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PROSPECTS OF THE APPLICATION OF 1,2,4-TRIAZOLE DERIVATIVES OF 1,4-NAPHTHOQUINONE ANTIMICROBIAL AGENTS: IN SILICO AND IN VITRO STUDIES

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The physicochemical parameters and drug similarity of new [{(1H-1,2,4-triazol-5-yl)phenyl}amino]naphthalene-1,4-diones were calculated *in silico* using the online resource ADMETlab 3. Toxicity was predicted using the ProTox-II software, and the search for potential biological targets was carried out using SwissTarget Prediction. The *in vitro* antimicrobial activity of 1,2,4-triazole derivatives of 1,4-naphthoquinone against the bacterial cultures *Escherichia coli*, *Staphylococcus aureus*, and *Mycobacterium luteum*, as well as the fungi *Candida tenuis* and *Aspergillus niger*, was studied by the serial dilution method. It was established that triazole derivatives of 1,4-naphthoquinone 3a–g comply with Lipinski's rule, which indicates their potentially high oral availability. According to the ProTox-II prediction, compounds 3a–g can be attributed to toxicity class IV. Based on the analysis of pharmacokinetic characteristics, the prospects for [{1H-1,2,4-triazol-5-yl)phenyl}amino]naphthalene-1,4-diones as potentially biologically active agents were confirmed. The results of hepatotoxicity prediction by ADMETlab 3 and ProTox-II indicate the need for further *in vitro* studies. The significant antimicrobial activity of compound 3b can be considered for further *in vivo* studies.

Keywords: 1,4-naphthoquinone, 1,2,4-triazoles, drug similarity, pharmacokinetic parameters, ADMETlab 3, toxicity, antimicrobial activity.

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Introduction

Today, drug development uses a strategy of combining two or more bioactive pharmacophore fragments into one molecule. This approach makes it possible to design promising matrices for new drugs with improved pharmacokinetic profiles and fewer side effects. In this context, 1,4-naphthoquinone derivatives, given their prevalence in nature and broad spectrum of action, as are promising compounds [1,2]. It is known that they are secondary metabolites that can be produced by bacteria, fungi and higher plants, and additionally they have pronounced various biological activities: antibacterial, antifungal, anticancer, antiviral, anti-inflammatory, regenerative, etc. [2–4]. At the same time, 1,2,4-triazole fragments are key elements in the composition of highly effective therapeutic drugs. Thus, inovelon is used in the treatment of epilepsy, and to relieve seizures associated with the Lennox-Gastaut syndrome; voriconazole, posaconazole, fluconazole, and itraconazole have antifungal effects; trazodone is an anxiolytic; ribavirin is an antiviral drug. Importantly, those drugs, such as

vorozol, anastrozole and letrozole which are aromatase inhibitors are effectively fight breast cancer [5,6]. That is why obtaining new derivatives that contain a quinoid ring and 1,2,4-trizole fragments in their structure and studying their pharmacological profile is an important task.

When searching for new biologically active compounds, scientists face a number of problems that need to be solved. These are as follows: laboriousness, cost of the search process, as well as ethical aspects that arise when studying the *in vivo* biological activity of new compounds. The main stages of preclinical research are the identification and validation of newly synthesized substances, the selection of compounds with the so-called improved drug-like properties and *in vitro* and *in vivo* studies of their biological activity, which are completed by clinical trials. The use of *in silico* methods at the initial stage of drug development allows for the identification of biologically active compounds with a high probability, the effectiveness of which is often proven in experimental studies.

In this study, we used *in silico* methods such as ADMETlab 3.0 [10] and ProTox II [11]. With the help of ADMETlab 3.0, the absorption, distribution, metabolism and excretion (ADME) properties of [{(1*H*-1,2,4-triazol-5-yl)phenyl}amino]naphthalene-1,4-diones were predicted, and with the help of ProTox II [11], the acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity and immunotoxicity were predicted.

This work is a continuation of the studies of 1,2,4-triazole derivatives of 1,4-naphthoquinone as promising biologically active agents.

Experimental

Chemistry

The studied compounds – [{(1*H*-1,2,4-triazol-5-yl)phenyl}amino]naphthalene-1,4-diones (3a–g) – were obtained by the Michael addition reaction [12]. The synthesis was carried out by the interaction of 1,4-naphthoquinone with the corresponding (1H-1,2,4-triazol-5-yl)aniline (2a–g), with the formation of a 1,4-diol intermediate, which was then oxidized to the target quinones (scheme). A detailed description of the preparation of the studied compounds 3a–g is given in a previous work [12]. The establishment and confirmation of their structure was carried out using physicochemical methods: ^{1}H NMR, IR and chromatography-mass spectroscopy.

