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Computer screening of peptidomimetics and small-molecule ligands of B-cell membrane proteins for therapy of Burkitt lymphoma

Iryna Demianenko*

PhD in Technical Sciences, Assistant Professor
National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute"
03056, 37 Beresteiskyi Ave., Kyiv, Ukraine
https://orcid.org/0000-0002-0832-3619

Anastasiia Bakhmach

Master of Science
Grenoble Alpes University
38400, 621 Centrale Ave., Saint-Martin-d'Heres, France
https://orcid.org/0000-0003-3746-3853

Abstract. The capabilities of molecular modelling and docking allow for the discovery of new potential drug agents to improve the treatment of diseases, which is a current concern. The objective of this study was to conduct *in silico* screening for antibody mimetics to B-cell membrane proteins for the treatment of Burkitt lymphoma through virtual screening. In this work, a standard protocol for structure-based virtual screening was employed, with the distinction that pharmacophores for screening were built not based on small-molecule ligands but on selected amino acid residues of antibodies. Based on literature data and the presence of a mechanism of direct cytotoxic action, as well as the availability of 3D structures of complexes, three monoclonal antibodies were selected: obinutuzumab, epratuzumab, and atezolizumab. The identification of biological targets was carried out by searching for 3D structures of selected complexes with target proteins in the Protein Data Bank. For virtual screening, the web service Pharmit was chosen. Using the Molecular Operating Environment program, pharmacophore models were constructed for three complexes: CD20 and obinutuzumab, CD22 and epratuzumab, and PD-L1 and atezolizumab. Docking with the CD20, CD22, and PD-L1 proteins was conducted at the binding sites recognised by the original antibody. Through *in silico* virtual screening using the Molecular Operating Environment software, a search for antibody mimetics to B-cell membrane proteins for Burkitt lymphoma treatment was conducted, resulting in the selection of 5 potential anti-lymphoma agents: CHEMBL505179 for the CD20 receptor, an antagonist of the melanocortin receptor for CD20 (PubChem-44406884), an inhibitor of blood clotting Factor Xa for CD22 (PubChem-136510605), and a blocker of epithelial Na+ channels for CD22 (PubChem-126761430), and an agonist of the melanocortin receptor for PD-L1 (PubChem-25078192). The obtained results can be applied in the pharmaceutical industry and oncological practice to enhance therapeutic outcomes in the treatment of patients with Burkitt lymphoma

Keywords: molecular docking; pharmacophores; antibodies; mimetics; immunogenicity

INTRODUCTION

The search for new potential drug agents remains a relevant task. Modern bioinformatic methods and algorithms significantly reduce the time and cost of laboratory research, streamlining the search and development of new drugs. Computational drug discovery methods are more cost-effective than standard methods and can analyse a vast number of components under conditions that would be impossible to recreate in laboratories. These methods combine three components: biological data, chemical knowledge, and modelling algorithms, offering a wide range of tools and plugins for predicting the biological activity of both molecular structures and chemical compounds with high probability. Developed and validated models can be further used for virtual screening of a vast number of substances, enabling the quick identification of promising components. These virtual methods can be combined with

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*Corresponding author

classical molecular docking methods to confirm their activity, as mentioned by S. Ai *et al.* [1] and L. Kucherenko *et al.* [2].

Molecular modelling and docking methods allow the selection and assessment of the binding strength between interacting pairs, such as antibodies and proteins. For this task, various programs exist to prepare computer models of molecules for interaction and conduct their docking. One such program is Molecular Operating Environment (MOE), known for its intuitive interface, a wide range of functions, and plugins, providing accurate computational results. In the work of Y. Zhang *et al.* [3], virtual screening of pharmacophores and molecular docking were utilised to identify potential inhibitors of Src (proto-oncogene tyrosine-protein kinase). The researchers obtained 891 pharmacophores and selected 10 for further investigation based on the highest docking scores and calculated binding energy between components. An essential component of computational drug discovery methods is the availability of information in databases about the properties of components, including absorption, distribution in the body, metabolic pathways, and elimination routes, as well as toxic properties. Evaluating all these characteristics of selected components, researchers identified two molecules as potential inhibitors of the Src kinase family.

Frequently, the atomic structure of a pharmacologically relevant receptor is unknown. Three-dimensional alignment of potential ligands can be applied to establish structural requirements for their biological activity. Another strategy is the creation of pharmacophores, aligning ligands considering the minimum number of molecular properties of components [4]. Alternatively, searching databases for 3D molecular structures and their alignment can be employed [5]. The methodology based on aligning 3D structures to determine the biological activity of ligands assumes that if two ligands have similar biological activity and bind at the same points (pockets) to the receptor (protein), the connection between these conformations is strong [6].

