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THE CYTOTOXIC EFFECT OF SOME SYNTHETIC NITROGEN-CONTAINING HETEROCYCLIC COMPOUNDS ON CULTURES OF TUMOUR AND NORMAL CELLS AND THE CALCULATION OF THEIR ADME, QSAR, AND DFT PHARMACOLOGICAL PROPERTIES

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The cytotoxic effect of several synthetic nitrogen-containing heterocyclic compounds on cultures of tumour and normal cells and the calculation of their ADME, QSAR, and DFT pharmacological properties

The aim. *The purpose of our work was to investigate the cytotoxic influence of some synthetic nitrogen-containing heterocyclic compounds, namely imidazole, aurones, and triazole on the culture of tumour cells of melanoma mouse B16, human glioma U251 and normal HEK293 and their ADME, QSAR, and DFT pharmacological properties calculation.*

Materials and methods. *The estimation of cell viability in the conditions of influence of the investigated drugs was carried out by MTT. ADME data screening was performed by the SWISSADME server. QSAR calculations were performed on Way2Drug servers (cancerogenicity was predicted with ROSC-Pred, metabolism – with RA, side effects of drugs were investigated using AdverPred server, LD_{50} were predicted with Gusar software). The calculation of the functional density (DFT) was carried out using B3LYP and the functional of the exchange-correlation with the base set of 6–31 G (D, P) in the MMFF94 force field in the Avogadro program.*

Results. *It was found that compounds **1** and **2** are toxic for normal cells HEK293, compounds **3**, **4**, **6** and **7** are low-toxic, and **5** does not inhibit cell growth at all. Our study has demonstrated that in the case of tumour cell line U251 compounds **2**, **3** and **7** are non-toxic in general, and substances **1**, **4**, **5**, **6** and **7** have significant toxicity. In a case of cancer cell line B16, compounds **1**, **2**, **4**, **5**, and **6** are toxic, and compound 7 is cytotoxic at any concentration. The test compounds (**1–7**) possess drug-like properties. All compounds meet Lipinski's "rule of five" criteria. The BOILED-Egg model demonstrates that compound **3** may penetrate blood-brain barrier, all compounds except **1** can be absorbed in the intestine, **2** and **5** can be cleaved in the gastrointestinal tract and **3**, **4**, **6**, and **7** have resistance to digestive enzymes. The analysis of metabolism showed that these compounds can mainly be metabolized by mechanisms of N- and O-glucuronidation and C-oxidation. The obtained data indicate that the smallest toxic effect is achieved with intravenously introduced compounds, and the largest toxicity is achieved with oral administration for compounds **3**, **4**, **5** and **6**. The compounds **1** and **3** are completely noncarcinogenic, the other compounds can affect thyroid glands and hematopoietic system. This result requires further research when introduced into practical application. DFT calculations have shown that all investigated compounds are stable and reactive.*

Conclusions. *Differences in the sensitivity of cell lines and dose-dependent effects of compounds detected during the study should be considered when calculating the optimal working concentrations of drugs. The results of the study are necessary to understand toxic effects on the cell lines B16, HEK293, and U251 and their further use for preclinical studies*

Keywords: *imidazoles, aurones, triazoles, MTT, ADME, QSAR, and DFT*

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1. Introduction

Nitrogen-containing heterocyclic compounds have a wide range of biological applications, i.e., have antiviral, antifungal, antipyretic, antidepressant and inhibitory effects in various cancers. It is believed that the broad biological activity of these compounds is due to the presence of sp^2 hybridized donor nitrogen atoms. Over the years, heterocyclic compounds and their derivatives have become of great importance as binding blocks and ligands in the construction of various homogeneous and heterogeneous complexes with a wide range of applications in the field of biological science [1].

Imidazoles have a unique place in heterocyclic chemistry, and their derivatives have aroused considerable interest due to their universal properties in chemistry and pharmacology. Imidazole is a nitrogen-containing heterocyclic ring of biological and pharmaceutical importance. Thus, imidazole compounds have been an in-

teresting source for researchers for more than a century. The imidazole ring is part of several important natural products, including purine, histamine, histidine and nucleic acid. Being a polar and ionizing aromatic compound, it improves the pharmacokinetic characteristics of molecules and, thus, is used to optimize the solubility parameters and bioavailability of the proposed sparingly soluble molecules. There are several methods used for the synthesis of imidazole-containing compounds, as well as their various structural reactions open great opportunities in the field of medicinal chemistry. Imidazole derivatives have a wide range of biological activity, including antibacterial, antitumor, antituberculous, antifungal, analgesic and antiviral effects [2].

