Scientific article
UDC 615.015.11
DOI: 10.52957/2782-1900-2026-7-1-164-172

IN SILICO PROFILING OF 3,4-DIGHYDRO-1*H*-[1,4]OXAZINO[4,3-A]BENZIMIDAZOLE DERIVATIVES: PREDICTION OF CYTOTOXIC PROPERTIES AND ADME PROFILE

Yu.R. Aleksandrova[1,3], M.V. Kucherenko[1,2], I.A. Shagina[1,3], L.I. Savina[1], R.S. Begunov[2], A.A. Zubishina[1,2], M.E. Neganova [1,3]

Yulia Romanovna Aleksandrova, Candidate of Biological Sciences, Senior Scientific Researcher; **Maria Viktorovna Kucherenko**, Research Laboratory Assistant; **Inna Aleksandrovna Shagina**, Junior Scientific Researcher; **Luiza Ilinichna Savina**, Research Laboratory Assistant; **Roman Sergeevich Begunov**, Candidate of Chemical Sciences, Associate Professor; **Alla Alexandrovna Zubishina**, Candidate of Biological Sciences, Senior Researcher; **Margarita Evgenievna Neganova**, Doctor of Biological Sciences, Leading Researcher.

[1]Yaroslavl State Medical University, Yaroslavl, Russia, *mariiaku1505@gmail.com, luizasavina2000@mail.ru*

[2]P.G. Demidov Yaroslavl State University, Yaroslavl, Russia, *begunov@bio.uniyar.ac.ru*

[3]A.N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, Moscow, Russia *yulia.aleks.97@mail.ru, neganova83@mail.ru*

Keywords: *condensed benzimidazole derivatives, morpholine ring, prediction of cytotoxic profile, pharmaceutical activity, assessment of pharmacokinetic parameters*

Abstract. *Previously, we synthesised a series of 3,4-dihydro-1H-oxazino[4,3-a]benzimidazole derivatives by functionalising a benzimidazole derivative with an annulated morpholine ring in an S_EAr reaction. Some compounds were not previously described in the literature. To assess their potential as pharmaceutical substances, the authors conducted an in silico analysis of the cytotoxic profile and pharmacokinetic parameters. Predicted cytotoxicity revealed a high probability of toxic effects on the nervous and respiratory systems for compounds with toxicity classes II to IV and LD_{50} in the range of 10-1500 mg/kg. The data obtained suggest a potential antitumour effect of the compounds against gliomas and lung cancer. Analysis of the ADME profile of the compounds showed that all molecules comply with Lipinski's rule. Therefore, further investigation of their antitumour potential in in vitro studies is prospective.*

For citation:
Aleksandrova Yu.R., Kucherenko M.V., Shagina I.A., Savina L.I., Begunov R.S., Zubishina A.A., Neganova M.E. *In silico* profiling of 3,4-dihydro-1*H*-[1,4]oxazino[4,3-*a*]benzimidazole derivatives: prediction of cytotoxic properties and ADME profile // From Chemistry towards Technology Step-by-Step. 2026. Vol. 7, Iss. 1. P. 164-172. URL: https://chemintech.ru/ru/nauka/issue/7073/view

Introduction

The benzimidazole ring in the molecule structure is a bioisosteric analogue of indole and purine nuclei. It contributes to the substance's broad spectrum of biological activity [1-5]. Modification of the 1,2,5,6-positions of benzimidazole with various substituents, ranging from simple atoms or small groups to complex or cyclic fragments, significantly affects their biological activity [6]. The presence of an imidazole-annulated terminal heterocycle has a greater effect. Such condensed benzimidazole derivatives with a nodal nitrogen atom possess high antimicrobial [7, 8], antifungal [9], antiviral [10], antitumour [11-13], and anti-inflammatory activity [14, 15]. Therefore, the study of the structural diversity of substituted benzimidazoles is a topical issue of medicinal chemistry.