Burkitt lymphoma (BL) belongs to the group of non-Hodgkin lymphomas (NHL) and is the most aggressive malignancy, doubling its cell population every 24-48 hours [7]. BL is characterised by hyperinvasiveness and high lethality [8]. Intensive, prolonged combined chemotherapy regimens are used for BL treatment, demonstrating positive results (adult 5-year survival is 56-70%). However, 15% of patients develop chemotherapy-resistant or recurrent forms, requiring the development of new therapeutic agents [9].

In addition to chemotherapy, monoclonal antibody drugs are used for BL treatment, showing high effectiveness but having drawbacks such as the inability for oral intake, immunogenicity, and high production costs, as mentioned by T. Lutsenko & M. Chalenko [10]. The search for small-molecule antibody mimetics, devoid of these drawbacks, is relevant. Computer-aided screening methods, which significantly reduce the time and financial costs of finding potential therapeutic agents, are suitable for such a search.

The study aimed to search for *in silico* antibody mimetics to B-cell membrane proteins for Burkitt lymphoma treatment through virtual screening. To achieve this, virtual screening and molecular docking with BL B-cell target proteins based on known cytotoxicity properties of substances from open libraries are required. Promising complexes need to be selected, and their potential action as anti-lymphoma agents should be analysed.

MATERIALS AND METHODS

The research was based on the use of a standard protocol for structure-based virtual screening. However, the applied pharmacophores for screening were constructed not based on small-molecule ligands but on selected amino acid residues of antibodies [11-13]. The first step in computer-aided drug discovery is the identification of the biological target. 3D structures of selected complexes with target proteins were downloaded from the Protein Data Bank (PDB). Two conditions were considered for selecting a protein as the target:

1. The therapeutic antibody to the protein should act through the mechanism of direct cytotoxicity (neutralization).

2. The availability of a 3D structure of the antibody-protein complex in the PDB database. Performing computer-aided drug discovery is possible even without using the 3D structure – for example, based on the structure of homologous proteins, but it is less accurate.

An important second step in the search for a potential drug candidate is the construction of pharmacophoric hypotheses, or simply pharmacophores. The MOE software package was used to build the pharmacophore model. The "QuickPrep" function in the MOE program was used to prepare the complexes before constructing the pharmacophore. This program has several functions that allow the removal of unnecessary water molecules, addition of missing hydrogen atoms, and selection of antibody regions for further binding. For virtual screening, the Pharmit web service was chosen, containing more than 340 million compounds from databases such as CHEMBL25, ChemDiv, ChemSpace, MCULE, MCULE-ULTIMATE, MolPort, NCI Open Chemical Repository, PubChem, LabNetwork, ZINC, and three manually added libraries of peptide mimetics [14].

Pharmit uses the Pharmer technology as its search algorithm [15]. Pharmer decomposes the uploaded pharmacophore into individual triangles, then searches for matches between compounds from libraries and formed triangles, after which it combines the results. For each compound, the root-mean-square deviation (RMSD) value is computed using the formula [16] (1):

$$RMSD = \sqrt{\frac{\sum_i^n \left(\frac{d(c_i,q_i)}{r_i}\right)^2}{n}}, \quad (1)$$

where $d(c_i, q_i)$ is the distance between the corresponding feature of the loaded pharmacophore and the compound; r_i – the permissible radius of the connection; n – the number of pharmacophore signs.

This value is a measure of the similarity between a compound and the pharmacophore. Following the execution of the Pharmer search query, a table is generated with the found compounds arranged in decreasing order of the RMSD value.

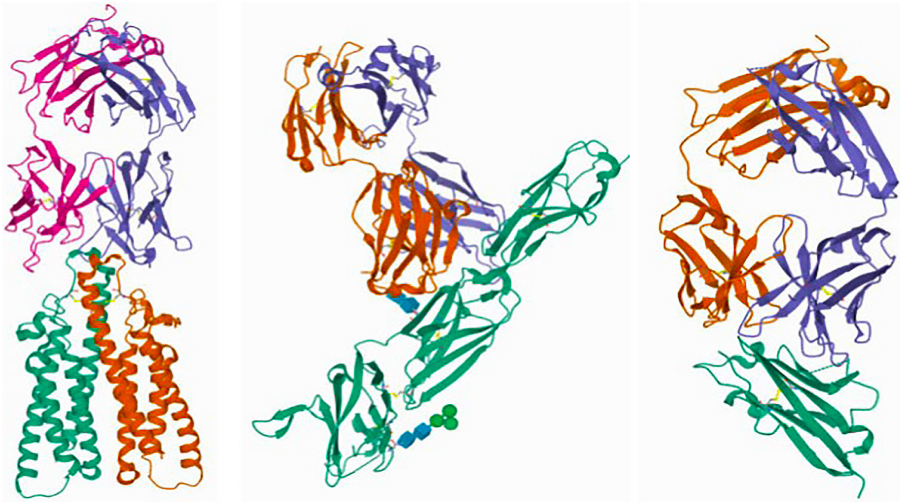
Based on literature data and the presence of a mechanism of direct cytotoxic action, as well as the availability of 3D structures of complexes, three monoclonal antibodies were selected:

1. Obinutuzumab [17] – a humanized monoclonal antibody to the CD20 protein, which is expressed on the surface of 93-100% of lymphoma cells [18, 19].

