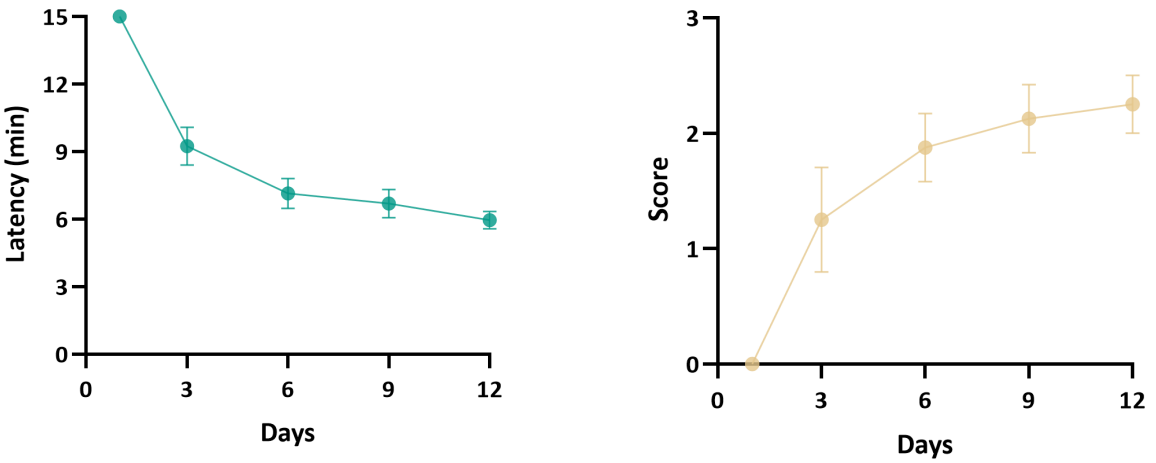


**Figure 6 – Dynamics of aggressive behavior indicators in rats induced by apomorphine (1 mg/kg) before the administration of substances with antipsychotic activity.**
Note: Data are presented as mean ± SEM.

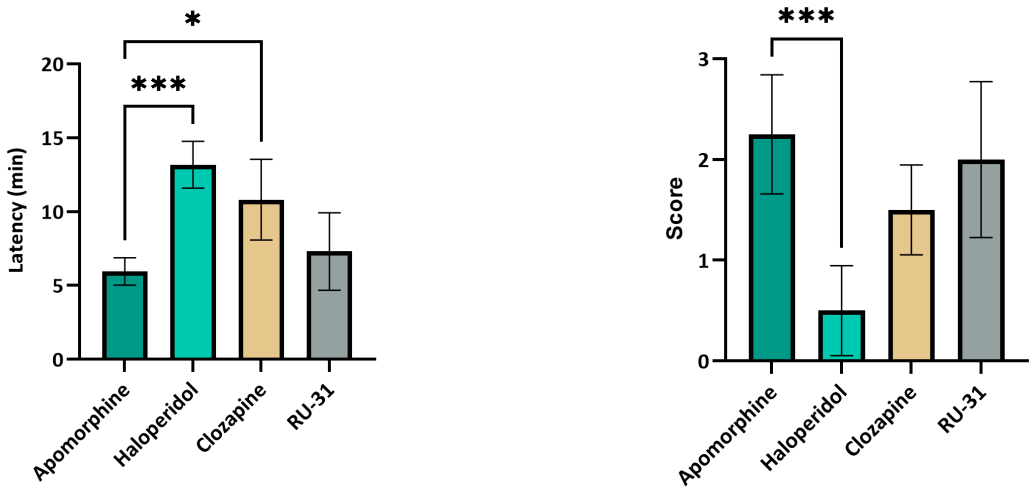


**Figure 7 – Effect of haloperidol (1 mg/kg), clozapine (7.5 mg/kg), and compound RU-31 (10 mg/kg) on aggressive behavior in rats induced by apomorphine (1 mg/kg).**
Note: Data are presented as mean ± 95% CI. Differences are statistically significant relative to the vehicle group: * — $p$ <0.05; *** — $p$ <0.001.