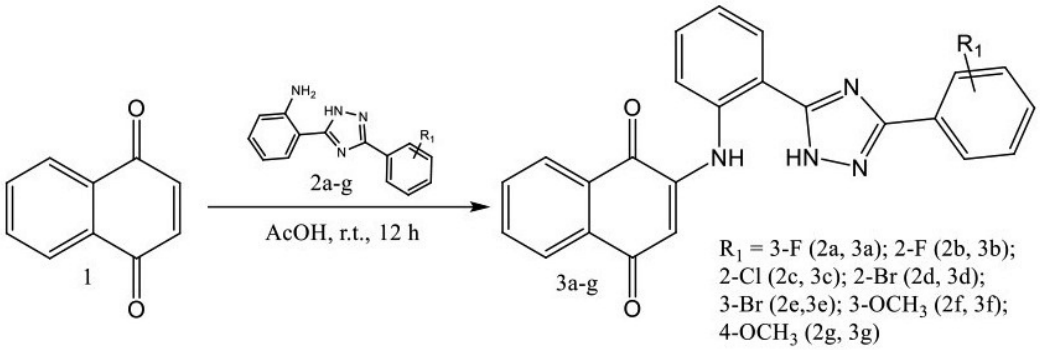
In silico experiment

ADMET profiling

An important step in the initial stage of research of new compounds is the global description of their molecular structure. To assess the therapeutic potential of the synthesized [{(1*H*-1,2,4-triazol-5-yl)phenyl}amino]naphthalene-1,4-diones (3a–g), *in silico* studies of physicochemical and pharmacokinetic parameters were performed using the ADMETab 3.0 online tools (http://www.swissadme.ch/) [10]. The water solubility of the compounds, their lipophilicity, topological polar surface area (TPSA), permeability through the blood-brain barrier and skin, as well as gastrointestinal absorption were calculated. The inhibition of cytochromes CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4, CYP2B6 and CYP2C8 was determined. Drug-like parameters of compounds 3a–g were predicted for the presence of violations of Lipinski's rule of five. Calculations of molecular descriptors were performed and predictions of the studied molecules were made, which are used by scientists in drug development.

Toxicological profile of [{(1H-1,2,4-triazol-5-yl)phenyl}amino]naphthalene-1,4-diones

The toxicity of compounds is one of the important parameters that is taken into account when creating new drugs and is based on the analysis of the similarity between compounds for which the median lethal dose (LD_{50}) values are known. *In silico* determination of the toxicity of the studied compounds was carried out using modern software ProTox-II [11], which is available online: https://tox-new.charite.de/protox_II. Hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, and immunotoxicity of 1,2,4-triazole derivatives of 1,4-naphthoquinone have been predicted [11].



Scheme

Study of antibacterial and antifungal activity

The antimicrobial activity of [{(1*H*-1,2,4-triazol-5-yl)phenyl}amino]naphthalene-1,4-diones (3a–g) was studied by the method of serial dilutions at the Department of Bioactive Compounds Technology, Pharmacy and Biotechnology of the National University «Lviv Polytechnic». The antibacterial and antifungal activities of the synthesized compounds were studied on test cultures of bacteria *Escherichia coli, Staphylococcus aureus, Mycobacterium luteum* and fungi *Candida tenuis,* and *Aspergillus niger* with the method of serial dilutions of the substance in a liquid nutrient medium (meat-peptone broth for bacteria and unhopped beer wort for fungi) in the range of 0.9–500 μg/ml using a previously prepared working solution of the substance in DMSO at a concentration of 10000 μg/ml. Bacterial and fungal inoculum was inoculated into the nutrient medium (microbial load 10^6 CFU (colony forming units) per 1 ml). The inoculated test tubes were kept in a thermostat at the appropriate temperature (37^0C for bacteria; 30^0C for fungi) for 24–72 hours. The results were evaluated for the presence or absence of microbial growth by visual inspection under transmitted light, comparing the degree of microbial turbidity of the nutrient medium with the «negative control». To determine the minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC), 0.02 ml of medium was taken from the tubes in which the medium solutions were visually transparent and applied to sterile meat-peptone agar (MPA) for bacteria or wort agar (WA) for fungi in sterile Petri dishes incubated in a thermostat. Results were evaluated after 24 hours for in test bacteria and 48–72 hours for test fungi. Based on the absence of growth of microbial colonies on the incubated Petri dishes, the MBC or MFC of the test substance was determined [13]. The experiment was reproduced three times. Nitrofural ((E)-2-[(5-nitro-furan-2-yl)methylene]hydrazine-1-carboxamide) and Ketoconazole (1-{4-(4-[(2-(1*H*-imidazol-1-yl)])methyl)-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl)methoxy)phenyl)piperazin-1-yl]ethan-1-one) were used as control compounds with proven antibacterial/antifungal activity. In addition, generally accepted methods were used for quality control of nutrient media and solvents [13].

