EasyChair Preprint

№ 4264

A Computational Approach in the Search of New Biologically Active 9,10-Anthraquinone Derivatives

Maryna Stasevych, Viktor Zvarych and Volodymyr Novikov

September 25, 2020

A computational approach in the search of new biologically active 9,10-anthraquinone derivatives

Maryna Stasevych, Viktor Zvarych and Volodymyr Novikov

Lviv Polytechnic National University, Bandera Str. 12, Lviv, 79013, Ukraine

Abstract

The results of using a computer approach in the search for new potential biologically active compounds in a series of 9,10-anthraquinone derivatives using free online programs *PASS Online, CLC-Pred (Cell Line Cytotoxicity Predictor), Acute Rat Toxicity* and determining the level of binding of the studied structures of anthraquinones with target proteins using the *Schrodinger* software package are generalized. The directions of experimental primary assessment of antimicrobial, antiplatelet, antioxidant, antiviral, anticonvulsant, antitumor action for selected objects of research are determined. Molecular docking shows the prospects for studies of the mechanisms of anticancer and antiplatelet agents.

Keywords 1

9,10-anthraquinone derivatives, *in silico* prediction, biological action

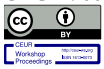
1. Introduction

Despite the significant achievements of modern medical chemistry and pharmacology, the search for new more effective and safer medicinal substances remains an actual problem [1-3]. The number of biological activities studied by modern pharmacology is more than three thousand, and the number of potential molecular targets of drugs is tens of thousands [4]. Experimental testing of tens / hundreds of millions of organic compounds for thousands of types of biological activity is practically not implemented. The basis of modern search and development of new drugs is the analysis of information about the mechanisms of disease development, molecular targets and substances with pharmacological activity, the action of which allows to normalize the pathological process [5].

A rational approach to the search for new biologically active substances with the desired properties is based on computer prediction of biological activity based on their structural formulas [6, 7]. The most promising substances for chemical synthesis are selected by researchers on the basis of computer prediction and determine the priorities of their experimental testing, which significantly reduces the cost of experimental research and eliminates unpromising substances in the earliest stages of research.

Computer methods are widely used to analyze the relationships "structure - biological activity" of organic compounds [8, 9]. Search and designing materials with desired properties and optimization of pharmacodynamic and pharmacokinetic characteristics of the basic structures of new biologically active compounds is carried out using them. Most computer programs designed for this purpose are distributed on a commercial basis by specialized firms (*Accelrys, Tripos, ACD Labs, ChemSoft*, etc.).

There are a relatively small number of computer programs available for free over the Internet and predicting pKa (http://vcclab.org/lab/alogps/start.html), some physicochemical properties

3rd International Conference on Informatics & Data-Driven Medicine, IDDM 2020, November 19-21, 2020, Växjö, Sweden
EMAIL: maryna.v.stasevych@lpnu.ua (A. 1); viktor.i.zvarych@lpnu.ua (A. 2); volodymyr.p.novikov@lpnu.ua (A. 3)
ORCID: 0000-0001-5042-4133 (A. 1); 0000-0003-3036-0050 (A. 2); 0000-0002-0485-8720 (A. 3)


(http://www.molecularknowledge.com/Online /Estimation/online1.htm), solubility, lipophilicity, and some types of biological activity (http://www.organic-chemistry.org/prog/peo/index.html, http://www.molinspiration.com/cgi-bin/properties). In recent years, foreign web services have appeared on the Internet that allow predicting the interaction of chemical compounds with target macromolecules based on structural similarity (http://bioinformatics.charite.de/superpred/, http://cpi.bio-x.cn/drar/). The information available on these websites does not allow us to assess the quality of the prediction provided by these web services.

The computer program *PASS Online* [10, 11] became the first of the free online services in the world, which allows you to predict 5066 types of pharmacological effects based on the structure of the molecule. Its effectiveness in finding new bioactive substances is constantly confirmed by the numerous works of more than 23,000 researchers from more than 100 countries [12], and the training sample is updated as new data on biologically active compounds for each type of biological activity [13]. In recent years, *PASS Online* has been added by a number of free online web services on the *Way2Drug* platform that predict more than 4,000 types of biological activity, including acute toxicity to rats with four routes of administration, effects on tumor and non-tumor cell lines interacting with antytargets and etc. [14].