Previously [16, 17], we synthesised a benzimidazole derivative containing a morpholine ring fused at positions 1 and 2 and functionalised it in an S_EAr reaction. As a result, a number of 3,4-dihydro-1*H*-[1,4]oxazino[4,3-*a*]benzimidazole derivatives were obtained, including do not described in the literature (Fig. 1).

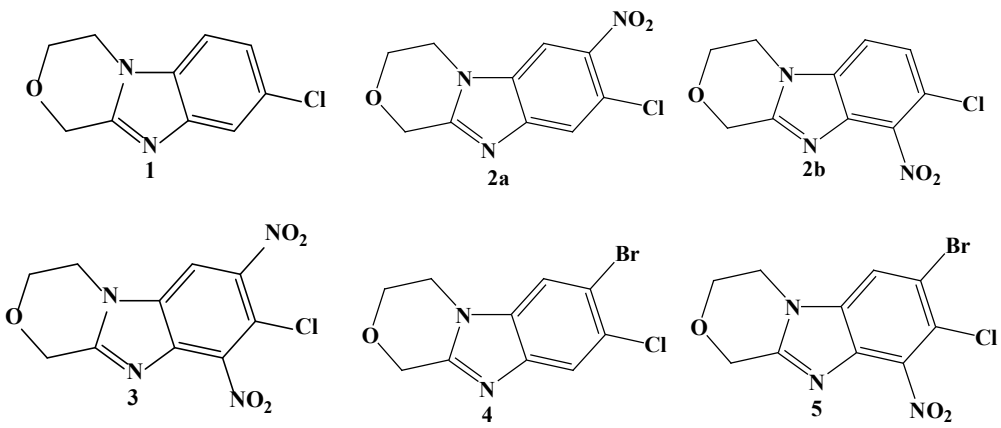

Fig. 1. Structure of 3,4-dihydro-1*H*-[1,4]oxazino[4,3-*a*]benzimidazole derivatives: 8-chloro-3,4-dihydro-1*H*-[1,4]oxazino[4,3-*a*]benzimidazole (**1**), 7-nitro-8-chloro-3,4-dihydro-1*H*-[1,4]oxazino[4,3-*a*]benzimidazole (**2a**), 9-nitro-8-chloro-3,4-dihydro-1*H*-[1,4]oxazino[4,3-*a*]benzimidazole (**2b**), 7,9-dinitro-8-chloro-3,4-dihydro-1*H*-[1,4]oxazino[4,3-*a*]benzimidazole (**3**), 7-bromo-8-chloro-3,4-dihydro-1*H*-[1,4]oxazino[4,3-*a*]benzimidazole (**4**) and 7-bromo-9-nitro-8-chloro-3,4-dihydro-1*H*-[1,4]oxazino[4,3-*a*]benzimidazole (**5**)

To assess the potential use of these compounds for the development of active pharmaceutical substances by *in silico* methods, we assessed the cytotoxic profile and pharmacokinetic parameters – absorption, distribution, metabolism, and excretion (ADME) – of the synthesised compounds.

Main body

To predict the cytotoxic activity of the synthesised molecules and assess the effects of structurally similar compounds described in the literature, an *in silico* cytotoxicity prediction procedure was performed using the ProTox 3.0 web server.

According to the ProTox 3.0 database, all compounds are highly likely to have a significant effect on the nervous and respiratory systems, but with varying degrees of toxicity. The probability of a toxic effect on the nervous system for compounds **1**, **2a**, **2b**, **4,** and **5** was 0.87, 0.57, 0.55, and 0.87, respectively. The probability value for substance **3** was below the acceptable range. The probability of a toxic effect on the respiratory system for compounds **1**, **2a**, **2b**, **3**, **4,** and **5** was 0.94, 0.79, 0.79, 0.77, 0.92, and 0.78, respectively.

Compounds **1**, **2a** and **4** were characterised by a toxicity level of 4; compounds **2b** and **3** were characterised by a toxicity level of 3. Compound **5** proved to be the most toxic, possessing a 2nd degree of oral toxicity.