2. Epratuzumab – a humanized monoclonal antibody to the CD22 protein, which is expressed on the surface of 47-70% of lymphoma cells [18, 20].

3. Atezolizumab – a humanized monoclonal antibody to the PD-L1 protein, which is expressed on the surface of 80% of lymphoma cells [21].

The 3D structures of the complexes CD20 and obinutuzumab, CD22 and epratuzumab [22], and PD-L1 and atezolizumab [23] were downloaded from the PDB with the following codes: 6Y9A (resolution 4.20 Å), 5VL3 (resolution 3.10 Å), and 5X8L (resolution 3.10 Å), respectively (Fig. 1).



a) b) c)

Figure 1. 3D structures of the proteins used in this work

Notes: a) 3D structure of CD20 and obinutuzumab complex; b) 3D structure of CD22 and epratuzumab complex; c) 3D structure of PD-L1 and atezolizumab complex

Source: [22, 23]

To prepare structures for docking in the MOE program, files in PDB format were loaded, and heavy and light chains of processed antibodies were removed. Using the "Dock" function, docking was performed with compounds loaded in SDF format with selected regions of the target protein. The choice of the program is explained by its broader and more convenient set of tools compared to other programs such as LigandScout and Maestro. The virtual screening method based on the pharmacophore involves comparing compounds from a molecular library with a pharmacophore hypothesis, allowing the automated identification of compounds likely to have biological activity in a relatively short time. The obtained activity will be like the activity of compounds used to model the current pharmacophore. Obinutuzumab was chosen because it has a Fc fragment with increased affinity for FcγRIIIa receptors on the surface of effector cells. These target cells include neutrophils and macrophages, so this antibody has an enhanced ability for antibody-dependent cellular cytotoxicity in the immune response. Its direct cytotoxic action also has a lysosomal nature and does not depend on additional apoptotic pathways [24]. As for epratuzumab, it initiates phosphorylation of CD22 with subsequent internalization of CD22 and the CD79α protein, which is part of the B-cell receptor and disrupts intracellular signalling during immune reactions. The action of epratuzumab almost entirely relies on direct cytotoxicity through negative regulation of BCR signalling and to a tiny extent on complement-dependent cytotoxicity (CDC) and Antibody-dependent cellular cytotoxicity (ADCC) [25]. Atezolizumab, in turn, blocks the interaction of PD-L1 with PD-1 and CD80, reactivating the immune response against tumour cells [26, 27]. The choice of Pharmit is explained by the fact that among all web applications for virtual screening, it contains the most comprehensive compound database and allows users to add their own compound libraries. The exclusion of the use of desktop programs is explained by the limited computational capabilities during the research; it is indicative that Pharmit works 10-1000 times faster than MOE [16].

RESULTS AND DISCUSSION

Using the MOE computer program, pharmacophore models were constructed for three complexes. For the CD20 and obinutuzumab complex, a pharmacophore model was built based on the amino acid residues Ser30, Tyr31, Trp33, Arg52, Gly54, and Asp55 in the heavy chain of obinutuzumab. The obtained model contains a total of 17 features, including two aromatic groups, five hydrogen bond acceptors, six hydrogen bond donors, and five hydrophobic groups. The pharmacophore model for the CD22 and epratuzumab complex was constructed based on the amino acid residues Ser31, Trp33, Arg53, Tyr56, Glu58 in the heavy chain of epratuzumab. The resulting model contains a total of 8 features, including one aromatic group, three hydrogen bond acceptors, and four hydrogen bond donors. The pharmacophore model for the PD-L1 and atezolizumab complex was constructed based on the amino acid residues Trp50, Ser52, Tyr54, Gly55, Ser57, Thr58, Tyr59 in the heavy chain of atezolizumab. The obtained model contains only nine features, including one aromatic group, four hydrogen bond acceptors, and four hydrogen bond donors (Fig. 2).