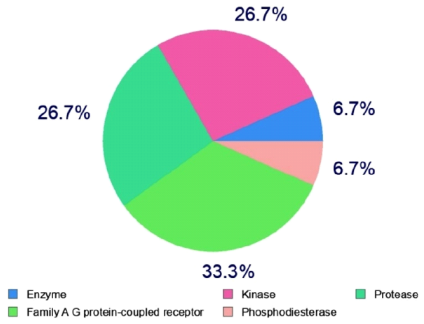
Results and discussion

To assess the pharmacological and pharmacokinetic potential of [{(1*H*-1,2,4-triazol-5-yl)phenyl}amino]naphthalene-1,4-diones (3a–g), the online software resource ADMETlab 3.0 [10] was used.

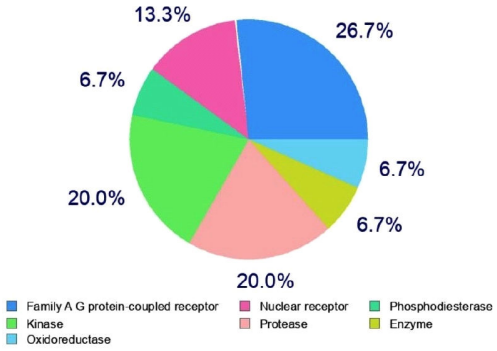
At the beginning of the study, potential targets for [{(1*H*-1,2,4-triazol-5-yl)phenyl}amino]naphthalene-1,4-diones (3a–g) were predicted using the online program SwissTarget Prediction (Fig. 1). Analysis of the obtained data showed that the kinase indices are the highest for all studied compounds (46.7% to 20.0%) and contribute to the formation of a significant part of the targets. Given the ability of kinases to regulate the cell cycle and signal transduction, their inhibition is used to stop uncontrolled cell division, and therefore this property is important in the development of new anticancer drugs.

For 3a, 3b, 3c, 3f, the distribution of target protein classes in the pie chart indicates a large proportion of receptors associated with AG family proteins (33.3% to 26.7%). This emphasizes their significant role as drug targets. Our results coincide with published data about the activity of naphthoquinones as potential modulators of biological signals [14]. A significant part is made up of proteases (33.0% to 13.3%), which play a crucial role in the vital activity of pathogens and tumor invasion. The effect on them indicates a potential antimicrobial and additional anticancer effect. Enzymes are found in insignificant quantities (13.3% to 6.7%), however their role in biochemical processes in cells is significant. Phosphodiesterases (13.3% to 6.7%) for 3a–f demonstrate the importance of cell signaling and regulation in the target profile. 3e and 3b contain a small percentage of oxidoreductases, unclassified proteins are contained in 3c, 3d and 3e, which indicates the ongoing investigation of new and less characterized targets. The high prediction result for 3a and 3f shows a 33.3% probability of interaction with kinase receptors. Compound 3e demonstrates the highest affinity for the protease, indicating a high inhibitory effect.

Therefore, the predicted high biological potential, namely the interaction with kinases and enzymes, prompted an assessment of the «likelihood» of the studied compounds, namely the biological availability to screen out compounds that have poor pharmacokinetics. At the same time, the analysis of the calculated physicochemical parameters of the studied molecules showed (Table 1) that they are within acceptable limits (MW<500, TPSA<140, nRot<11, nHA<10, nHD<5, LogP<5), i.e. they have a correlation with permeability through biological membranes. The lipid solubility indices at physiological pH=7.4 for all studied substances 3a–g slightly exceed the specified norm. Thus, the minimum value of LogD=3.226 for 3d was obtained, the largest value for 3a=3.542 at the norm LogD<3.0. In addition, all selected compounds 3a–g are consistent with the Lipinski's rule, therefore they can be potential candidates for drug development.