The predicted median lethal doses (LD_{50}) for **1**, **2a**, **2b**, **3**, **4,** and **5** were 1000 mg/kg, 1500 mg/kg, 91 mg/kg, 91 mg/kg, 1000 mg/kg, and 10 mg/kg, respectively. Nevertheless, the position of the nitro group is altered in compounds **2a** and **2b**; the LD_{50} is significantly lower for compound **2b**.

Thus, based on the data obtained using the ProTox 3.0 web server, compounds **1**, **2a**, **2b**, **3**, **4** and **5** may potentially have an antitumour effect against brain and lung tumours.

Indeed, for a drug to be effective, the active molecule should reach the target site in the body in sufficient concentration and remain in a bioactive form for the duration required to produce the desired biological effects. The drug development process involves the early assessment of pharmacokinetic parameters during the candidate screening phase. However, the number of compounds under study is high and access to physical samples is limited [18].

To assess the potential of the synthesised molecules as drug candidates, an analysis of their pharmacokinetic properties was conducted using software available on the SwissADME online portal [19].

To visualise the results of the study into the pharmacokinetic properties of the compounds, Figure 2 shows the 'Boiled egg' predictive models. This model enables the assessment of passive absorption in the gastrointestinal tract and the penetration of potential drugs into the brain by calculating their lipophilicity and polarity.

Based on the calculated data, the value of the topological polar surface area (TPSA) – a descriptor used to characterise the ability of drugs to penetrate cells and cross biological barriers - ranged from 27.05 $Å^2$ to 118.69 $Å^2$, which falls within the acceptable range. As a result, all compounds can be formulated for oral use due to their high rate of absorption in the human gastrointestinal tract. Furthermore, all compounds except for compound **3** were predicted to be capable of crossing the blood-brain barrier, as indicated by the presence of points in the yellow region. This clearly suggests they could be used for therapeutic intervention in cases of malignant tumours of the central nervous system. The data obtained correlate with the results from the previous experiment, in which the probability of toxic effects on the nervous system was below acceptable levels, specifically for compound **3**.