In the early twentieth century, Gustav Klein coined the term anthochlor (*anthos* = flower, *chlōrós* = =yellowish) to define a class of water-soluble pigments that give colour to plants capable of synthesizing them as secondary metabolites [3]. Distinguished by a bright yellow colour, aurones play a key role in pollination, attracting vectors to flowers and pollen. The visual contrast that is perceived is the result of different UV absorption spectra. Flavones, isoflavones and flavanones have UV absorption at 350 nm and therefore have no visible colour. In contrast, aurones have an absorption spectrum in the range of 390–430 nm, which results in a more intense colour than the original chalcones, demonstrating ultraviolet absorption in the range of 365–390 nm. Aurones are part of a broad family of polyphenols. They were first characterized in 1940 in plants of the genus *Asteraceae*. Considered poorly represented in nature, aurones are often ignored by researchers compared to other members of the flavonoid superfamily. However, over the past two decades, the scientific community has overestimated them, as aurones could modulate various biological activities.

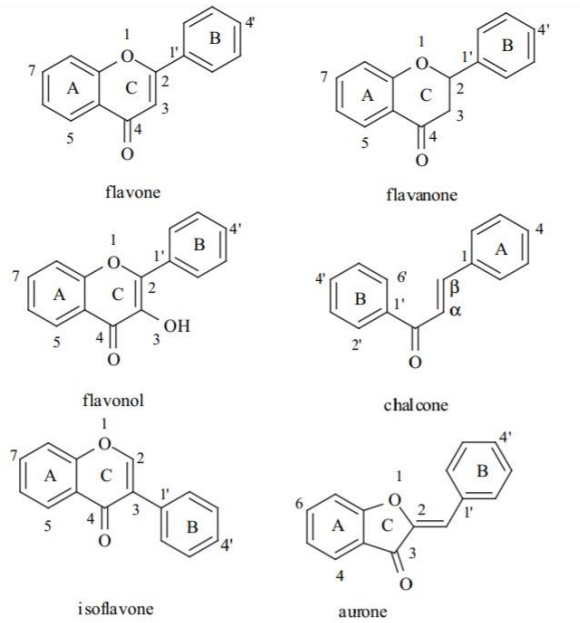


Fig. 1. A variety of flavonoids [4]

Unlike alkaloids or terpenoids, which have a large variety of chemical structure, flavonoids have a limited number of scaffolds (Fig. 1) and their diversity is due to substitutions such as hydroxylation, methoxylation, prenylation or glycosylation [5].

As an integral part of the diet, they have a wide range of positive effects on human health, including protection against cardiovascular disease and various forms of cancer. They probably have such biological effects due to antioxidant activity and the ability to chelate metal ions. They have been extensively studied for their antitumor diseases in several modes of action: aromatase inhibition, topoisomerase, protein kinase C, several protein tyrosine kinases, and cyclin-independent kinase [4].

Aurones could be obtained (Fig. 2) synthetically in the process of oxidative cyclization reactions of chalcones and 1- (2-hydroxyphenyl) -3-phenylpropinols; by cyclization of chalcones in DMSO and mercury acetate; by the condensation reaction between benzofurans and benzaldehyde derivatives in an acidic medium or in a basic medium [6].

A series of (Z) -2-benzylidenebenzofuran-3-(2H)-ones (aurones) containing various substituents on rings A and B have also been reported and their antiparasitic activity against the intracellular form of *Leishmania infantum* has been evaluated and their cytotoxicity against THP1-differentiated human macrophages [8]. A series of simple analogues of aurones were also synthesized and tested for antifungal activity against *Candida spp.* [9]. According to the literature, aurone derivatives have been identified as potential antitumor agents that exhibit selective cytotoxicity. Interestingly, flow cytometry found that this growth inhibition was achieved by blocking the tumour cell cycle in the G_2 / M phase. This behaviour is consistent with the tubulin-binding effect, which is supported by the inhibitory properties of one aurone derivative against tubulin polymerization. More recently, there have been derivatives of aurones in which the B-ring has been replaced by a piperazine moiety, able to induce cell cycle stop in the G_0/G_1 phase and demonstrated apoptosis-inducing effect [7].

Also in our study we consider compounds containing a triazole ring, they are found in nature in some microorganisms, fungi and marine organisms. Triazoles are widely studied for their healing properties. They have a variety of biological and pharmacological properties, such as antitumor, anti-inflammatory, cyclooxygenase (COX) inhibition, anti-obesity, antiviral and antibacterial activity. One of the deferasirox drugs on the market, used as an iron chelator, contains diaryl triazole. Triazoles have been shown to be promising as tubulin polymerization inhibitors, COX-2 inhibitors, and CB1 receptor antagonists. These scaffolds can be further used to develop new and better pharmacologically active compounds [10].

The aim of the study was to investigate the cytotoxic effect of some synthetic nitrogen-containing heterocyclic compounds among imidazole derivatives, triazole auronate on cultures of tumour cells of mouse B16 melanoma and human glioma U251 and normal Hek293 cells and calculation of their ADME, QSAR and DFT pharmacological properties.