3a



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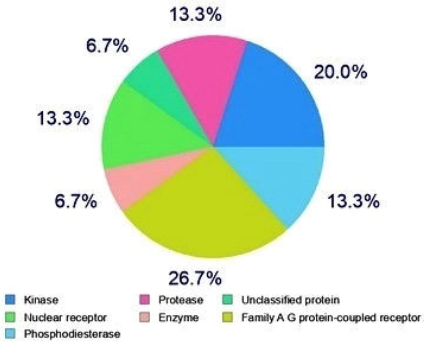

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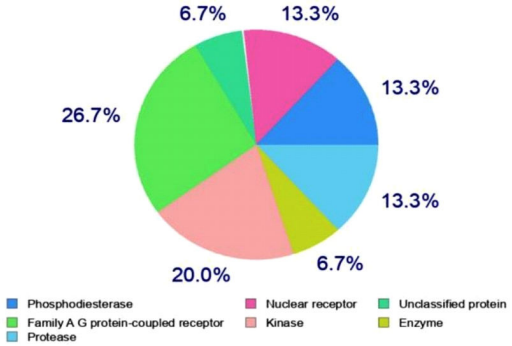
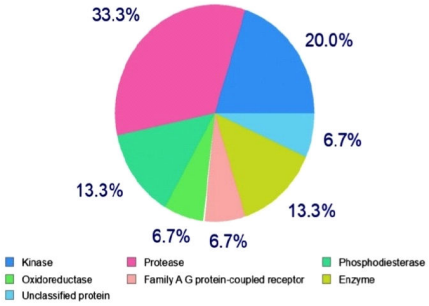
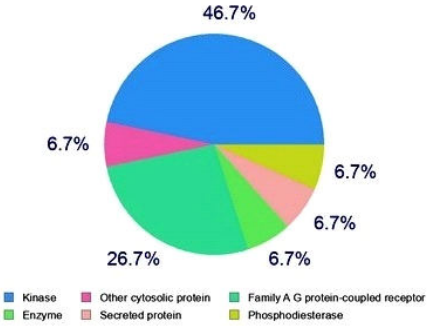


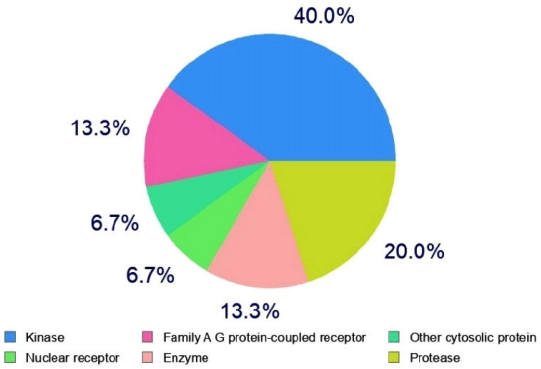
3d



3e



3f



3g

Probable targets for [{(1H-1,2,4-triazol-5-yl)phenyl}amino]naphthalene-1,4-diones (3a–g) predicted using the online program SwissTarget Prediction

When determining the pharmacokinetic properties of compounds 3a–g (Table 2), it was found that all compounds have good intestinal absorption (NIA<0.3), high penetration through colon adenocarcinoma cell lines (Caco-2>5.15), and high penetration rate through the blood-brain barrier (BBB) in the range of 0.0 to 0.002. These data indicate the need for further studies of [{(1*H*-1,2,4-triazol-5-yl)phenyl}amino]naphthalene-1,4-diones as promising candidates acting on the CNS. The bioavailability of all studied compounds is 0.55 units, which confirms their similarity to drugs (Table 2). The ability of compounds to inhibit and induce cytochrome P450 (CYP) enzymes plays an important role in drug metabolism and detoxification of the body. Thus, CYP inhibitors affect the metabolism of drugs by reducing the activity of CYP enzymes involved in their metabolism. The rate and extent of metabolism of CYP substrates are also affected by the presence of CYP inhibitors. As can be seen from Table 2, compounds 3a–f inhibit CYP A2, C19, C9, A4 and C8, and therefore affect the rate and extent of metabolism of their substrates. Compound 3g acts as a substrate for CYP A2, C19, and D6 [15].