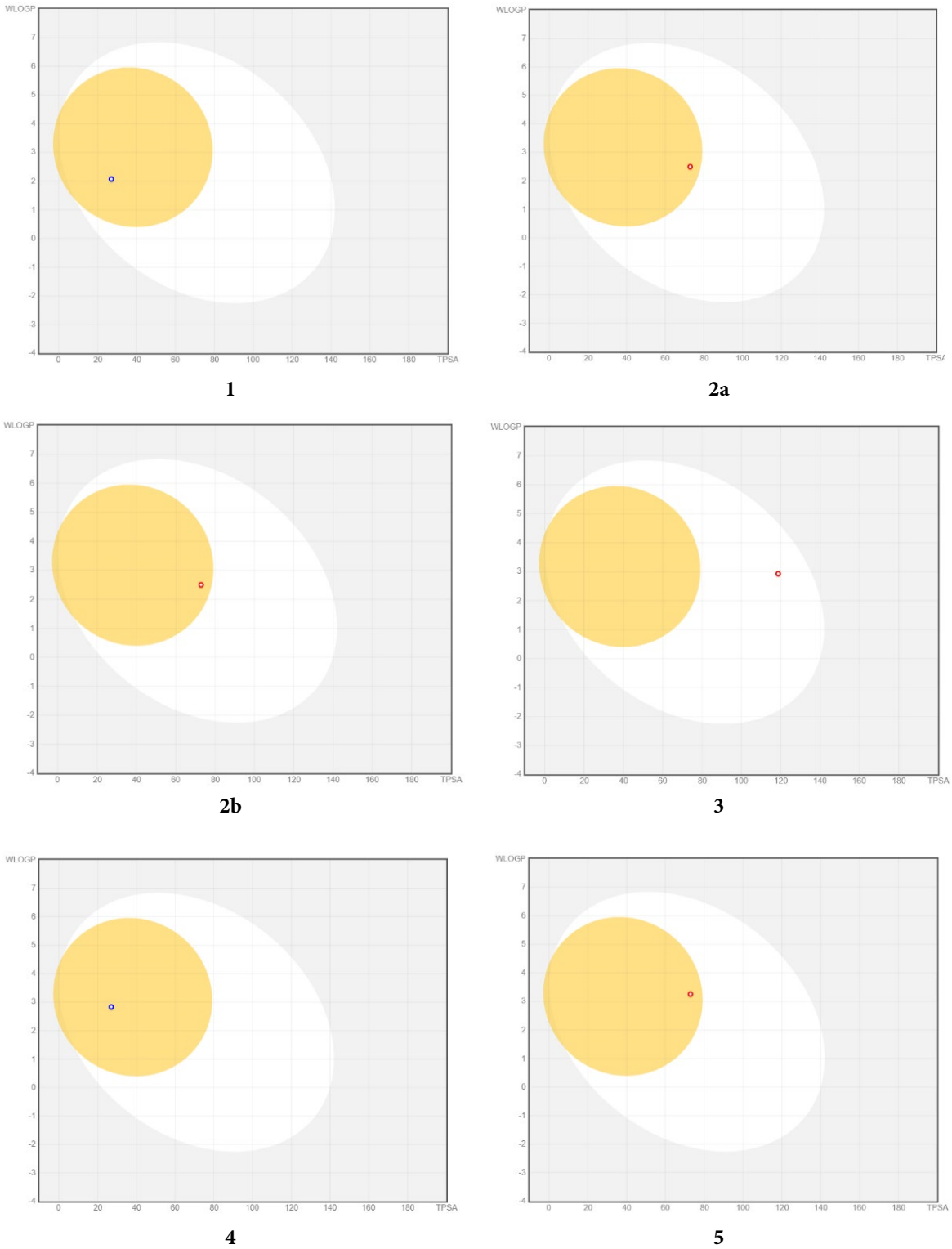

Fig. 2. The 'Boiled egg' model for predicting the passive absorption of compounds in the gastrointestinal tract (white area) and their penetration into the brain (yellow area). The blue dots indicate molecules that are predicted to be substrates of P-glycoprotein and are therefore actively transported out of the brain or into the lumen of the gastrointestinal tract. If a molecule is not a substrate for P-glycoprotein, the corresponding point is coloured red.

Furthermore, the results of the calculations for the key pharmacokinetic parameters, presented in Table 1, show the molecule fully complies with Lipinski's rule, falling within the range of parameters expected for a pharmaceutical product, and also has a good predicted bioavailability index (0.55).

Table 1. ADME parameters and bioavailability of the test compounds **1–5**

Compound	MW (g/M)	Donors	Acceptors	TPSA, Å²	Log Po/v	BA
1	208.64	0	2	27.05	1.92	0.55
2a	253.64	0	4	72.87	1.29	0.55
2b	253.64	0	4	72.87	1.27	0.55
3	298.64	0	6	118.69	0.65	0.55
4	287.54	0	2	27.05	2.54	0.55
5	332.54	0	4	72.87	1.88	0.55

Abbreviations: MW – molecular weight (less than 500 daltons); donors – number of hydrogen bond donors (no more than 5); acceptors – number of hydrogen bond acceptors (no more than 10); TPSA – total polar surface area (no more than 150); Log Po/v – octanol-water partition coefficient (not more than 5); BA – bioavailability assessment.

We also analysed the data obtained regarding the inhibitory potential of molecules against cytochrome P450 (CYP) isoenzymes. It plays a key role in phase I metabolism, primarily in the liver [20]. As shown in Table 2, all the compounds tested demonstrate predictable inhibitory activity exclusively against the CYP1A2 isoform; inhibition of other clinically relevant isoforms (CYP2C19, CYP2C9, CYP2D6, CYP3A4) is not predicted. This finding suggests a minimal risk of significant adverse effects (in particular, cardiovascular and neuroleptic effects, and increased myotoxicity) associated with these cytochrome P450 isoforms.