Molecular docking (molecular modeling) is actively used to solve virtual screening problems [15]. Its essence is to model the relative position of the studied molecule and the target protein. The docking program tests the studied structures using a special scoring function (affinity), which roughly describes the energy of interaction of the molecule with the target protein. It is possible to reject from further consideration a substance with poor values of the scoring function using the results of docking. Modeling of ligand-receptor interactions is carried out using a variety of different software packages (*AutoDock, AMBER, eHiTS, Surflex-Dock, Schrödinger, etc.*) [16-19], each of which has its own advantages and disadvantages, including accessibility via the Internet.

Considering the above, an *in silico* approach was used in the search for new derivatives of 9,10-anthraquinone using the latest resources to determine the experimental directions of research on their pharmacological activity.

2. Materials and methods

Evaluation of the pharmacological activity of new functionalized derivatives of 9,10-anthraquinone (Fig. 1) was performed using online services *PASS Online, CLC-Pred, Acute Rat Toxicity* of web portal *Way2Drug*. As an initial information, the structural formula of the substance in MOL or SDF file format was used to obtain prediction data in each of the mentioned programs. The result of the forecast was a list of possible types of biological activity with estimates of two probabilities - the presence of Pa activity and the absence of Pi activity (Fig. 2-4).

In addition to the above-mentioned free online access programs for predicting the pharmacological effects of the studied structures, molecular modeling of affinity (binding) with the corresponding target protein site was also used to determine probable mechanisms of action using *Small-Molecule Drug Discovery Suite* of *Schrödinger* within the test access [20] and *AutoDock Vina* [21].