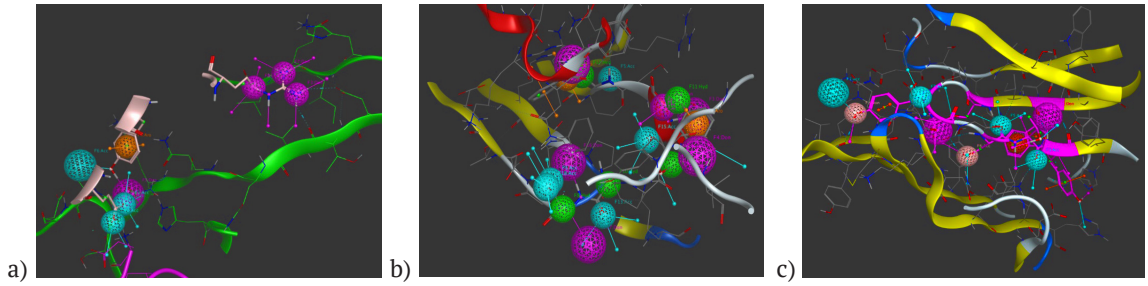


Figure 2. Pharmacophore protein-binding site of antibodies to (a) CD20; (b) CD22; (c) PD-L1
Notes: a) the CD20 pharmacophore model consists of amino acid residues Ser30, Tyr31, Trp33, Arg52, Gly54 and Asp55 of the heavy chain of obinutuzumab; b) the CD22 pharmacophore model consists of amino acid residues Ser31, Trp33, Arg53, Tyr56, Glu58 of the heavy chain of epratuzumab; c) PD-L1 pharmacophore model consists of amino acid residues Trp50, Ser52, Tyr54, Gly55, Ser57, Thr58, Tyr59 of the heavy chain of atezolizumab
Source: compiled by the authors

As a result of the library screening, the following results were obtained: for the CD20 and obinutuzumab pair – 157 compounds, for the CD22 and epratuzumab pair – 117 compounds, and for the PD-L1 and atezolizumab pair – 205 compounds. Guided by the threshold value of the RMSD indicator (0.8 Å), 15 compounds were selected for the CD20 protein, 15 compounds for CD22, and 35 compounds for PD-L1 for further work. Obinutuzumab was chosen based on its Fc fragment with increased affinity for FcγRIIIa receptors on the surface of effector cells. Epratuzumab was selected based on its ability to initiate phosphorylation of CD22 with subsequent internalization of CD22 and CD79α, which is part of the B-cell receptor and disrupts intracellular signalling during immune reactions.

Docking with the CD20 protein was performed at the binding site recognised by the original antibody. The binding site consists of amino acids in chain A, namely Ala170, Asn171, Pro172, Ser173, Glu174, Kys175, Asn176, Ser177, and Pro178. Docking with the CD22 protein was performed with the amino acids Ile 145, Ser 211, His 213, Gly 214, Asp 232, Thr 233, Gln 235, Val 267, Asn 271, and Tyr 274. Docking with the PD-L1 protein was conducted with the amino acids Ala18, Glu45, Asp49, Ala51, Ala52, Ile54, Tyr56, Glu58, Glu60, Asp61, Asn63, Qln66, Val68, His69, Val111, Arg113, Met115, Ser117, Tyr118, Gly119, Ala121, Tyr123, and Arg125. Based on the docking results with the minimum score (S) values, which characterize the binding affinity, 5 potential anti-lymphoma agents were selected (Fig. 3).