Table 2. Pharmacokinetic parameters of compounds **1–5**

Compound	Inhibition				
	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4
1	+	-	-	-	-
2a	+	-	-	-	-
2b	+	-	-	-	-
3	+	-	-	-	-
4	+	-	-	-	-
5	+	-	-	-	-

Abbreviations: '+' acts as an inhibitor; '-' does not act as an inhibitor.

Figure 3 also clearly represents the results obtained. It shows the bioavailability radars for the compounds under study. It illustrates the compounds' compliance with drug-likeness criteria across six key physicochemical properties, including lipophilicity (from -0.7 to +5.0), size (molecular weight from 150 to 500 g/mol), polarity (from 20 to 130 Å²), solubility (no more than 6), saturation (proportion of carbon atoms in sp^3 hybridisation of at least 0.25), and conformational flexibility (no more than nine rotating bonds) [19]. The molecules agree with all the necessary criteria, confirming their potential as bioavailable agents. Based on the data presented in Table 1 and Figures 2 and 3, we assume the compounds possess good ADME properties and high bioavailability.

Thus, an *in silico* analysis of the synthesised 3,4-dihydro-1*H*-oxazino[4,3-a]benzimidazole derivatives revealed their cytotoxic potential against the nervous and respiratory systems. It suggests these compounds may be effective against gliomas and lung cancer.