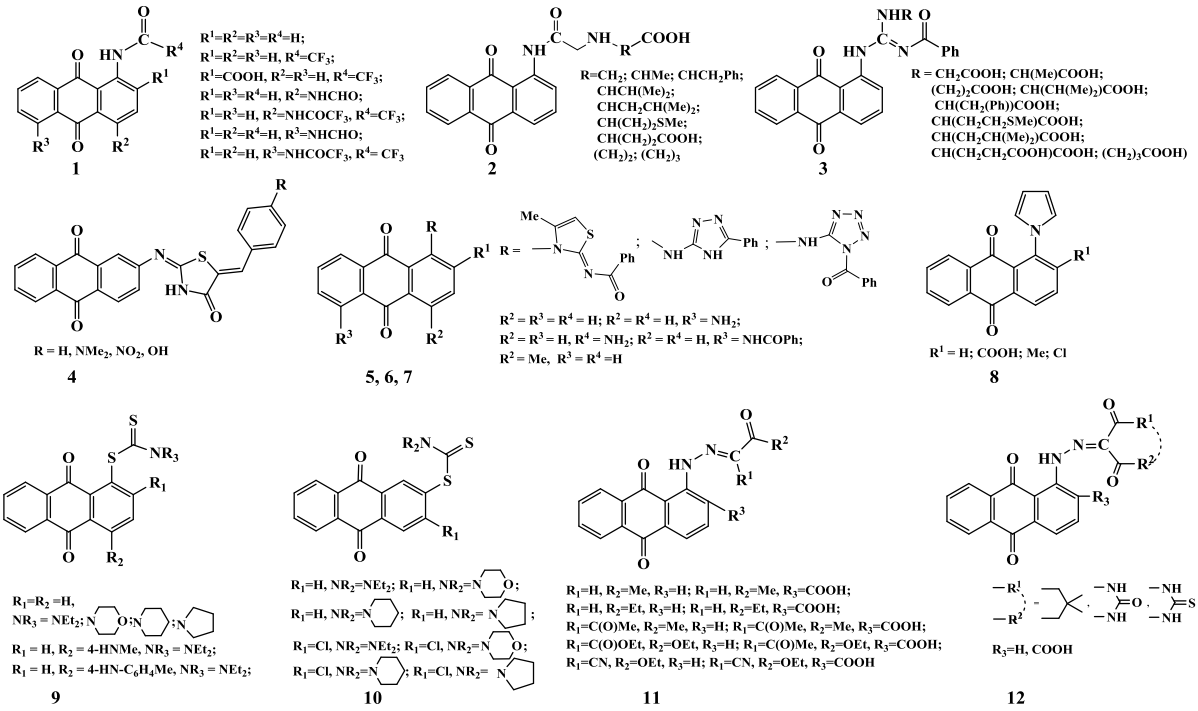


Figure 1: Objects *in silico* study

All | Pa>Pi | Pa>0,3 | Pa>0,7 | ok

Pa	Pi	Activity
0,639	0,021	Neurotransmitter uptake inhibitor
0,631	0,025	Alkane 1-monooxygenase inhibitor
0,626	0,028	Aldehyde oxidase inhibitor
0,601	0,026	27-Hydroxycholesterol 7alpha-monooxygenase inhibitor
0,591	0,022	3-Hydroxybenzoate 6-monooxygenase inhibitor
0,585	0,021	Ovulation inhibitor
0,602	0,041	Nicotinic alpha4beta4 receptor agonist
0,582	0,033	Thioredoxin inhibitor
0,571	0,028	(R)-6-hydroxynicotine oxidase inhibitor

Figure 1: Example of prediction results of *PASS Online*

Cancer cell line prediction result

Pa	Pi	Cell-line	Cell-line full name	Tissue	Tumor type
0.392	0.043	HeLa	Cervical adenocarcinoma	Cervix	Adenocarcinoma
0.235	0.007	U2OS	Osteosarcoma	Bone	Sarcoma
0.316	0.130	RKO	Colon carcinoma	Colon	Carcinoma
0.316	0.198	Hs 683	Oligodendroglioma	Brain	Glioma
0.243	0.128	HOP-18	Non-small cell lung carcinoma	Lung	Carcinoma
0.111	0.017	MEXF276L	Xenograft melanoma	Skin	Melanoma
0.111	0.017	MEXF989	Xenograft melanoma	Skin	Melanoma
0.111	0.017	PAXF546	Pancreatic carcinoma	Pancreas	Carcinoma

Figure 2: Example of prediction results of *CLC-Pred*

Rat acute toxicity predicted by GUSAR

Rat IP LD50 Log10(mmol/kg)	Rat IV LD50 log10(mmol/kg)	Rat Oral LD50 log10(mmol/kg)	Rat SC LD50 log10(mmol/kg)
0,307 in AD	-0,382 in AD	0,330 in AD	0,272 in AD

Rat IP LD50 (mg/kg)	Rat IV LD50 (mg/kg)	Rat Oral LD50 (mg/kg)	Rat SC LD50 (mg/kg)
717,400 in AD	146,500 in AD	755,000 in AD	661,200 in AD

Acute Rodent Toxicity Classification of Chemicals by OECD Project

Rat IP LD50 Classification	Rat IV LD50 Classification	Rat Oral LD50 Classification	Rat SC LD50 Classification
Class 5 in AD	Class 4 in AD	Class 4 in AD	Class 4 in AD

Figure 3: Example of prediction results of *Acute Rat Toxicity*

3. Results and Discussion

Analysis of prediction data for all new structures of functionalized 9,10-anthraquinone derivatives showed that the main, expected, direction of experimental studies is to determine the antitumor effect. Taking this into account, cytotoxicity was assessed against cancer cell lines using the web service *PASS CLC-Pred (Cell Line Cytotoxicity Predictor*) [22]. The analysis of the obtained results showed that the most probable effect of the studied compounds is on cancer cell lines of the lungs, lymphoid tissue, glandular tissue of the breast, and for some compounds is on also tissues of the cervix, brain, and colon.

The introduction of new biophore fragments into the 9,10-anthraquinone molecule allowed to expand the range of *in vitro* and *in vivo* tests, in addition to the study of the antitumor effect, in the following areas. In particular, antimicrobial and antioxidant activityies are predicted for *N*-acylamino-9,10-anthraquinones **1**, amino acid derivatives of 2-chloro-*N*-(9,10-dioxy-9,10-dihydroanthracen-1-yl) acetamide **2**. 1,2,3-Substituted guanidine **3** would be interesting subjects to test for antianginal, anti-ischemic, cardiotonic effects. The direction of research on hypoglycemic activity is interesting for 5-arylidene derivatives **4**. Iminothiazoles **5**, 1,2,4-triazoles **6** and tetrazoles **7** can be studied for antimicrobial, anti-inflammatory, anti-allergic effects and effects on vascular processes. An additional area of testing for pyrrolylantracenediones **8** is to determine the level of antibacterial and antifungal activity. According to Pa prediction data for dithiocarbamate derivatives **9, 10** antitumor activity is supplemented by antimicrobial, antioxidant, antiplatelet, antiviral, anticonvulsant effects. Dithiocarbamates **9, 10** and hydrazone derivatives **11, 12** can be further investigated for antiviral properties.