Among all models, the NN demonstrated the best performance on both the test and validation datasets. It provided the lowest MAE of 0.01405 and 0.01920, respectively, and the highest $R^2$ — 0.92 and 0.85. This indicates the NNs ability to most accurately capture complex non-linear dependencies in the data.

The RF and SVR models showed comparable results, slightly inferior to the NN. The PLSR and MLR methods demonstrated the least accurate results among the models considered.

It is worth noting that all models showed some performance degradation on the validation set compared to the test set, which is expected and indicates the absence of significant overfitting. Overall, the results suggest the superiority of more complex non-linear models, such as NNs and ensemble methods, for solving this prediction task.

The results in Table 2 reflect the predicted activity values ($IC_{50}$) for three compounds (RU-31, RU-30, and RU-204; Table 3), obtained using five different machine learning models (MLR, PLSR, SVR, RF, and NN). The table also includes experimental $IC_{50}$ values for comparison.

Analyzing the predicted $IC_{50}$ values, it can be noted that all models provide quite similar results for each compound. For compound RU-31, the predicted values range from 0.062 to 0.067 μM, for RU-30 — from 0.074 to 0.081 μM, and for RU-204 — from 0.164 to

0.167 μM. For compounds RU-31, RU-30, and RU-204, the difference between predicted and experimental values is 0.018–0.023, 0.005–0.012 and 0.014–0.017 μM, respectively.

Among the models considered, the RF and NN models provide the most accurate predictions. Their predicted values are closest to the experimental data for all three compounds. This is consistent with the results obtained on the test and validation sets, where these models also showed the best performance.

### Pharmacophore modeling

The five-feature (DHHRR) pharmacophore model, derived using the protein-ligand complex of the serotonin 2A receptor and zotepine (PDB ID: 6A94), is presented in Figure 1.

Table 4 presents the screening results of benzimidazole derivatives for compliance with the pharmacophore hypothesis. Ligands were selected based on matching sites and fitness scores. Compound RU-31 demonstrated the best fitness score of 1.013 with three matching ligand sites. Compound RU-30 also had three matching ligand sites and showed a fitness score of 0.989. Compound RU-204 had two matching ligand sites and a fitness score of 0.208. These data show that benzimidazole derivatives such as RU-31 and RU-30 have a high degree of compliance with the pharmacophore hypothesis compared to RU-204. Figure 2 also shows the alignment of the pharmacophore hypothesis with the studied ligands.

### Effect on apomorphine-induced climbing

In the experiment, dose-response curves were obtained for haloperidol, clozapine, and compound RU-31 in the apomorphine-induced climbing model in mice (Fig. 3). The potential antipsychotic activity of the compounds was assessed based on the suppression of stereotypical behavior induced by apomorphine.

Haloperidol showed the highest efficacy among the tested compounds, with an $ED_{80}$ of 0.9753 mg/kg and a $R^2$ of 0.8917, indicating a high correlation between dose and effect. Clozapine demonstrated intermediate efficacy with an $ED_{80}$ of 7,659 mg/kg and $R^2$ = 0.8924. Compound RU-31 showed activity close to that of clozapine, with an $ED_{80}$ = 10,16 mg/kg and a coefficient of determination of 0.8150.

These results demonstrate that haloperidol, as a typical antipsychotic, most effectively suppressed dopamine-mediated stereotyped behavior, while clozapine and compound RU-31 showed a less pronounced effect. The obtained $ED_{80}$ values for all compounds were used for further pharmacological studies.

### Apomorphine-induced hyperactivity in rats in the Open field test

Figure 4 presents the results of the study on apomorphine-induced hyperactivity in rats in the Open field test after compound administration. The graph shows the level of motor activity in rats, expressed as the number of lines crossed on the field's arena. In the vehicle-treated group, high motor activity was observed. The administration of haloperidol (1 mg/kg) significantly reduced the number of lines crossed, indicating its pronounced inhibitory effect on motor activity, likely due to the blockade of dopamine receptors. In the group receiving clozapine (7.5 mg/kg), motor activity was not reduced compared to the vehicle group. The administration of compound RU-31 (10 mg/kg) also did not lead to a significant change in the number of lines crossed compared to the vehicle group. This may suggest its neutral effect on the motor activity of rats in this experiment, a characteristic of substances with atypical antipsychotic activity similar to clozapine.