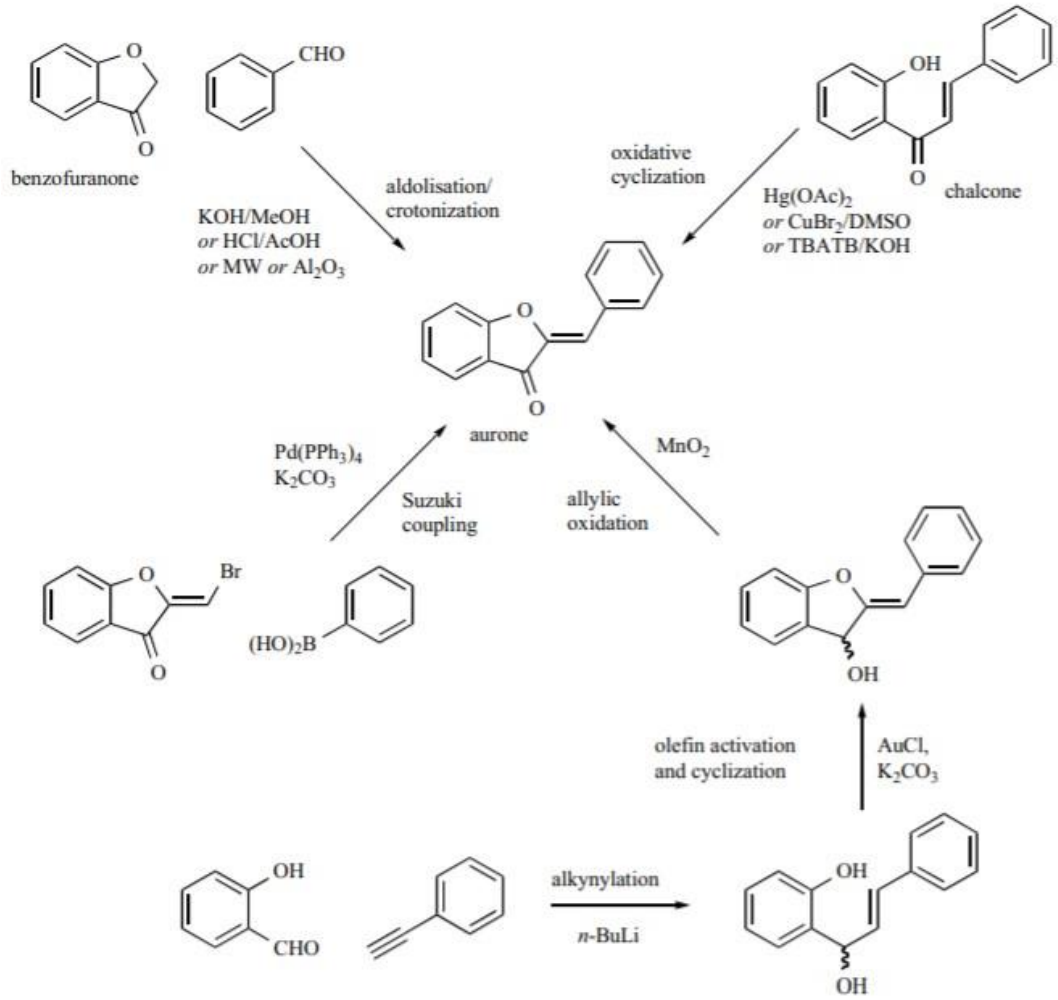


Fig. 2. Ways to obtain aurones [7]

2. Materials and methods

To evaluate the antiproliferative activity of the compounds as a marker of their potential antitumor efficacy, the synthesized compounds were tested for induction of apoptosis by MTT analysis using apoptosis-competent cell culture cells of mouse B16 melanoma, human glioma U251 and to determine toxicity in human embryonic control culture29 (Table 1).

Table 1

Characteristics of cell lines

Characteristic	B16	U251	HEK293
Biological source	Mouse	Human brain	Human kidneys (embryonic)
Description	Melanoma of a mouse that produces melanin	Tumor glioma	Non-tumor cell culture
Form	Liquid	Liquid	Liquid
Growth mode	Adhesive	Adhesive	Adhesive
Karyotype	Not specified	2n=46	2n=46, hypotriploid
Morphology	Epithelial	Pleomorphic / astrocytoid	Epithelial
Product	Melanin	Contains GFAP positive cells	Not specified
Receptors	Not specified	PDGFR Alpha and EGFR	Not specified
Growing technique	Mammalian cell culture	Mammalian cell culture	Mammalian cell culture