The analysis of the prediction results showed that, in the overwhelming majority, the Pa values for the structures under study fluctuate in the range 0.3-0.6 and, taking into account the categorization of the Pa values, testifies in favor of the originality of molecular structures.

The probable acute toxicity of LD_{50} using the online resource *Acute Rat Toxicity* [23] for promising compounds selected by experimental tests was evaluated in a rat model with four different routes of administration: Intraperitoneal, intravenous, oral and subcutaneous. The results showed that the test compounds can be classified as medium-, low- and non-toxic compounds according to the classification [24].

For molecular docking as target proteins were selected: receptor proteins-tyrosine kinases cyclooxygenase-1 (*COX-1*) - *3N8X*, glycoprotein-IIb/IIIa (*GPIIb /IIIa*) - *2VDM*, glycoprotein-VI (*GP-VI*) - *2G17*, purine receptor *P2Y12* - *4PXZ*, prostacyclin receptor (*PG-I2*) - *4F8K*, protein-activated receptor-1 (*PAR-1*) - *3VW7*, antithrombin III (*ATIII*), factor-X (*FX*), factor-II (*F-II*), factor -IX (*F-IX*) and vitamin K-epoxy reductase (*VKOR*) - *1NQ9, 1KSN, 5JZY, 1RFN* and *3KP9* [25], *c-Kit, B-Raf, EGFR* (*1NQL, 1IVO, 1M17, 2GS6*) and *PDGF (1T46, AKT1, ERK2)*, non-receptor tyrosine kinases *SRC (1SKJ)*, nonspecific tyrosine kinases *ABL* (*3OXZ, 3QRJ, 2ABL*) [26].

As a result, the values of G_{score} scoring functions were determined, which showed anthracenedione derivatives with high, medium and low levels of binding to a specific target protein among the studied molecular structures. Examples of visualization of binding of hit compounds in the corresponding active zone of the protein are given below (Fig. 2-7).

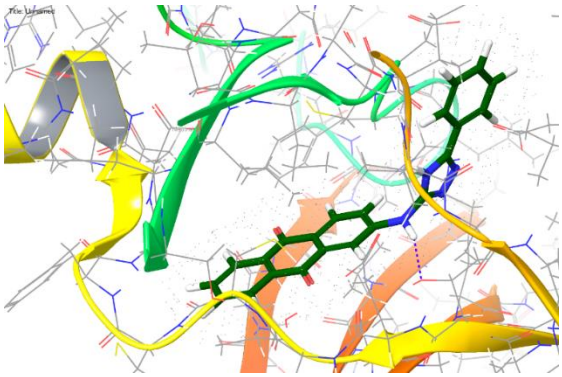


Figure 2: Visualization of the binding of the hit compound triazole anthracenedione in the active zone of the protein *1T46* (G_{score}= -10.7)

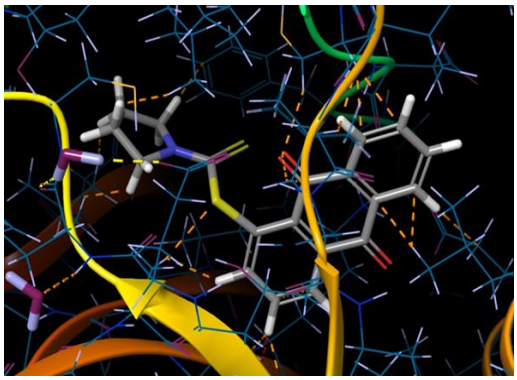


Figure 3: Visualization of the binding of the hit compound pyrrolidine dithiocarbamate in the active zone of the protein *1T46* (G_{score}= -10.92)

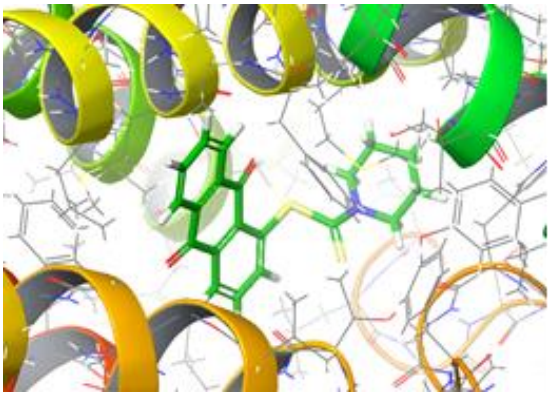


Figure 4: Visualization of the binding of the hit compound piperidine dithiocarbamate in the active zone of the protein *3KP9* (G_{score} = -10.39)