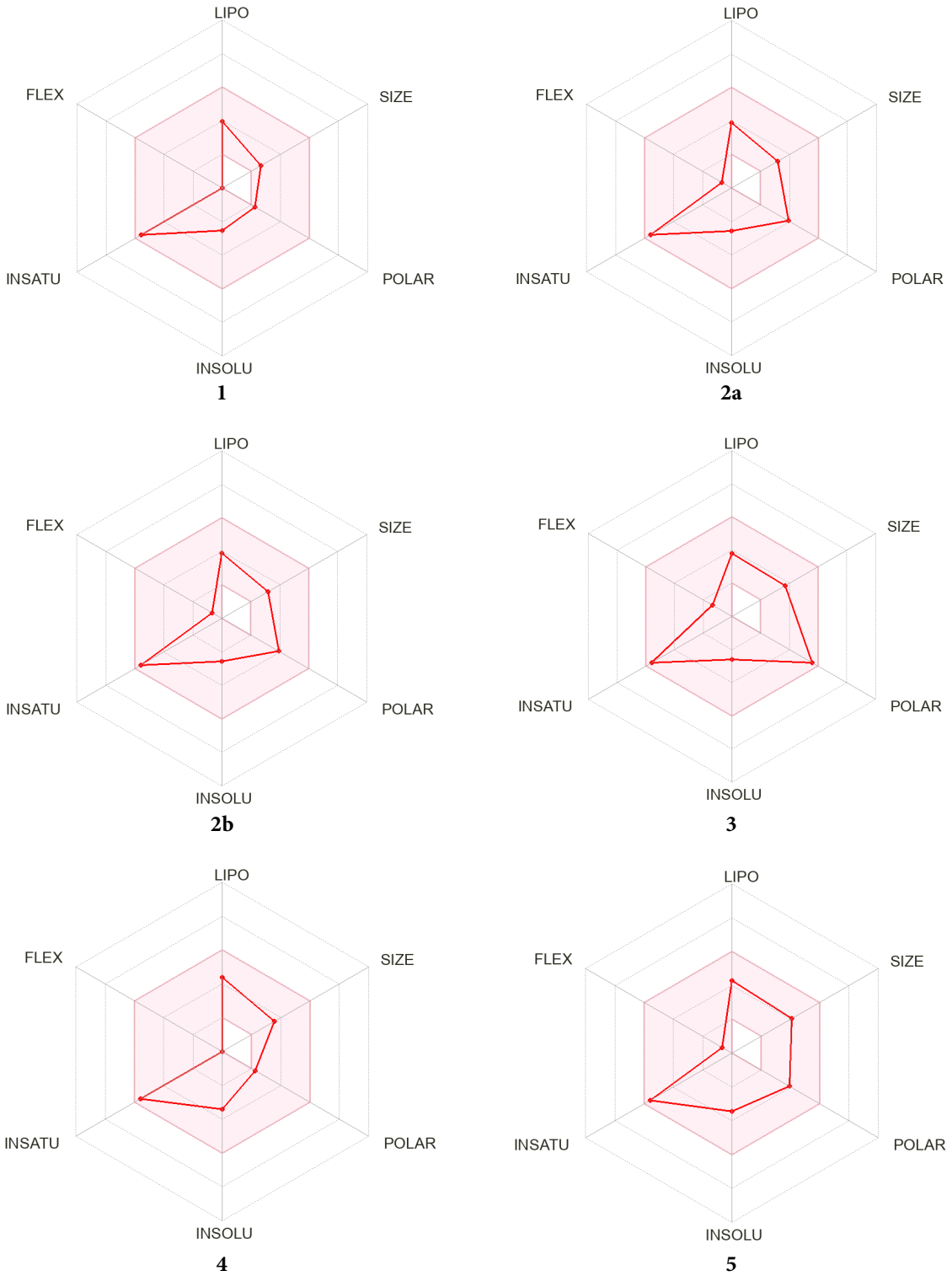

Fig. 3. Radar chart of the bioavailability of compounds **1**, **2a**, **2b**, **3**, **4,** and **5.** The graphs show the parameters LIPO (lipophilicity), SIZE (molecular weight), POLAR (polarity), INSOLO (insolubility), INSATU (unsaturation), and FLEX (conformational flexibility). The pink area corresponds to the ideal bioavailability values for oral administration; the red line indicates the actual value for the compound under study.

At the same time, full compliance with Lipinski's rule, a favourable ADME profile, and the ability of most compounds to cross the blood-brain barrier confirm the promise of their further investigation as oral agents, primarily for the treatment of central nervous system tumours. Those *in silico* molecular profiling plays a key role in the early stages of drug candidate

development, enabling the elimination of unpromising structures and the optimisation of ligands before resource-intensive *in vitro* experiments are conducted. The use of this approach demonstrated the value of further biological studies on 3,4-dihydro-1*H*-oxazino[4,3-a]benzimidazole and suggested that these compounds are the candidates for potential anticancer agents.

Experimental part

In silico assessment of the toxicological and cytotoxic profile of condensed benzimidazole derivatives 1–5.

The toxicological and cytotoxic profiles of the condensed benzimidazole derivatives 1–5 were assessed using the ProTox 3.0 web server (https://tox.charite.de). This program uses molecular similarity models and machine learning to predict 61 toxicity indicators, such as acute toxicity, organotoxicity, clinical toxicity, molecular initiating events (MIE), adverse effects (Tox21), a number of other toxicological endpoints, and non-target toxicity parameters [21].

In silico assessment of the toxicological and cytotoxic profile of condensed benzimidazole derivatives 1–5.

The pharmacokinetic properties of the condensed benzimidazole derivatives **1–5** were analysed using software from the Swiss Institute of Bioinformatics, available on the SwissADME online portal (http://www.swissadme.ch) [19].

The SwissADME program is based on a combination of empirical models, quantitative structure-activity relationships (QSARs), and physicochemical descriptors for predicting the pharmacokinetic properties of small molecules. It integrates algorithms such as BOLED (for assessing lipophilicity based on logP), iLOGP (a physical model based on free energy), the Lipinski, Ghose, Weber and Muegge rules for assessing drug-like properties, the models for solubility, absorption (including Caco-2 and P-gp), hepatotoxicity and synthetic accessibility (SAscore). These methods rely on extensive databases of experimental data and machine learning.

Conflict of interest

The authors declare that there are no conflicts of interest to report in this article.