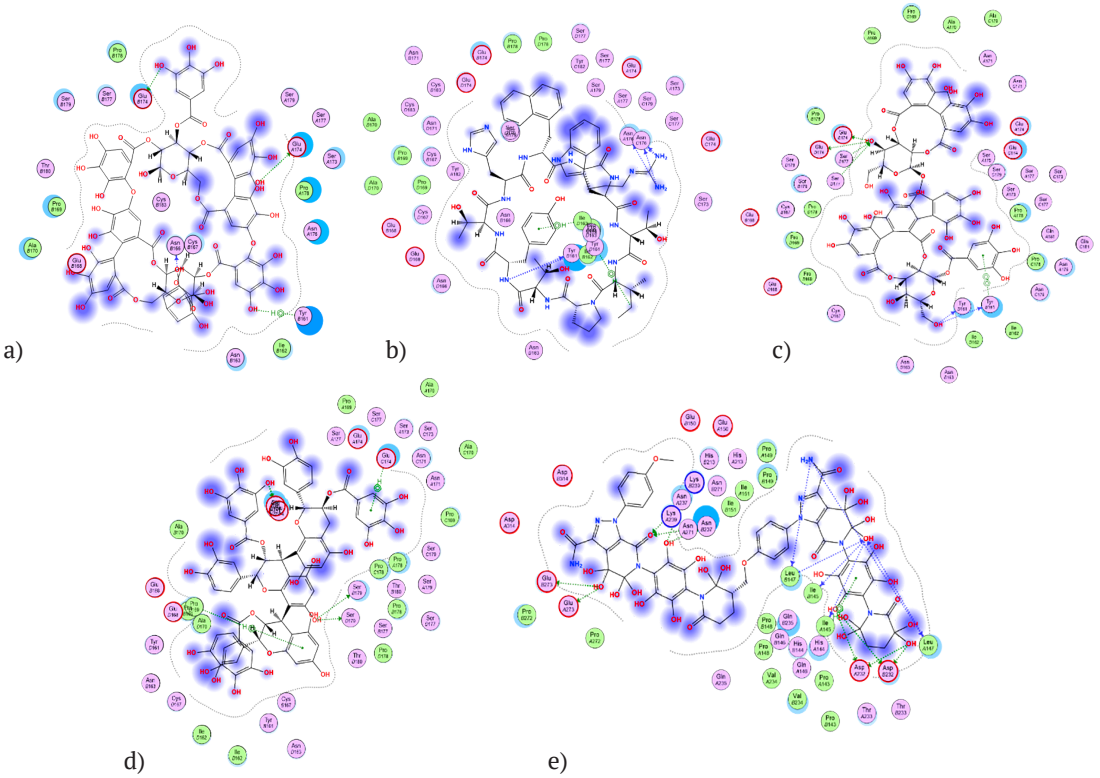


Figure 3. Agents obtained as a result of docking
Notes: a) CHEMBL505179 in the binding site of obinutuzumab with CD20; b) PubChem-44406884 in the binding site of obinutuzumab to CD20; c) PubChem-101933623 in the binding site of obinutuzumab with CD20; d) PubChem-44249983 in the binding site of obinutuzumab to CD20; e) PubChem-136510605 in the CD22 binding site of epratuzumab
Source: compiled by the authors

According to the standard parameters of Pharmit, it is recommended to filter compounds based on a value of 2 Å, or vary it in the range of 1.5-2.5 Å. By filtering at 0.8 Å, the authors applied more stringent computational constraints to select potential compounds. Among all the selected compounds suitable for testing *in vitro* anti-lymphoma activity are the following:
CHEMBL505179, PubChem-101933623, PubChem-16135635, PubChem-44249983, PubChem-16209234, PubChem-135854913, PubChem-44256795, MolPort-046-788-300, PubChem-45359413, MCULE-5553462135. MolPort-046-857-137 (Table 1).

Their activity can be assessed on BL cell lines such as Raji, Ramos, and Akata. This can be achieved by evaluating cell viability and survival using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) after incubating cells with the selected compounds. The analysis of these potential anti-lymphoma agents was conducted using PubChem data, revealing that five of the selected compounds already exhibit documented biological activity. Four of them, namely PubChem-44406884, PubChem-136510605, PubChem-126761430, and PubChem-25078192, are experimentally confirmed *in vitro* ligands for proteins distinct from the chosen biological targets typical for B-cell BL.

Table 1. Summarized data on the results of molecular docking of formed formacophores with ligands

White	Ligand	S score, kcal/mol	A/c with which it forms connections
CD20	CHEMBL505179	-9.9295	Glu A174, Tyr B161, Asn B166, Glu B174
	PubChem-44406884	-9.5939	Tyr B161, Ile B162, Asn B176
	PubChem-101933623	-9.3220	Tyr B161, Glu B174, Ser B177
	PubChem-16135635	-8.8296	Tyr B161, Glu B174, Glu B168, Cys B183, Asn A176
	PubChem-44249983	-8.4617	Pro B169, Glu B174, Ser B179
CD22	PubChem-16209234	-10.807	Asn 235, Asn 271
	PubChem-136510605	-10.5678	Ile 145, Leu 147, Asp 232, Lys 239, Asn 271, Glu 273
	PubChem-135854913	-10.1344	Ile 145, Leu 147, Lys 215, His 213, Ser 269, His 213, The 233, Asn 237
	PubChem-44256795	-10.0038	His 213, Val 234, Gln 235, Glu 266, Val 267, Glu 273
	PubChem-126761430	-9.9341	Asp 232, Ser 269, Ser 270
PD-L1	MolPort-046-788-300	-10.953	Val 76, Arg 113, Asp 122
	PubChem-45359413	-10.8218	Gln 66, Arg 125
	MCULE-5553462135	-9.9989	Glu 58, Arg 113, Ser 117, Arg 125
	MolPort-046-857-137	-9.8211	Glu 58, Met 59, Glu 60, Arg 113, Arg 125
	PubChem-25078192	-9.207	Asp 26, Glu 60, Lys 124, Arg 125

Source: compiled by the authors

The compound PubChem-44406884 (Table 2) acts as an antagonist to the melanocortin receptor. This receptor is specific to the heptapeptide core, which is common to adrenocorticotropic hormone and alpha-, beta-, and gamma-MSH. PubChem-44406884 plays a central role in energy homeostasis and somatic cell growth in the body. The melanocortin 4 receptor (MC4R) is expressed in hypothalamic cells and is essential for regulating appetite and energy expenditure in the body. Dysfunction of MC4R in humans leads to hyperphagia, impaired satiety sensation, and the development of obesity [28-30]. A similar compound is the investigated PubChem-25078192. At this stage of scientific research, the compound PubChem-136510605 is not sufficiently studied in terms of its impact on Burkitt lymphoma cells. Scientific sources about PubChem-25078192 currently indicate that it is analogous in action to the inhibitor of blood clotting factor Xa. Similar novel substituted carboxamidic compounds and pharmaceutical compositions made based on them are mainly used to inhibit the activity of factor Xa in patients for specific indications to treat disorders such as deep vein thrombosis, pulmonary artery embolism, cerebrovascular ischemia, coronary artery disease, and oncological diseases [31].