An important characteristic of the molecules is their ability to be a substrate for P-glycoprotein (P-gp). P-gp is a transmembrane protein that actively transports a large number of structurally different compounds out of the cell and is the so-called efflux pump [16]. P-gp main function is to reduce the toxicity of xenobiotics. As can be seen from Table 2, none of the studied compounds 3a–g is a P-gp substrate. However, the P-gp inhibition values are close to 1 (from 0.905 to 0.977), which suggests their ability to inhibit the function of P-gp. Therefore, we can conclude that the potential effect of [{(1*H*-1,2,4-triazol-5-yl)phenyl}amino]naphthalene-1,4-diones is facilitating penetration through the BBB.

The half-life of a drug is the time required to reduce the amount of the active component in the drug by half of its initial dose [10]. According to *in silico* results (Table 2), compounds 3a–g have a short half-life ($T_{1/2}$ ranging from 0.572 for 3f to 0.807 for 3d).

The results obtained indicate that the studied compounds can potentially be used as oral agents and provide a basis for further pharmaceutical development of new drugs.

As is known, at the initial stage of designing new drugs, it is important to evaluate the toxicological parameters of the compounds [17]. The evaluation of [{(1*H*-1,2,4-triazol-5-yl)phenyl}amino]naphthalene-1,4-diones (3a–g) for toxicological profile and acute toxicity was carried out using ADMETab3.0 (Table 3) and ProTox-II (Table 4). It is important to note that among the tested compounds 3a–g, the rates of liver injury (DILI), so-called hepatotoxicity, and carcinogenicity (CARC) are high (Table 3). The predicted mutagenicity (AMES) scores for 3a–3c and 3f, 3g are high, and for 3c, 3d and 3e the scores are medium, 0.714, 0.698 and 0.61 respectively. The acute oral toxicity scores for rats of all compounds are in the range of moderate to moderate risk, and the moderate skin sensitivity (skin sensitization) scores are 0.592, 0.568 and 0.575 for compounds 3a, 3f and 3g, respectively. Compounds 3c–3e are predicted to have a fairly high potential for skin damage.

Whereas, the predicted oral toxicity of the 1,2,4-triazole derivatives of 1,4-naphthoquinone obtained via the ProTox-II online platform (Table 4) differs significantly from the ADMETab 3.0 predictions. As can be seen from Table 4, all studied compounds belong to toxicity class IV (LD_{50}=1000 mg/kg) and are low-toxic. However, in high doses they can be harmful when swallowed (300<LD50≤2000). Analyzing other potential toxicological risks, it can be concluded that compounds 3b, 3c, 3d and 3g have high predicted immunotoxicity of 0.9, 0.95, 0.84 and

Table 1

Physicochemical parameters of the studied [{(1H-1,2,4-triazol-5-yl)phenyl}amino]naphthalene-1,4-diones (3a–g)

Parameter	Value						
	compound						
	3a	3b	3c	3d	3e	3f	3g
MW	410.12	410.12	426.09	470.04	470.04	422.14	422.14
TPSA	87.74	87.74	87.74	87.74	87.74	96.97	96.97
nRot	4.0	4.0	4.0	4.0	4.0	5.0	5.0
nHA	6.0	6.0	6.0	6.0	6.0	7.0	7.0
nHD	2.0	2.0	2.0	2.0	2.0	2.0	2.0
LogP	4.235	3.932	4.126	4.839	4.839	3.898	3.881
LogD	3.542	3.367	3.348	3.226	3.308	3.335	3.334
L.V.[h]	0	0	0	0	0	0	0

0.88, respectively. Compounds 3a and 3b are moderately cytotoxic. All compounds have negligible hepatotoxicity, compared to the ADMETab3.0 results (Table 3), which indicate high hepatotoxicity. The obtained toxicity results of the studied compounds by ADMETab3.0 and ProTox-II require additional *in vitro* studies of their toxicity.