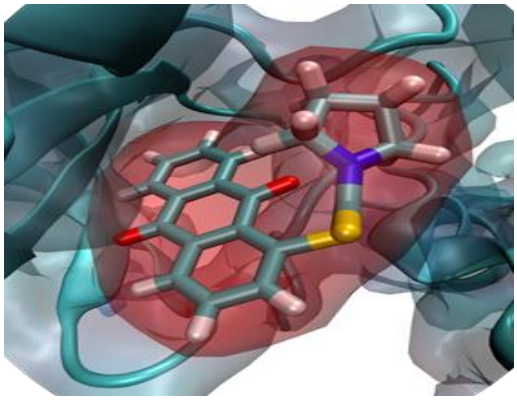


Figure 5: Visualization of the binding of the hit compound pyrrolidine dithiocarbamate in the active zone of the protein *c-Kit* (G_{score}= -9.1)

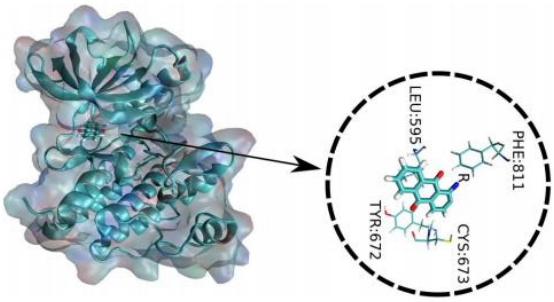


Figure 6: Visualization of the binding of the hit compound anthratriazinone in the active zone of the protein *c-Kit* (G_{score} = -11.8)

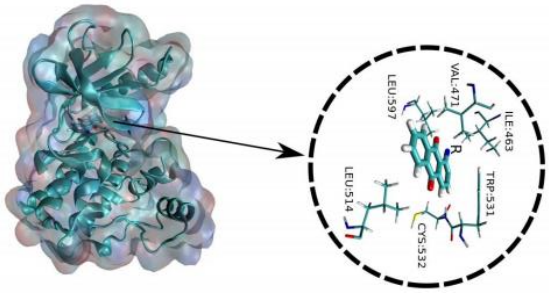


Figure 7: Visualization of the binding of the hit compound anthratriazinone in the active zone of the protein *B-Raf* (G_{score} = -11.3)

The obtained results of molecular modeling showed the prospects of studies of mechanisms, including simultaneously several mechanisms, antitumor and antiplatelet action of new functionalized derivatives of 9,10-anthracenedione [25, 26].

It should be noted that the results of prediction using the available online programs *PASS Online, CLC-Pred, Acute Rat Toxicity* regarding the probable manifestation of antimicrobial, antiplatelet, antioxidant, antiviral, anticonvulsant, antitumor action and indicators of acute toxicity, as well as molecular docking data using *Small-Molecule Drug Discovery Suite* by *Schrödinger* and *AutoDock Vina* have been experimentally confirmed by *in vitro* and *in vivo* studies [27-30].

4. Conclusion

In this article, we present the generalized results of using in silico tools in the search for new potential biologically active compounds in the series of 9,10-anthraquinone derivatives. The used computer approach carried out by free online programs *PASS Online, CLC-Pred, Acute Rat Toxicity* allowed to determine the directions of the experimental primary assessment of antimicrobial, antiplatelet, antioxidant, antiviral, anticonvulsant, antitumor action for the selected objects of study. Molecular docking data on biotargets of pathological processes allowed to define compounds with a high degree of affinity and showed the prospect of studying the mechanisms of anticancer and antiplatelet agents from this class of compounds by modifying the ligand with various pharmacophore fragments. The results of *in silico* approach allowed experimentally identify promising compounds in the series of 9,10-anthraquinone.

5. Acknowledgements

This research was funded by the Ministry of Education and Science of Ukraine, Project number: 0119U002252.

6. References

[1] S. P. Elliott, T. Holdbrook, S. D. Brandt, Prodrugs of New Psychoactive Substances (NPS): A New Challenge, J. Forensic Sci. 65 (2020) 913–920. doi:10.1111/1556-4029.14268.