Funding

The research was performed by Yaroslavl State Medical University, a federal state budgetary educational institution of higher education under the Ministry of Health of the Russian Federation as part of a state assignment for scientific research and development for 2025. The topic of the research is: 'Development of new drugs for targeted chemotherapy of oncological diseases based on condensed benzimidazole derivatives with a nodal nitrogen atom.'

References

1. **Ersan R.H., Kuzu B., Yetkin D., Alagoz M.A., Dogen A., Burmaoglu S.** 2-Phenyl substituted Benzimidazole derivatives: Design, synthesis, and evaluation of their antiproliferative and antimicrobial activities. *Med. Chem. Res*, 2022, 16(**31**), 1192-1208. DOI: 10.1007/s00044-022-02900-3.
2. **Pham E.C., Le Thi T.V., Ly Hong H.H., Vo Thi B.N., Vong L.B., Vu T.T., Vo D.D., Tran Nguyen N.V., Bao Le K.N., Truong T.N.** *N*,2,6-Trisubstituted 1*H*-benzimidazole derivatives as a new scaffold of antimicrobial and anticancer agents: design, synthesis, *in vitro* evaluation, and *in silico* studies. *RSC Adv.*, 2022, 13(**1**), 399-420. DOI:10.1039/d2ra06667j.
3. **Bansal S., Gaur R., Bhardwaj H., Kumar N., Sharma G.K., Mishra S.S.** Synthesis, biological evaluation, and molecular docking studies of benzimidazole derivatives substitutes as new antimicrobial agents. *JDDMC,* 2024, 10(**2**), 54-66. DOI: 10.11648/j.jddmc.20241002.12.
4. **Ahmed A.E.R., Hassan Y.A.-E.** Benzimidazole derivatives as potential chemotherapeutic agents. *Curr. Drug Ther.* 2013, 8(**1**), 1-14. DOI: 10.2174/1574885511308010001
5. **Akhtar W., Khan M.F., Verma G., Shaquiquzzaman M., Rizvi M.A., Mehdi S.H., Akhter M., Alam M.M.** Therapeutic evolution of benzimidazole derivatives in the last quinquennial period. *Eur. J. Med. Chem.*, 2017, 126, 705-753. DOI: /10.1016/j.ejmech.2016.12.010
6. **Begunov R.S., Egorov D.O., Chetvertakova A.V., Savina L.I., Zubishina A.A.** Antibacterial activity of the halogenand nitro derivatives of benzimidazole against *Bacillus Subtilis*. *Antibiot. Khimioter.*, 2023, 68(**3-4**), 19-24. DOI: 10.37489/0235-2990-2023-68-3-4-19-24.
7. **Begunov R.S., Zaitseva Y.V., Sokolov A.A., Egorov D.O., Filimonov S.I.** Synthesis and Antibacterial Activity of 1,2,3,4-Tetrahydro- and Pyrido[1,2-a]benzimidazoles. *Pharm. Chem. J.*, 2022, 56(**1**), 22–28. DOI: 10.1007/s11094-022-02596-0
8. **Nandwana N.K., Singh R.P., Patel O.P.S., Dhiman S., Saini H.K., Jha P.N., Kumar A.** Design and Synthesis of Imidazo/Benzimidazo[1,2-*c*]quinazoline Derivatives and Evaluation of Their Antimicrobial Activity. *ACS Omega*, 2018, 3(**11**), 16338-16346. DOI: 10.1021/acsomega.8b01592.
9. **Mohamed B.G., Abdel-Alim A.M., Hussein M.A.** Synthesis of 1-acyl-2-alkylthio-1,2,4-triazolobenzimidazoles with antifungal, anti-inflammatory and analgesic effects. *Acta Pharm.* 56(**1** 31-48.
10. **Huo X., Hou D., Wang H., He B., Fang J., Meng Y., Liu L., Wei Z., Wang Z., Liu F.W.** Design, synthesis, *in vitro* and *in vivo* anti-respiratory syncytial virus (RSV) activity of novel oxizine fused benzimidazole derivatives. *Eur. J. Med. Chem.*, 2021, 224, Article 113684. DOI: 10.1016/j.ejmech.2021.113684.
11. **Wang H., Meng Y., Yang J., Huang H., Zhao Y., Zhu C., Wang C., Liu F.W.** Design, synthesis and antitumour activity of novel 5(6)-amino-benzimidazolequinones containing a fused morpholine. *Eur. J. Med. Chem.*, 2022, 238, Article 114420. DOI: 10.1016/j.ejmech.2022.114420.
12. **Aleksandrova Y., Savina L., Shagina I., Lyubina A., Zubishina A., Makarova S., Bagylly A., Khokhlov A., Begunov R., Neganova M.** New Dihalogenated Derivatives of Condensed Benzimidazole Diones Promotes Cancer Cell Death Through Regulating STAT3/HK2 Axis/Pathway. *Molecules*, 2025, 30(**21**), 4150. DOI: 10.3390/molecules30214150
13. **Begunov R.S., Aleksandrova Y.R., Shagina I.A., Kucherenko M.V., Pelyovin P.S., Khokhlov A.L., Neganova M.E.** Cytotoxicity of a new condensed benzimidazoledione derivative against tumor and normal cell lines. *Acta Biomedica Scientifica,* 2025;10(**6**):12-19. (In Russ.) DOI: 10.29413/ABS.2025-10.6.2
14. **Sondhi S.M., Rajvanshi S., Johar M., Bharti N., Azam A., Singh A.K.** Anti-inflammatory, analgesic and antiamoebic activity evaluation of pyrimido[1,6-a]benzimidazole derivatives synthesized by the reaction of ketoisothiocyanates with mono and diamines. *Eur. J. Med. Chem.,* 2002, 37(**10**), 835-843. DOI:10.1016/s0223-5234(02)01403-4.
15. **Sondhi S. M., Rani R., Singh J., Roy P., Agrawal S. K., Saxena A. K.** Solvent free synthesis, anti-inflammatory and anticancer activity evaluation of tricyclic and tetracyclic benzimidazole derivatives. *Bioorg. Med. Chem. Lett.,* 2010, 20(**7**), 2306-2310. DOI:10.1016/j.bmcl.2010.01.147.