2.1. Cultivation of cells

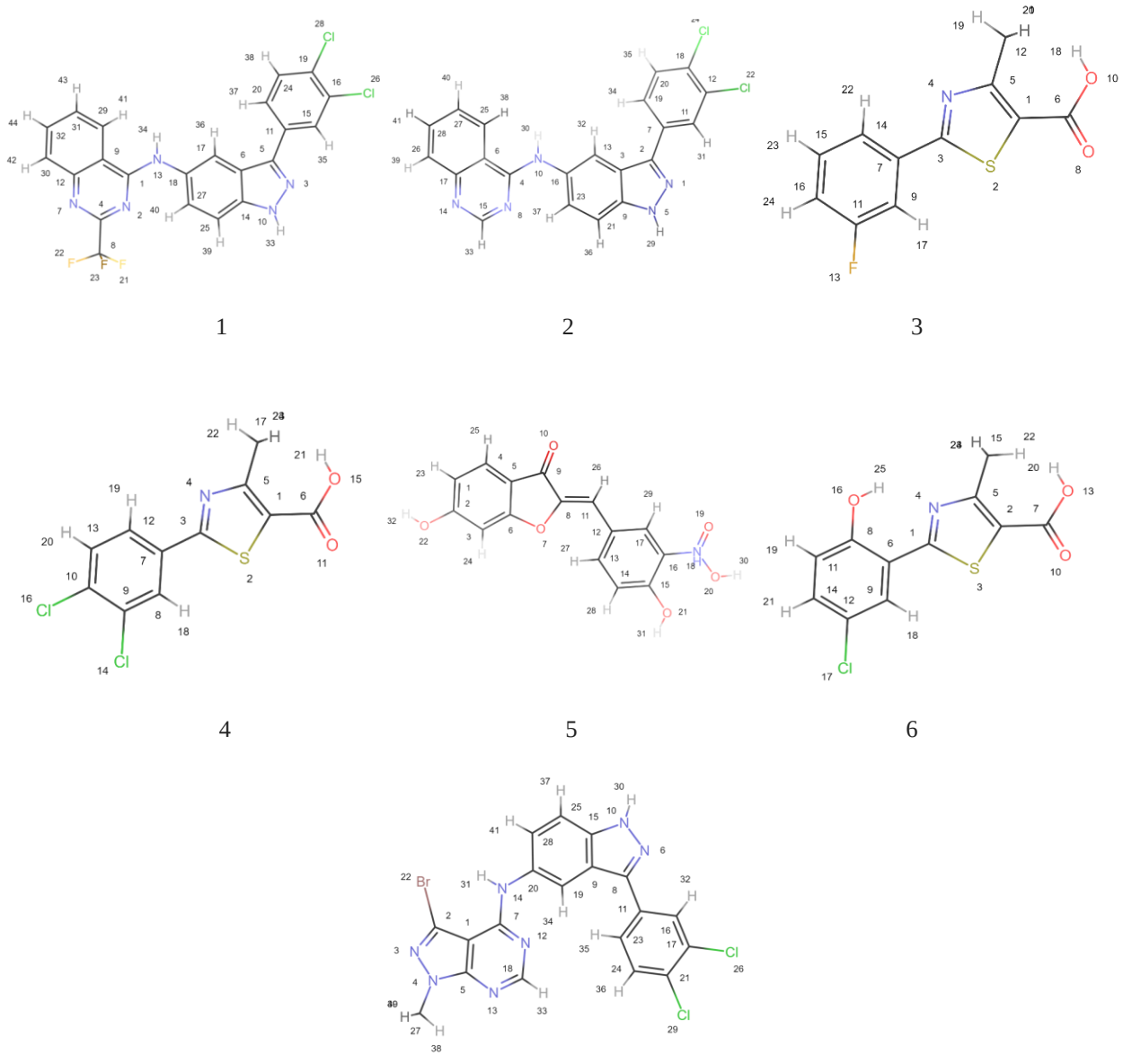
The inhibitory effect of substances at the Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine in 2021–2022 (Fig. 3) on the viability of cell culture of melanoma B16, human glioma U251 and human embryonic kidney Hek293 in the concentration range 0.02–2000 μg. The B16 cell line was cultured in RPMI medium (Gibco, USA) and U251 in DMEM (Gibco, USA) with the addition of FBS to a final concentration of 10 %, penicillin (100 IU / ml) and streptomycin (100 μg/ml) (PAA). at 37 °C in a humidified incubator with 5 % CO_2. Evaluation of cell viability under the influence of the studied drugs was performed by the MTT method. Cells were seeded on a 96-well plate at a concentration of 8x103 cells / well and cultured for 12 hours. The culture medium was then replaced with

serum-free nutrient medium with test substance dissolved therein and incubated for 24 hours.