[2] S. Mignani, S. Huber, H. Tomás, J. Rodrigues, J.-P. Majoral, Why and how have drug discovery strategies in pharma changed? What are the new mindsets?, Drug Discovery Today 21 (2016) 239–249. doi: 10.1016/j.drudis.2015.09.007.

[3] J. Strovel, S. Sittampalam, N. P. Coussens, M. Hughes, J. Inglese, A. Kurtz, A. Andalibi, L. Patton, C. Austin, M. Baltezor, M. Beckloff, M. Weingarten, S. Weir, Early drug discovery and development guidelines: for academic researchers, collaborators, and start-up companies, in: S. Markossian, G. Sitta Sittampalam (Eds.), Assay Guidance Manual, Eli Lilly & Company and the National Center for Advancing Translational Sciences, Bethesda (MD), 2016, pp. 3–37.

[4] G. Mueller, Medicinal chemistry of target family-directed masterkeys, Drug Discovery Today 8 (2003) 681–691. doi: 10.1016/S1359-6446(03)02781-8.

[5] S. Marshall, R. Madabushi, E. Manolis, K. Krudys, A. Staab, K. Dykstra, S. A. G. Visser, Model-Informed Drug Discovery and Development: Current Industry Good Practice and Regulatory Expectations and Future Perspectives, CPT: Pharmacometrics & Systems Pharmacology 8 (2019) 87–96. doi:10.1002/psp4.12372.

[6] S. M. Ivanov, A. A. Lagunin, D. A. Filimonov, V. V. Poroikov, Computer prediction of adverse drug effects on the cardiovascular system, Pharm. Chem. J. 52 (2018) 758–762. doi: 10.1007/s11094-018-1895-1.

[7] R. K. P. Tripathi, S. R. Ayyannan, Evaluation of 2-amino-6-nitrobenzothiazole derived hydrazones as acetylcholinesterase inhibitors: in vitro assays, molecular docking and theoretical ADMET prediction, Med. Chem. Res. 27 (2018) 709–725. doi: 10.1007/s00044-017-2095-3.

[8] D. R. Flower (Ed.), Drug Design Cutting Edge Approaches, RSC Publishing, London, 2002. doi: 10.1039/9781847550705.

[9] H. Jhoti, A. R. Leach (Eds.), Structure-based Drug Discovery, Springer, Netherlands, 2007. doi: 10.1007/1-4020-4407-0.

[10] A. Lagunin, A. Stepanchikova, D. Filimonov, V. Poroikov, PASS: prediction of activity spectra for biologically active substances, Bioinformatics 16 (2000) 747–748. doi: 10.1093/bioinformatics/16.8.747.

[11] PASS Online Home Page, 2020. URL: https://www.way2drug.com/passonline.

[12] V. V. Poroikov, D. A. Filimonov, T. A. Gloriozova, A. A. Lagunin, D. S. Druzhilovskiy, A. V. Rudik, L. A. Stolbov, A. V. Dmitriev, O. A. Tarasova, S. M. Ivanov, P. V. Pogodin, Computer-aided prediction of biological activity spectra for organic compounds: the possibilities and limitation, Russ. Chem. Bull. 68 (2019) 2143–2154. doi: 10.1007/s11172-019-2683-0.

[13] V.V. Poroikov, Computer-Aided Drug Design: from Discovery of Novel Pharmaceutical Agents to Systems Pharmacology, Biochemistry (Moscow) Supplement Series B: Biomedical Chemistry 14 (2020) 216–227. doi: 10.1134/S1990750820030117.

[14] Way2Drug Predictive Service Home Page, 2020. URL: http://www.way2drug.com.

[15] X. Y. Meng, H. X. Zhang, M. Mezei, M. Cui, Molecular docking: a powerful approach for structure-based drug discovery, Curr. Comput. Aided Drug Des. 7 (2011) 146–157. doi:10.2174/157340911795677602