16. **Begunov R.S., Savina L.I., Astafieva D.A.** Intramolecular amination of *ortho*-nitro-*tert*-anilines as a method for the synthesis of condensed benzimidazole derivatives with a nodal nitrogen atom. *From Chemistry Towards Technology Step-by-Step,* 2025, 6(**1**), 88-98. DOI:10.52957/2782-1900-2025-6-1-88-98
17. **Kucherenko M.V., Savina L.I., Begunov R.S., Zubishina A.A., Gracheva E.L.** Regioselectivity of the S_EAr reaction of 8-chloro-3,4-dihydro-1H-[1,4]oxazino[4,3-a]benzimidazole. *From Chemistry Towards Technology Step-by-Step*, 2025, 6(4), 126-136. DOI: 10.52957/2782-1900-2025-6-4-126-136
18. **Balani S.K., Miwa G.T., Gan L.S., Wu J.T., Lee F.W.** Strategy of utilizing in vitro and in vivo ADME tools for lead optimization and drug candidate selection. *Curr Top Med Chem*. 2005, 5(11), 1033-1038. DOI:10.2174/156802605774297038
19. **Daina A., Michielin O., Zoete V.** SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci. Rep.*, 2017, 7, Article 42717. DOI: 10.1038/srep42717
20. **Manikandan P., Nagini S.** Cytochrome P450 Structure, Function and Clinical Significance: A Review. *Curr Drug Targets*. 2018, 19(1), 38-54. DOI: 10.2174/1389450118666170125144557
21. **Banerjee P., Kemmler E., Dunkel M., Preissner R.** ProTox 3.0: a webserver for the prediction of toxicity of chemicals. *Nucleic Acids Res.*, 2024, 52(W1), W513-W520. DOI: 10.1093/nar/gkae303

Received 28.01.2026
Approved after reviewing 25.02.2026
Accepted 05.03.2026