2.2. Basic cytotoxicity

Analysis of the cytotoxic effect of test substances on the cells of these lines was performed using the MTT test, using the reagent 3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT), which crystallizes into formazan inside the cell. Measurement of the concentration of formazan in solution after interaction with dimethyl sulfoxide (DMSO) allows to estimate the number of viable cells, and in cytotoxic studies – specific cell death induced by one or another agent [11].

After treatment, the medium was collected and replaced with MTT solution (0.75 mg/ml) (Sigma-Aldrich, USA), which was incubated for 3 hours. The crystals of the formed formazan were dissolved in DMSO. The concentration of formazan in the wells was determined with a microphotometer ELx800 Absorbance Microplate Reader (BioTek, USA) by optical absorption at a wavelength of 570 nm. The results were processed using OriginPro 8 software. The percentage of viable cells in each test well was calculated relative to the positive control wells, the viability of which was assumed to be 100 %. Next, we plotted the dependence of cell viability, expressed as a percentage relative to the decimal logarithm of the concentrations of added compounds. The resulting curve is subjected to analysis "Fit Sigmoidal – DoseResp" in the software OriginPro 8, find the logarithm of the concentration at the point of 50 % cell viability. Calculate the concentration of semi-maximal inhibition of cell growth (IC_{50}) [12].



1 2 3

4 5 6

7

Fig. 3. Compounds used in the study: 1 – AS32079; 2 – 1655036; 3 – 0610-131; 4 – 797902; 5 – 441450; 6 – 949808; 7 – 3644584

2.3. Calculation methods

ADME data was screened by a SwissADME server. QSAR calculations were performed on Way2drug servers (prediction of carcinogenicity in ROSC-Pred, transformation in the body was studied using RA, prediction of drug side effects was done in ADVERPred server, prediction *in silico* LD_{50} values – using GUSAR software). Functional density (DFT) calculations were performed using B3LYP and exchange correlation functional with a base set of 6–31 G (d, p) in the MMFF94 force field in the Avogadro program.

3. Research results and their discussion

A well-documented MTT assay was introduced by Tim Mosman in 1983. This colorimetric analysis is used to determine the proliferation, viability and cytotoxicity of cells [13]. Traditionally, the reduction of tetrazolium salts to their equivalent formazan precipitates has been used to histochemically demonstrate the activity of oxidative and non-oxidative enzymes in mitochondria by light and electron microscopy. In the MTT analysis, tetrazolium MTT (3- [4,5-dimethylthiazol-2-yl] -2,5-diphenyltetrazolium bromide) is reduced in a mitochondrial- dependent reaction to insoluble violet formazan by cleavage of the tetrazolium ring succinate dehydrogenase in mitochondria (Fig. 4).

Yellow MTT is metabolized only by living mitochondria, producing purple formazan. Formazan accumulates inside the cell because it cannot pass through the cell membrane. When dimethyl sulfoxide (DMSO), isopropanol or other suitable solvent is added, formazan dissolves and is released and is easily quantified colorimetrically. The number of living cells (in percent) was determined by the ratio of the value of absorption between the cells exposed to the test drug and the control intact in serum-free medium. A substance is considered to have inhibitory activity if the percentage of living cells does not exceed 70 %. Cytotoxic concentration (IC_{50}) is usually defined as the concentration that kills 50 % of cells.

The graph (Fig. 5) shows the dependence of cell viability on the logarithm of the concentration of the test substance.