[16] G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell, A. J. Olson, Autodock4 and AutoDockTools4: automated docking with selective receptor flexiblity, J. Computational Chem. 16 (2009) 2785–2791. doi: 10.1002/jcc.21256.
[17] R. Salomon-Ferrer, D. A. Case, R. C. Walker, An overview of the Amber biomolecular simulation package, WIREs Comput. Mol. Sci. 3 (2012) 198–210. doi: 10.1002/wcms.1121.
[18] Z. Zsoldos, D. Reid, A. Simon, S. B. Sadjad, A. P. Johnson, eHiTS: a new fast, exhaustive flexible ligand docking system, J. Mol. Graph Model. 26 (2007) 198–212. doi: 10.1016/j.jmgm.2006.06.002.
[19] R. Spitzer, A.N. Jain, Surflex-Dock: Docking benchmarks and real-world application, J. Comput. Aided Mol. Des. 26 (2012) 687–699. doi: 10.1007/s10822-011-9533-y.
[20] R. A. Friesner, R. B. Murphy, M. P. Repasky, L. L. Frye, J. R. Greenwood, T. A. Halgren, P. C. Sanschagrin, D. T. Mainz, Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes, J. Med. Chem. 49 (2006), 6177–6196. doi: 10.1021/jm051256o.
[21] O. Trott, A. J. Olson, Autodock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, J. Comput. Chem. 31 (2010), 455–461. doi: 10.1002/jcc.21334.
[22] A. A. Lagunin, V. I. Dubovskaja, A. V. Rudik, P. V. Pogodin, D. S. Druzhilovskiy, T. A. Gloriozova, D. A. Filimonov, N. G. Sastry, V. V. Poroikov, CLC-Pred: A freely available web-service for in silico prediction of human cell line cytotoxicity for drug-like compounds, PLOS One 13 (2018) e0191838. doi: 10.1371/journal.pone.0191838.
[23] A. Lagunin, A. Zakharov, D. Filimonov, V. Poroikov, QSAR modelling of rat acute toxicity on the basis of PASS prediction, Mol. Inf. 30 (2011) 241–250. doi: 10.1002/minf.201000151.
[24] J. Švarc-Gajić, General toxicology, Nova Science Publishers Inc., New York, 2010.
[25] M. V. Stasevych, V. I. Zvarych, V. P. Novikov, Computer-aided prediction in search of new antiplatelet agents with antioxidant effect among sulfur-containing derivatives of 9,10-anthraquinonone, Chemistry, Technology and Application of Substances 2 (2019) 115–121. doi: 10.23939/ctas2019.02.115.
[26] M. V. Stasevych , V. I. Zvarych , V. V. Lunin , N. A. Kopak, V. P. Novikov, Prediction in silico biological activity of derivatives of refunctionalized amino-9,10-anthracenediones, Visnyk Natsional′noho universytetu „L′vivs′ka politekhnika", Khimiya, tekhnolohiya rechovyn ta yikh zastosuvannya 868 (2017) 203–215
[27] V. I. Zvarich, M. V. Stasevich, O. V. Stan'ko, E. Z. Komarovskaya-Porokhnyavets, V. V. Poroikov, A. V. Rudik, A. A. Lagunin, M. V. Vovk, V. P. Novikov, Computerized prediction, synthesis, and antimicrobial activity of new amino-acid derivatives of 2-chloro-*N*-(9,10-dioxo-9,10-dihydroanthracen-1-yl)acetamide, Pharm. Chem. J. 48 (2014) 584–588. doi: 10.1007/s11094-015-1174-3.
[28] M. V. Stasevich, V. I. Zvarich, V. P. Novikov, S. D. Zagorodnyaya, O. Yu. Povnitsa, M. A. Chaika, M. V. Nesterkina, I. A. Kravchenko, D. S. Druzhilovskii, V. V. Poroikov, 9,10-Anthaquinone dithiocarbamates as promising pharmaceutical agents with pleiotropic action: computer prediction of biological activity and experimental validation, Pharm. Chem. J. 53 (2020) 905–913. doi: 10.1007/s11094-020-02098-x.
[29] M. V. Stasevych, V. I. Zvarych, D. R. Spreis, O. S. Yaremkevych, Computer prediction and verificationof antioxidative activity of exo-functionalizedderivatives of 9,10-antraquinone, Chemistry, Technology and Application of Substances 2 (2019) 83–91. doi: 10.23939/ctas2019.01.083.
[30] M. Stasevych, V. Zvarych, V. Lunin, T. Halenova, O. Savchuk, O. Dudchak, M. Vovk, V. Novikov, Novel anthraquinone-based derivatives as potent inhibitors for receptor tyrosine kinases, Indian J. Pharm. Sci. 77 (2015) 634–637. doi: 10.4103/0250-474X.169062.