Thus, the obtained results of activity prediction and pharmacokinetic properties indicate the promising potential of the compounds as anticancer and antimicrobial and the need for further studies of their biological activity. To validate the *in silico* results for compounds 3a–g, we conducted *in vitro* studies of their antimicrobial activity using the serial dilution method. Analysis of the results of antimicrobial activity showed (Table 5) that 2-((2-(3-(3-fluorophenyl)-1*H*-1,2,4-triazol-5-yl)phenyl)amino)naphthalene-1,4-dione (3a) exhibits insignificant activity against *E. coli* (MIC=500.0 μg/ml), moderate activity against test cultures *S. aureus* and *C. tenuis*

Table 2

Pharmacokinetic properties of compounds 3a–g

Pharmacokinetic property	Value						
	compounds						
	3a	3b	3c	3d	3e	3f	3g
Caco-2	–4.963	–5.08	–5.061	–5.031	–4.958	–4.95	–4.966
HIA	0.001	0.001	0.0	0.001	0.003	0.003	0.047
BBB permeant	0.0	0.0	0.002	0.002	0.0	0.0	0.0
CYP1A2 inhibitor	1.0	1.0	1.0	1.0	1.0	1.0	1.0
CYP1A2 substrate	0.999	1.0	1.0	1.0	0.994	0.999	1.0
CYP2C19 inhibitor	0.998	0.983	0.991	0.995	1.0	1.0	0.0
CYP2C19 substrate	0.0	0.0	0.0	0.0	0.0	0.005	0.999
CYP2C9 inhibitor	0.996	0.991	0.987	0.965	1.0	1.0	0.027
CYP2C9 substrate	0.009	0.04	0.012	0.001	0.001	0.023	0.286
CYP2D6 inhibitor	0.332	0.47	0.412	0.847	0.306	1.0	0.002
CYP2D6 substrate	0.002	0.0	0.0	0.0	0.0	0.439	0.798
CYP3A4 inhibitor	0.903	0.892	0.899	0.977	0.985	1.0	0.0
CYP3A4 substrate	0.0	0.0	0.0	0.0	0.0	0.0	0.0017
CYP2B6 inhibitor	0.002	0.001	0.005	0.053	0.001	0.009	0.0
CYP2B6 substrate	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CYP2C8 inhibitor	1.0	1.0	1.0	1.0	1.0	1.0	1.0
$T_{1/2}$	0.77	0.775	0.766	0.807	0.728	0.572	0.616
P-gp substrate	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pgp-inhibitor	0.972	0.95	0.907	0.977	0.958	0.958	0.905
LogK_p (skin permeation)	–5.47	–5.47	–5.20	–5.43	–5.43	–5.64	–5.64
Bioavailability score	0.55	0.55	0.55	0.55	0.55	0.55	0.55

Table 3

Toxicological properties of compounds 3a–g

Compound	Normative indicator*				
	DILI	AMES	CARC	Rat Acute Toxicy	Skin Sensitization
3a	0.965	0.79	0.828	0.6	0.592
3b	0.967	0.817	0.806	0.54	0.765
3c	0.982	0.714	0.81	0.534	0.833
3d	0.982	0.698	0.835	0.62	0.899
3e	0.987	0.61	0.798	0.589	0.863
3f	0.981	0.797	0.873	0.445	0.568
3g	0.983	0.795	0.871	0.415	0.575

Note: * – (0–0.3 excellent; 0.3–0.7 medium; 0.7–1.0 poor). For classification endpoints, the prediction probability values are converted to six characters: 0–0.1 (– – –), 0.1–0.3 (– –), 0.3–0.5 (–), 0.5–0.7 (+), 0.7–0.9 (+ +) and 0.9–1.0 (+ + +).

(MIC=125.0 μg/ml, MBC=250.0 μg/ml and MIC=250.0 μg/ml, MFC=500.0 μg/ml, respectively). While 2-[(2-(3-(2-fluorophenyl)-1*H*-1,2,4-triazol-5-yl)phenyl)amino]naphthalene-1,4-dione (3b) showed significant activity against *C. tenuis* (MIC=15.6 μg/ml, MFC=62.5 μg/ml), it was inactive against *E. coli, S. aureus, M. luteum* and the fungus *A. niger*, indicating its selectivity of action. Compounds 3c and 3d were equally active against *S. aureus* (MIC=250.0 μg/ml, MBC=500.0 μg/ml) and inactive against the remaining test cultures at the tested concentrations. As can be seen from Table 5, 2-((2-(3-(3-bromophenyl)-1*H*-1,2,4-triazol-5-yl)phenyl)amino)naphthalene-1,4-dione (3e) is inactive against all test cultures at the tested concentrations, and 3f has moderate selective activity against *C. tenuis* (MIC=125.0 μg/ml, MFC=250.0 μg/ml). Moderate activity of compound 3g was found against test cultures *S. aureus*, *M. luteum* and *C. tenuis* (MIC=250.0 μg/ml, MBC=500.0 μg/ml; MIC=125.0 μg/ml, MBC=250.0 μg/ml and MIC=125.0 μg/ml, MFC=250.0 μg/ml, respectively).