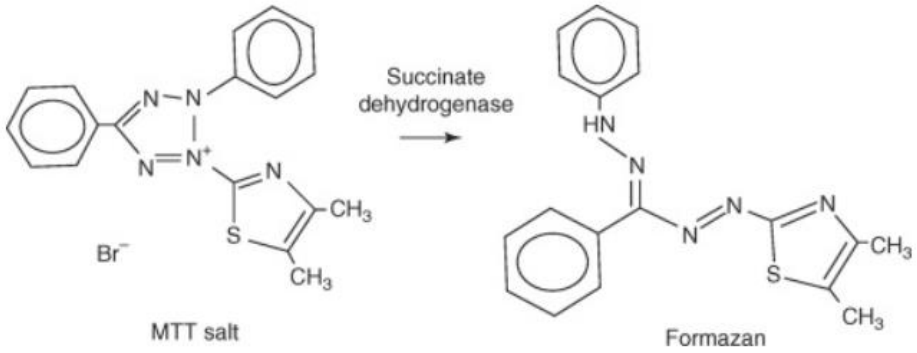
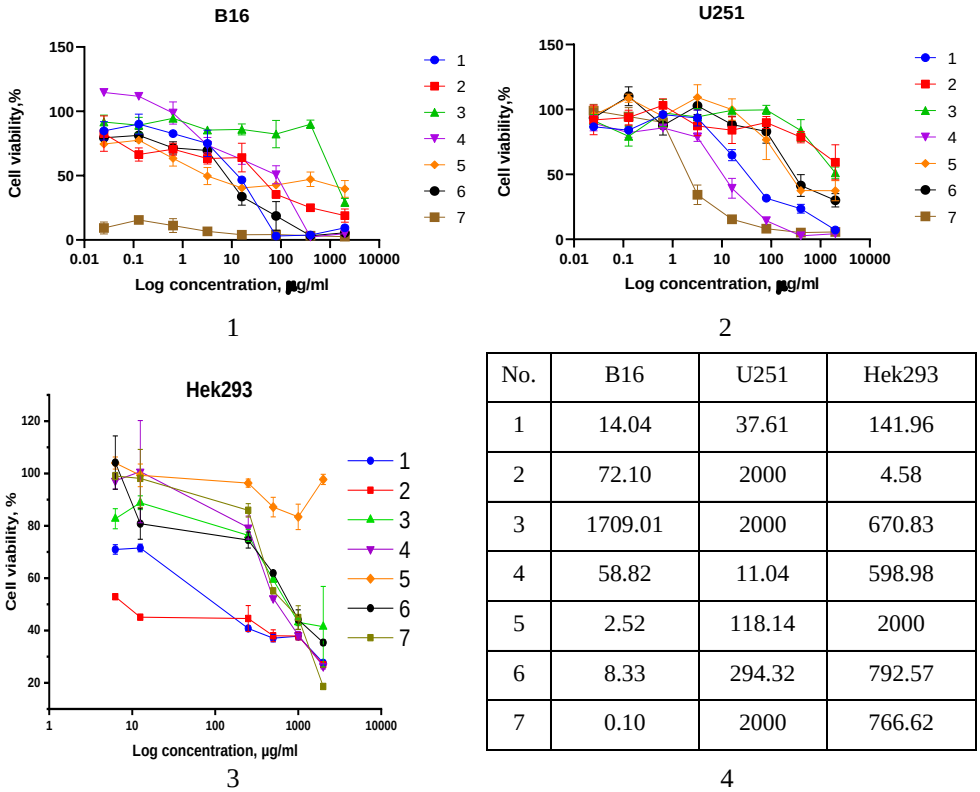


Fig. 4. The reaction underlying the MMT analysis



1 2 3

No.	B16	U251	Hek293
1	14.04	37.61	141.96
2	72.10	2000	4.58
3	1709.01	2000	670.83
4	58.82	11.04	598.98
5	2.52	118.14	2000
6	8.33	294.32	792.57
7	0.10	2000	766.62

4

Fig. 5. Analysis of cytotoxicity of test substances: 1 – inhibitory effect of substances on the viability of mouse melanoma cell culture B16, 2 – glioma human U251 and 3 – human embryonic kidney Hek293, 4 – IC_{50} values

According to the results of previous experiments, the study compounds did not show a clear time-dependent effect.

According to the MTT test, the cytotoxicity curves of the three cell lines have different dynamics, which indicates different sensitivity of normal and different tumour cells to the test compounds.

According to the IC_{50}, the following conclusions could be drawn: compounds **1** and **2** are toxic to normal HEC293 cells at doses of 141.96 and 4.58 μg/ml culture, respectively, compounds **3** (670.83 μg/ml), **4** (598.98 μg/ml), **6** (792.57 μg/ml), and **7** (766.62 μg/ml), low toxicity, and **5** – does not inhibit cell growth. Our study showed that compounds **2**, **3** and **7** were non-toxic for the U251 tumour line, and substances **1** (37.61 μg/ml), **4** (11.04 μg/ml), **5** (118.14 μg/ml) and **6** (294.32 μg/ml) have significant toxicity. For tumour line B16, compounds **1** (14.04 μg/ml), **2** (72.1 μg/ml), **4** (58.82 μg/ml), **5** (2.52 μg/ml), and **6** (8, 33 μg/ml), toxic, and compound **7** is cytotoxic at any concentration, compound **3** (1709.01 μg/ml) is almost non-toxic. This indicates a slightly higher sensitivity of B16 and U251 cells to the action of drugs, which may be due to the presence in the cell of hypersensitivity to these compounds. The sources of such hypersensitivity of transformed cells could be different. It could be assumed the induction of oxidative stress caused by the introduction into the cell of synthetic compounds with the production of reactive oxygen species, in particular singlet oxygen. It is likely that these factors underlie the reduction of the resistance of tumour lines. Compound **3** showed low toxicity in both normal and tumour cells, suggesting that this compound may have an antioxidant effect, which leads to high cell viability.