### Effect on stereotyped behavior induced by apomorphine

Figure 5 shows the effects of haloperidol (1 mg/kg), clozapine (7.5 mg/kg), and compound RU-31 (10 mg/kg) on apomorphine-induced stereotypy in animals over 120 min. In the vehicle group, a gradual decrease in the level of stereotypy is observed, with maximum values in the first 15 min and a gradual reduction to minimum values by the 120 min.

At 15 min after haloperidol administration, the level of stereotypy is significantly reduced ($p < 0.05$ compared to vehicle). At 30 min, this reduction becomes even more pronounced ($p < 0.01$ compared to vehicle). Throughout the observation period, haloperidol maintains a reduced level of stereotypy, most pronounced in the first 30 min. During the first 45 min of observation, clozapine shows a slight tendency to reduce the level of stereotypy compared to the vehicle. After 45 min, the levels of stereotypy under the influence of clozapine approach the vehicle values.

Compound RU-31 has no statistically significant

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effect on the level of stereotypy. Throughout the observation period, the levels of stereotypy under the influence of RU-31 remain close to the vehicle values, with minor deviations at 30 min.

### Effect on the effects of low (presynaptic) doses of apomorphine

According to the obtained data (Table 5), in the group receiving haloperidol (1 mg/kg), the number of yawns decreased by 81.5%, which is significantly less than the vehicle level ($p < 0.05$). In the group receiving clozapine (7.5 mg/kg), the number of yawns decreased by 65.7%, which is also significantly lower compared to the vehicle group ($p < 0.05$). In the group receiving compound RU-31 (10 mg/kg), the average number of yawns was 49.3% less than in the vehicle group ($p < 0.05$). Thus, all studied compounds statistically significantly reduced the number of yawns compared to the vehicle group, indicating the presence of antipsychotic activity. The high specificity of the test is explained by the fact that serotonin receptor antagonists do not have a direct effect on dopamine receptors but can influence the level of dopamine in synapses by reducing its secretion. This effect is not apparent in tests with high doses of apomorphine, as in that case, the effect on mediator release is not significant.

### Effect of substances on aggressive behavior

Figure 6 shows the dynamics of changes in the aggressive behavior of rats under the influence of apomorphine over 12 days. The left graph shows the latency period (time to the first aggressive reaction), which gradually decreases from 15 to 6 min. The right graph illustrates the severity of aggression in scores, which increases from 0 to 2 or more points by day 12, also indicating an intensification of aggressive reactions.

Figure 7 shows the effect of the test compounds on aggressive behavior on day 15 of the experiment. On the left diagram, it can be noted that haloperidol (1 mg/kg) significantly increases the latency period compared to the vehicle ($p < 0,001$). Clozapine (7.5 mg/kg) also significantly increases the latency period ($p < 0,05$), but this increase is less pronounced compared to haloperidol. The latency period in the group receiving compound RU-31 (10 mg/kg) does not differ significantly from the vehicle.

The right diagram shows the effect of the substances on the intensity of aggression. Haloperidol significantly reduces aggression scores compared to the vehicle ($p < 0.001$), while clozapine and compound RU-31 do not have a significant effect on this indicator.

## DISCUSSION

The obtained results demonstrate high predictive efficacy of machine learning models in assessing the 5-$HT_{2A}$-antagonistic activity of benzimidazole derivatives. In particular, the NN and RF methods showed the best performance in terms of accuracy and predictive significance among all tested models. This suggests the utility of employing more complex algorithms for analyzing data on the biological activity of substances, as they are capable of accounting for complex non-linear dependencies in molecular descriptors, which significantly enhances the quality of prediction [18, 19].

The significant correlation between the predicted and experimental $IC_{50}$ values for compounds RU-31, RU-30, and RU-204 confirms the validity of the developed models. However, some deviations from the experimental data indicate the potential for further optimization of the models and the inclusion of additional factors that influence the activity of the substances.