As can be seen from the presented results, 2-[(2-(3-(2-fluorophenyl)-1*H*-1,2,4-triazol-5-yl)phenyl)amino]naphthalene-1,4-dione (3b) may be a good candidate as a potential antifungal agent against *C. tenuis*. These data correlate with the above-mentioned ratio of 3b to the class of target proteins and the proportion of protease in it (20%). The ratio of the studied compound 3b to the IV toxicity class (LD_{50}=1000 mg/kg)) proves the need for its further

Table 4

Prediction of the toxicity of substances

Compound	Oral toxicity			Prediction**: active, probability of 1				
	TI*	LD_{50}, mg/kg	Prediction accuracy, %	HT	CG	IT	MG	CT
3a	4	1000	54.26	0.68	0.53	0.59	0.54	0.70
3b	4	1000	54.26	0.68	0.53	0.90	0.54	0.70
3c	4	1000	54.26	0.64	0.57	0.95	0.50	0.63
3d	4	1000	54.26	0.66	0.54	0.84	0.53	0.54
3e	4	1000	54.26	0.66	0.54	0.54	0.53	0.54
3f	4	1000	67.38	0.63	0.50	0.55	0.62	0.55
3g	4	1000	54.26	0.63	0.50	0.88	0.62	0.55

Notes: * – Class I: fatal if swallowed (LD_{50}≤5); Class II: fatal if swallowed (5<LD_{50}≤50); Class III: Toxic if swallowed (50<LD_{50}≤300); Class IV: harmful if swallowed (300<LD_{50}≤2000); Class V: may be harmful if swallowed (2000<LD_{50}≤5000); Class VI: not toxic (LD_{50}>5000); ** – TI (toxicity Index); HT (Hepatotoxicity), CG (Carcinogenicity), IT (Immunotoxicity), MG (Mutagenicity) and , CT (Cytotoxicity).

Table 5

Antimicrobial activity of [{(1*H*-1,2,4-triazol-5-yl)phenyl}amino]naphthalene-1,4-diones

Compound	Bacterial cultures						Fungi cultures			
	E. coli		*St. aureus*		*M. luteum*		*C. tenuis*		*A. niger*	
	MIC, μg/ml	MBC, μg/ml	MIC, μg/ml	MBC, μg/ml	MIC, μg/ml	MBC, μg/ml	MIC, μg/ml	MFC, μg/ml	MIC, μg/ml	MFC, μg/ml
3a	500.0	*	125.0	250.0	–	–	250.0	500.0	–	–
3b	–	–	–	–	–	–	31.2	62.5	–	–
3c	–	–	250.0	500.0	–	–	–	–	–	–
3d	–	–	250.0	500.0	–	–	–	–	–	–
3e	–	–	–	–	–	–	–	–	–	–
3f	–	–	–	–	–	–	125.0	250.0	–	–
3g	–	–	250.0	500.0	125.0	250.0	125.0	250.0	–	–
Nitrofuralum	1.50	+	6.25	+	6.25	+	+	+	+	+
Ketoconazole	+	+	+	+	+	+	25.0	50.0	25.0	50.0

Note: * – in the studied concentrations, the biocidal effect was not established; «–» – in the studied concentrations, the biocidal effect was not observed (growth of the microorganism was observed); «+» – the comparison drugs for MIC and MFK were not studied.

study as a potential antifungal agent.

Conclusions

The predictions of the biological activity of [{(1*H*-1,2,4-triazol-5-yl)phenyl}amino]naphthalene-1,4-diones (3a–3g) indicate their possible multitargeting, and the priority targets include proteins of the AG family, kinases and proteases. Analysis of the physicochemical parameters of drug similarity showed that compounds 3a–3g have drug-like characteristics, and also have satisfactory pharmacokinetic parameters, and therefore, prospects for their further study. It was established, using the online program ProTox-II, that all [{(1*H*-1,2,4-triazol-5-yl)phenyl}amino]naphthalene-1,4-diones are low-toxic compounds, belonging to the IV toxicity class (LD_{50}=1000 mg/kg). High immunotoxicity risk was found for 3b, 3c, 3d, 3g, and conflicting hepatotoxicity results by ADMETab3.0 and ProTox-II, which require additional verification. The initial screening for antimicrobial and antifungal activity allowed to identify 2-[(2-(3-(2-fluorophenyl)-1*H*-1,2,4-triazol-5-yl)phenyl)amino]naphthalene-1,4-dione (3b), which may be a good candidate as a potential antifungal agent against *C. tenuis*. The obtained results also provide basis for further studies of anticancer activity.