3.1. Toxicity analysis *in silico*

Computer modelling is extremely important for predicting the properties of compounds in drug development. In addition to efficacy and toxicity, many drug development failures are related to pharmacokinetics, i.e., the fate of the compound in the body. Starting in 2017, the quality of clinical candidates could be improved by monitoring the physicochemical profiles of compounds. Individual consideration of absorption, distribution, metabolism, and excretion behaviours (ADMEs) in the early stages of drug discovery has reduced the proportion of global pharmacokinetic failures in the later stages of development. As a result, candidate drugs enter the market more efficiently [14].

ADME properties were screened, and drug-like test compounds were identified for a number of parameters. Compounds **1–7** passed the drug similarity criteria (Figs. 6, 7), according to the calculated ADME parameters using the SwissADME server. Radar graphs of compounds showing drug similarity are important for the identification of compounds with poor absorption and penetration. Each developed compound meets Lipinsky RO5 criteria (not more than 5 hydrogen bond donors; not more than 10 hydrogen bond acceptors; molecular weight of compound less than 500, octanol-water partition coefficient (log P) should not exceed 5 [15–17].

The test compounds fully meet the requirements of ADME for drug ligands. However, compounds **1**, **2** and **7** have a slightly weak dissolution and go beyond lipophilicity, which could be corrected by the introduction of additional agents.

Parameters in Table 2, indicating good oral absorption of drugs. LIPO – lipophilicity in the range from -0.7 to 5, SIZE – molecular weight from 150 to 500, POLAR – polarity from 20 to 130, INSOLU – LogS solubility from 0 to 6 per module, INSATU – saturation from 0.25 to 1, FLEX – the number of flexible connections from 0 to 9.

The boiled egg model demonstrates that compound **3** is able to cross the blood-brain barrier, all but **1** could be absorbed in the gut, **2** and **5** could be broken down in the gastrointestinal tract, and **3, 4, 6** and **7** are resistant to digestive enzyme.

Table 2

ADME parameters of test compounds

No.	LIPO	SIZE	POLAR	INSOLU	INSATU	FLEX
1	6.88	474.24	66.49	–7.43	0.05	4
2	5.96	406.27	66.49	–6.58	0	3
3	2.98	237.25	78.43	–3.57	0.09	2
4	4.13	288.15	78.43	–4.58	0.09	2
5	2.8	300.24	116.42	–3.74	0	2
6	3.15	267.7	98.66	–3.84	0.09	2
7	5.63	489.16	84.13	–6.83	0.05	3