Table 2. Data on biological activity of selected ligands

White	Ligand	Data on biological activity
CD20	CHEMBL505179	Immunoregulatory, antiproliferative action
	PubChem-44406884	Melanocortin cell receptor antagonist
CD22	PubChem-136510605	Inhibitor of blood coagulation factor Xa
	PubChem-126761430	A blocker of epithelial Na^+ channels of the plasmolemma
PD-L1	PubChem-25078192	Melanocortin membrane receptor agonist

Source: compiled by the authors based on [32-34]

Compound PubChem-126761430 (Table 2) has undergone a limited and insufficient amount of scientific research to understand the full spectrum of its action on normal and tumour cells in the body. It has been established that PubChem-126761430 is structurally similar to the blocker of plasma membrane sodium channels in epithelial cells. One approach to restoring the protective layer of fluid on the mucous membrane surface is the “rebalancing” of the

system by blocking sodium channels in the cell membrane and corresponding fluid absorption. The protein in the membranes of epithelial cells that mediates the inhibition of sodium ion movement and fluid absorption is the epithelial sodium channel (ENaC). ENaC is located on the apical surface of epithelial cells lining mucous membranes, at the interface of the mucosal surface and the surrounding environment (or the organ cavity). Under normal conditions, to suppress ENaC-mediated sodium ion and fluid absorption, an ENaC blocker of the amiloride class should be delivered to the mucous membrane surface and maintained in place to achieve maximum therapeutic benefit and effect.

Of all the investigated compounds listed, the most promising is the substance CHEMBL505179, which is a macrocycle from the ellagitannin group. CHEMBL505179 is extracted from plant raw materials of the genera *Oenothera, Epilobium,* and *Eucalyptus*. This substance demonstrates a broad spectrum of immunoregulatory effects, including reducing the production of reactive oxygen species by mouse lymphocytes, inducing neutrophil migration *in vitro* and *in vivo*, activating NF-κB factor in monocyte culture, and more. Additionally, this compound exhibits high anti-proliferative activity in experimental studies on prostate carcinoma cells, hepatocellular carcinoma, and other tumours [28], making it promising for the development of a new drug for targeted BL treatment in patients.

Research on NHL group representatives [35], including follicular lymphomas, marginal cell lymphomas, and BL as the main part of malignant neoplasms of mesenchymal cells, focuses on targeted treatment with courses of monoclonal antibody drugs. Due to their high specificity, these drugs are targeted at key markers of malignant B-cells, enhancing the patient's immune system defence mechanisms, leading to apoptosis of malignant cells.

In the conducted study, obinutuzumab was chosen because it has a Fc fragment with increased sensitivity to FcγRIIIa receptors on the surface of effector cells (specifically neutrophils and macrophages) and enhanced ability for antibody-dependent cellular cytotoxicity in the lysosomal immune response, independent of additional apoptotic pathways. The effectiveness of obinutuzumab in lymphomas of various origins is confirmed by other contemporary studies [24, 27, 36]. Obinutuzumab is a type II anti-CD20 monoclonal antibody belonging to the immunoglobulin G2 class. Published data by A. Prica & M. Crump [36] show that targeted therapy against CD20 activity using the monoclonal antibody rituximab led to significant improvement in overall post-therapeutic survival of patients with B-cell NHL and BL. Despite the identified improvements, some patients were diagnosed with relapse or refractoriness after rituximab treatment. Obinutuzumab, despite its recent discovery, is recognised as a promising humanized anti-CD20 monoclonal antibody currently studied in late-phase clinical trials compared to rituximab. Preclinical safety experiments by A. Prica & M. Crump [36] demonstrate that obinutuzumab is a more effective anti-CD20 monoclonal antibody than rituximab, especially in assessing its role in initiating antibody-dependent cellular cytotoxicity and direct cell apoptosis in BL. Obinutuzumab is safe and effective for CD20-positive NHL, including BL, and requires further investigation. As noted by A. Prica & M. Crump [36] and A. Fekih *et al.* [37], obinutuzumab has been recommended for use in clinical treatment protocols for lymphomas since 2013, specifically in combination with chlorambucil; since 2016, it has been recommended for lymphoma treatment in combination with bendamustine followed by maintenance therapy with obinutuzumab every two months for two years in patients refractory to rituximab. The use of obinutuzumab after completion of clinical studies that justify its safety and efficacy compared to or with the use of other drugs will help improve the tactics of targeted and personalised BL therapy.