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ПЕРСПЕКТИВИ ЗАСТОСУВАННЯ 1,2,4-ТРИАЗОЛЬНИХ ПОХІДНИХ 1,4-НАФТОХІНОНУ ЯК ПРОТИМІКРОБНИХ ЗАСОБІВ: IN SILICO ТА IN VITRO ДОСЛІДЖЕННЯ

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У даній роботі проведено *in silico* оцінювання фармакологічного потенціалу похідних [{(1*H*-1,2,4-тріазол-5-іл)феніл}аміно]нафталін-1,4-діону. Здійснено прогноз фармакокінетичних показників, токсичності та біологічних мішеней досліджуваних сполук за допомогою ресурсів ADMETlab 3.0, ProTox-II та SwissTarget Prediction відповідно. Встановлено, що досліджувані сполуки відповідають правилу Ліпінського, мають високу ймовірність пероральної доступності та здатність проникати крізь гематоенцефалічний бар'єр. Пріоритетними біологічними мішенями визначено кінази та протеази. За результатами прогнозування 1,2,4-триазолові похідні 1,4-нафтохінону віднесено до IV класу токсичності (LD_{50}=1000 мг/кг). Виявлено розбіжності у результатах прогнозування гепатотоксичності за ADMETlab 3 та ProTox-II, що вказує на потребу проведення *in vitro* досліджень. Досліджено протимікробну та протигрибкову активність 1,2,4-триазолових похідних 1,4-нафтохінону методом серійних розведень. Встановлено значну протигрибкову активність 2-[(2-(3-(2-фторфеніл)-1*H*-1,2,4-триазол-5-іл)феніл)аміно]нафтален-1,4-діону проти *Candida tenuis* (МІК=15,6 мкг/мл). Результати проведеного *in silico* та *in vitro* скринінгу [{(1*H*-1,2,4-тріазол-5-іл)феніл}аміно]нафталін-1,4-діонів свідчать про доцільність подальших експериментальних досліджень їх протиракової та протимікробної активності.

Ключові слова: 1,4-нафтохінон, 1,2,4-триазоли, лікарська подібність, фармакокінетичні параметри, ADMETlab 3, токсичність, антимікробна активність.

PROSPECTS OF THE APPLICATION OF 1,2,4-TRIAZOLE DERIVATIVES OF 1,4-NAPHTHOQUINONE ANTIMICROBIAL AGENTS: IN SILICO AND IN VITRO STUDIES

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The physicochemical parameters and drug similarity of new 1H-1,2,4-triazol-5-yl)phenyl)amino)naphthalene-1,4-diones were calculated in silico using the online resource ADMETlab 3. Toxicity was predicted using the ProTox-II software, and the search for potential biological targets was carried out using SwissTarget Prediction. The in vitro antimicrobial activity of 1,2,4-triazole derivatives of 1,4-naphthoquinone against the bacterial cultures Escherichia coli, Staphylococcus aureus, and Mycobacterium luteum, as well as the fungi Candida tenuis and Aspergillus niger, was studied by the serial dilution method. It was established that triazole derivatives of 1,4-naphthoquinone 3a–g comply with Lipinski's rule, which indicates their potentially high oral availability. According to the ProTox-II prediction, compounds 3a–g can be attributed to toxicity class IV. Based on the analysis of pharmacokinetic characteristics, the prospects for 1H-1,2,4-triazol-5-yl)phenyl)amino)naphthalene-1,4-diones as potentially biologically active agents were confirmed. The results of hepatotoxicity prediction by ADMETlab 3 and ProTox-II indicate the need for further in vitro studies. The significant antimicrobial activity of compound 3b can be considered for further in vivo studies.

Keywords: 1,4-naphthoquinone; 1,2,4-triazoles; drug similarity; pharmacokinetic parameters; ADMETlab 3; toxicity; antimicrobial activity.

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