Pharmacophore analysis revealed important structural features of benzimidazole derivatives that mediate interaction with 5-HT2A receptors [20]. Compounds RU-31 and RU-30 showed the highest degree of compliance with the pharmacophore model, which corroborates their high affinity for this receptor. These results are consistent with the *in vivo* experimental data, where compound RU-31 demonstrated significant antipsychotic activity.

The obtained QSAR and pharmacophore models have high potential for future use in the search for new active ligands with antagonistic activity towards 5-$HT_{2A}$ receptors. In particular, these models can be successfully applied for the rational design and optimization of new benzimidazole derivatives capable of interacting with 5-$HT_{2A}$ receptors.

Experiments with apomorphine, aimed at assessing the antipsychotic activity of the tested compounds, confirmed the efficacy of RU-31 in several behavioral tests in animals. Compound RU-31 significantly reduced apomorphine-induced climbing in mice, indicating its ability to block the dopaminergic influence. However, in the apomorphine-induced stereotypy experiment,

the effect of compound RU-31 and clozapine did not differ from the vehicle, which corresponds to previously obtained data for the compound MDL 100 907 [21, 22] and trazodone [23]. In the Open field test, compound RU-31 did not exert a significant effect on the motor activity of rats. Similar results were obtained for ketanserin [24]. This suggests that RU-31 may have a more favorable side-effect profile compared to typical antipsychotics like haloperidol, which often cause pronounced motor depression [25].

In contrast to haloperidol, which significantly suppressed aggressive reactions, RU-31 did not have a significant impact on this indicator. This result may be explained by the fact that compound RU-31 affects the dopaminergic system through pathways different from those of typical antipsychotics, which is characteristic of atypical antipsychotic agents. However, the lack of effect on aggression may indicate a limitation of its action regarding certain behavioral aspects associated with psychoses and requires further investigation.

The obtained results also demonstrate the potential of RU-31 as an antipsychotic with a low risk of extrapyramidal side effects [26]. In tests with low doses of apomorphine, aimed at assessing presynaptic effects, compound RU-31 statistically significantly reduced the number of yawning movements. However, this effect was less pronounced compared to haloperidol and clozapine, which is likely due to the lack of direct action on dopamine receptors and, consequently, a lower risk of developing extrapyramidal disorders. The obtained results are consistent with a previous study where it was shown that the serotonin receptor antagonists ritanserin and ketanserin do not affect aggressive behavior [27, 28].

Comparing the results obtained for RU-31 with those for haloperidol and clozapine allows us to conclude that compound RU-31 has characteristics similar to atypical antipsychotics. The studied substance reduces the severity of climbing and yawning behavior, while not significantly affecting motor activity and aggression. In aggregate, these properties make compound RU-31 a promising candidate for further preclinical research.

A logical next step for this research could be to employ longitudinal technologies to measure morphofunctional effects at the level of synaptic structures in the neocortex of laboratory animals using confocal microscopy. These technologies would allow for the assessment of the temporal dynamics of both the development of psychosis (under chronic apomorphine administration) and its mitigation (with the administration of benzimidazole derivatives), including the characterization of individual animal responses in the experimental model.

## CONCLUSION

In the present study, a comprehensive analysis of benzimidazole derivatives was conducted to search for substances with atypical antipsychotic activity. The use of QSAR modeling and pharmacophore analysis allowed for the identification of the most promising compounds, among which RU-31 demonstrated high affinity for the 5-$HT_{2A}$ receptor and significant antipsychotic activity in *in vivo* experiments. The results showed that RU-31 possesses the characteristics of an atypical antipsychotic with a low risk of extrapyramidal side effects, making it a promising candidate for further study and the development of new drugs for treating psychoses.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHORS' CONTRIBUTION

Konstantin Yu. Kalitin — statement of key objectives, analysis of scientific and methodical literature, data processing, writing, and editing of the manuscript; Olga Yu. Mukha — data collection and processing, writing, editing and formatting of the manuscript; Viktor B. Voynov — literature review, discussion of results, editing and formatting of the manuscript. All authors confirm that their authorship meets the international ICMJE criteria (all authors made a significant contribution to the development of the concept, conduct of the study and preparation of the article, read and approved of the final version before the publication).

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