Among other potential ligands for targeted BL treatment, the study identified the melanocortin receptor antagonist (PubChem-44406884) to the CD20 receptor. Several studies support the use of the melanocortin receptor antagonist for lymphocytic malignancies, particularly mantle cell lymphoma [37]. Melanocortin receptor 1, investigated as a target in modern clinical studies by M. Li *et al.* [38], is being studied for delivering active components to treat metastatic melanoma and other malignant neoplasms. Increased expression of melanocortin receptor 1 is detected *in situ* in histological samples of diagnosed melanoma. B-Raf proto-oncogene (BRAF) inhibitors and histone deacetylase inhibitors significantly increase the expression of melanocortin receptor 1 through MITF-dependent pathways. This effect was enhanced by ligand conjugation to the cell glycoalkalix. The obtained data indicate that the use of BRAF inhibitors and histone deacetylase inhibitors significantly enhances the effectiveness of melanocortin receptor 1-targeted therapy by activating the delivery of active chemotherapy components through regulated expression of melanocortin receptor 1.

Among all the compounds identified during the study, the substance CHEMBL505179, belonging to the ellagitannin group of macrocycles, is the most promising. CHEMBL505179 is extracted from plant raw materials of the *Oenothera* genus and demonstrates a broad spectrum of immunoregulatory action by inhibiting the production of reactive oxygen species by mouse B-lymphocytes and activating the NF-κB factor in monocyte culture [28]. A study by S. Yoshikawa *et al.* [39] on high-molecular-weight polyphenolic molecules in *Barringtonia racemosa* leaves allowed the identification of approximately five previously unexplored ellagitannins. One such compound is barringtonin M1, which is a hydrolysed monomer of tannin. Barrycyclin D1 has a macrocyclic organization formed from casuarictin and tellimagrandin.

From scientific works on PubChem-25078192, it is known that this compound has a similar effect to the blood clotting factor Xa inhibitor, which is focused on malignant lymphomas [40]. Blood clotting factor Xa is a serine protease that has a central role in the mechanism of activating the physiological blood clotting cascade and is therefore investigated as a promising compound for antithrombotic and antitumor drugs. Inhibitors of blood clotting factor Xa can affect the initiation cascades of thrombotic enzymes and catalytic activity. In contemporary research by T. Schmitz *et al.* [41], peptide inhibitors of tridegin and transglutaminase-inhibiting acceptors of Michael are the most promising and potentially successful candidates for developing clinically effective inhibitors of blood clotting factor Xa due to their specific selectivity for activated blood clotting factor Xa.

Rituximab, a CD20 monoclonal antibody, is historically the first immunotherapeutic agent ever used in oncology, and to this day, it remains a cornerstone of lymphoma therapy. Since then, scientists have witnessed the development of humanized antibodies, next-generation anti-CD20 monoclonal antibodies targeting other tumour markers (CD19 and CD22), their microenvironment (PD-1, CD47), antibody-drug conjugates, and bispecific T-cell engagers [42, 43]. Considering their activity, safety, and specificity, mAbs can remain crucial therapeutic tools for treating NHL and other malignant neoplasms.

The activity of the programmed cell death protein 1 (PD-1) ligand is a key mechanism for preserving the tumour from the patient's own immune defence. In malignant B-cell lymphoma cells and their tumour histological microenvironment, PD1-L conjugates with PD-1, leading to the inhibition of cytotoxic T-cell signalling in the immune response and, as a result, to their exhaustion. According to P. Willard *et al.* [43], complete blocking of PD-1 and PD1-L function provided an innovative approach to lymphoma therapy, as pathways for implementing PD-1 inhibitors into B-cell lymphoma treatment protocols were identified and developed. Experimental studies on classical Hodgkin's lymphoma cells and primary mediastinal B-cell lymphomas revealed the pathogenetic basis for blocking the immune response by increasing PD-1L regulation. Two inhibitors of PD-1 from the PD-1 inhibitor group demonstrated high clinical activity and were approved for two subtypes of lymphoma. These registered drugs include nivolumab and pembrolizumab, which are fully humanized monoclonal antibodies, immunoglobulins of class G4, blocking the binding of PD-1L to malignant lymphoma cells.

CONCLUSIONS

Through virtual screening using *in silico* methods with the MOE software, a search for antibody mimetics to B-cell membrane proteins for the treatment of Burkitt lymphoma was conducted, resulting in the selection of five potential anti-lymphoma agents. From the entire pool of screened chemical compounds suitable for *in vitro* anti-lymphoma activitytesting,onlyCHEMBL505179,PubChem-101933623, PubChem-16135635, PubChem-44249983, PubChem-16209234, PubChem-135854913, PubChem-44256795, MolPort-046-788-300, PubChem-45359413, MCULE-5553462135, and MolPort-046-857-137 were chosen.

Docking with the CD22 protein was performed using the amino acids Ile 145, Ser 211, His 213, Gly 214, Asp 232, Thr 233, Gln 235, Val 267, Asn 271, Tyr 274. Docking with the PD-L1 protein was conducted using the amino acids Ala18, Glu45, Asp49, Ala51, Ala52, Ile54, Tyr56, Glu58, Glu60, Asp61, Asn63, Qln66, Val68, His69, Val111, Arg113, Met115, Ser117, Tyr118, Gly119, Ala121, Tyr123, Arg125. Based on the minimal score (S) values, which characterize the binding affinity, five potential anti-lymphoma agents were selected. The analysis of potential anti-lymphoma agents was conducted using data from the PubChem database, revealing that all five selected compounds have already registered biological activity. Four of them, including PubChem-44406884, PubChem-136510605, PubChem-126761430, and PubChem-25078192, are experimentally proven *in vitro* ligands for proteins different from the chosen biological targets characteristic of B-cells in Burkitt lymphoma. Among the identified potential ligands, the compound CHEMBL505179 targeting the CD20 receptor stood out as the most promising. It is a macrocyclic ellagitannin isolated from plant sources (genera *Oenothera, Epilobium, Eucalyptus*). Other potential ligands include an antagonist of the melanocortin receptor (PubChem-44406884), an inhibitor of blood clotting Factor Xa (PubChem-136510605) targeting the CD22 receptor, and a blocker of epithelial Na^+ channels (PubChem-126761430). For the PD-L1 receptor, an agonist of the melanocortin receptor (PubChem-25078192) was identified. Thus, the *in silico* mimetics of antibodies to B-cell membrane proteins that cause BL development obtained as a result of the search are a promising method for the treatment of BL using the virtual screening technique. Further directions of this research will be aimed at identifying additional ligands with anti-lymphoma properties that will have an anti-tumour effect for a wider range of non-Hodgkin lymphoma.

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Комп'ютерний скринінг пептидоміметиків та маломолекулярних лігандів мембранних білків в-клітин для терапії лімфоми Беркітта

Ірина Володимирівна Дем'яненко
Кандидат технічних наук, асистент
Національний технічний університет України
«Київський політехнічний інститут імені Ігоря Сікорського»
03056, просп. Берестейський, 37, м. Київ, Україна
https://orcid.org/0000-0002-0832-3619

Анастасія Василівна Бахмач
Магістр
Університет Гренобль Альпи
38400, просп. Сентраль, 621, м. Сен-Мартен-д'Ер, Франція
https://orcid.org/0000-0003-3746-3853

Анотація. Можливості молекулярного моделювання та докінгу дозволяють знайти нові потенційні лікарські агенти, які дозволяють покращити лікування хвороб, що є актуальним питанням сьогодення. Метою даного дослідження був пошук *in silico* міметиків антитіл до мембранних білків В-клітин для лікування лімфоми Беркітта методом віртуального скринінгу. В даній роботі було використано стандартний протокол структурно-залежного віртуального скринінгу з тією відмінністю, що фармакофори для скринінгу будували не на основі маломолекулярних лігандів, а на основі обраних амінокислотних залишків антитіл. За літературними даними та на основі наявності механізму прямої цитотоксичної дії та наявності 3D структур комплексу було обрано три моноклональні антитіла: обінутузумаб, епратузумабута та атезолізумабу. Ідентифікація біологічної мішені проводилися шляхом пошуку 3D-структур обраних комплексів з білками-мішенями в базі даних Protein Data Bank. Для проведення віртуального скринінгу обрано веб-сервіс Pharmit. За допомогою програми Molecular Operating Environment було побудовано моделі фармакофорів для трьох комплексів: CD20 і обінутузумабу, CD22 і епратузумабу та PD-L1 і атезолізумабу. Докінг з білком CD20, CD22 і PD-L1 проведено за сайтами зв'язування, який впізнає оригінальне антитіло. Методом віртуального скринінгу *in silico* за допомогою програмного продукту Molecular Operating Environment здійснено пошук міметиків антитіл до мембранних білків В-клітин для лікування лімфоми Беркітта, в результаті чого відібрано 5 потенційних антилімфомних агента: CHEMBL505179 до рецептора CD20, до рецептора CD20 – антагоніст рецептору меланокортину (PubChem-44406884), до рецептора CD22 – інгібітор фактору згортання крові Ха (PubChem-136510605) та блокатор епітеліальних Na+ каналів (PubChem-126761430), до рецептора PD-L1 – агоніст рецептору меланокортину (PubChem-25078192). Отримані результати можуть бути застосовані у фармакологічній промисловості та онкологічній практиці з метою покращення терапевтичних результатів лікування хворих з лімфомою Беркітта

Ключові слова: молекулярний докінг; фармакофори; антитіла; міметики; імуногенність