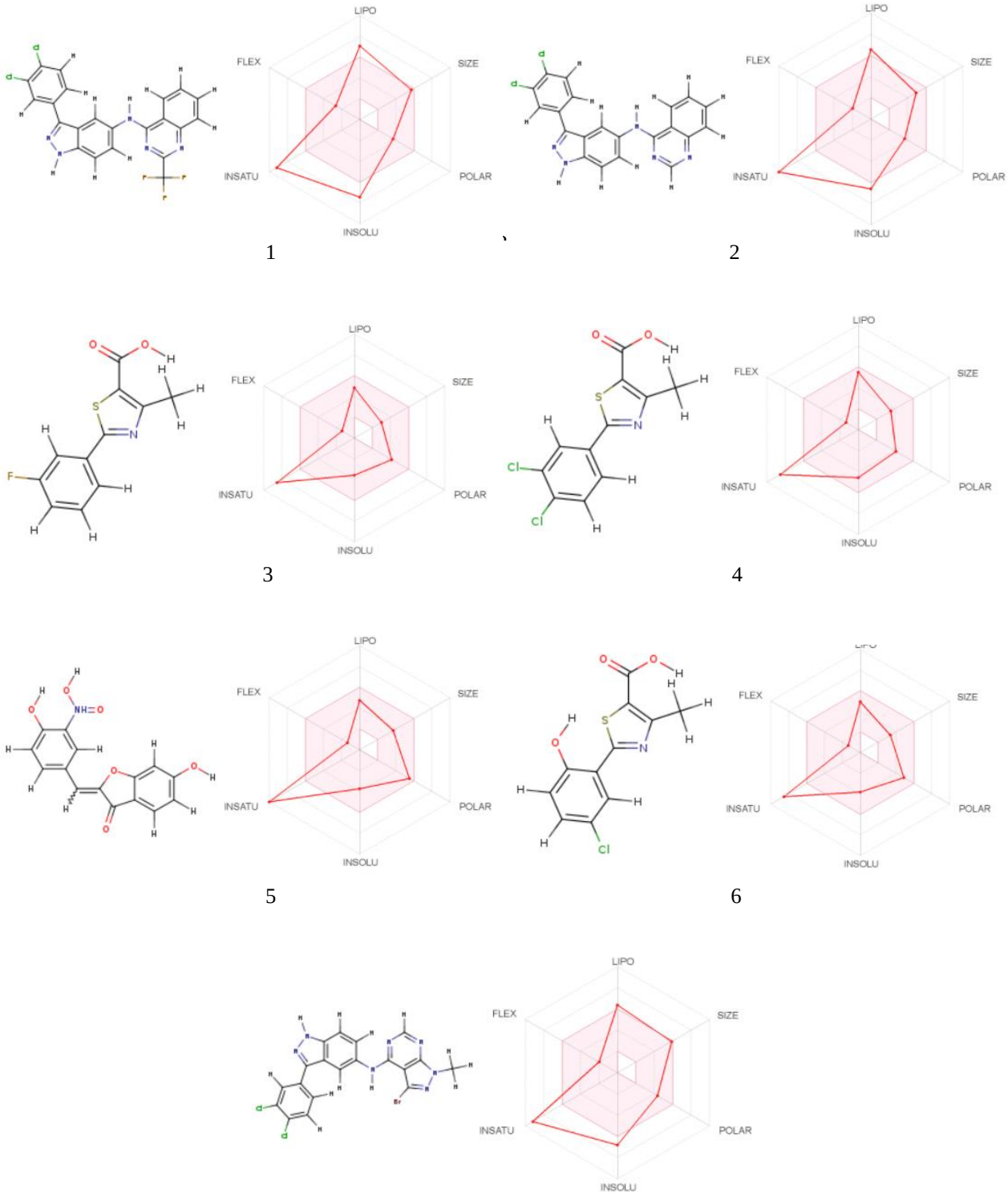


Fig. 6. Screening of the behavior of absorption, distribution, metabolism and excretion of test compounds

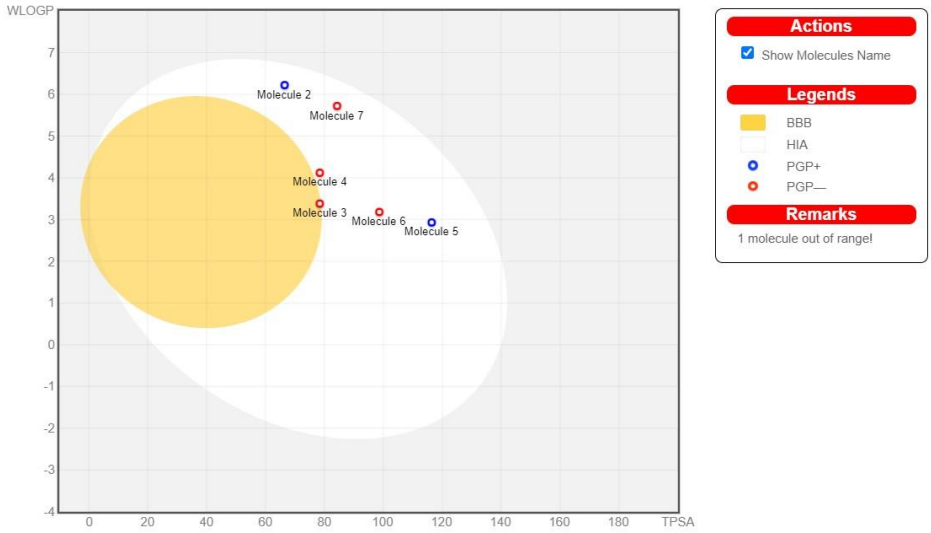


Fig. 7. Boiled egg (Yellow indicates the possibility of crossing the blood-brain barrier, white absorption in the intestine, blue the ability to be broken down in the gastrointestinal tract and red resistance to digestive enzymes)

3.2. Prediction of metabolism of the obtained compounds *in silico*

Transformations in the body were studied using RA – a web service for predicting reacting atoms in silico based on the concept of local correspondence, according to which the biological activity of a medicinal organic compound is based on molecular recognition between individual ligand and target atoms.

Prediction of drug conversion sites for nine reaction classes: aliphatic hydroxylation, aromatic hydroxylation, C-oxidation, N-oxidation, S-oxidation, N-glucuronidation, O-glucuronidation, N-dealkylation, O-dealkylation.

The forecast is based on PASS (Prediction of Activity Spectra for Substances) technology [16], LMNA descriptors and a training kit based on the main classes of biotransformation reactions mediated by five human cytochrome P450 isoforms and all UDP glucuronosyltransferase family isoforms. For evaluation were used the terms Pa – the possibility of a reaction and Pi – the possibility of no reaction, more than 0.5 low probability, more than 0.7 – medium probability, 0.9 – high probability.

For compound **1**, an N-glucuronidation reaction is possible with an estimate of Pa=0.690 and Pi=0.010 for N13 and with an estimate of Pa=0.690 and Pi=0.044 aliphatic hydroxylation with free hydrogen is possible.

For compound **2** in the process of N-glucuronidation according to N10 Pa=0.799 and Pi=0.006, as a result of N-oxidation N14 – Pa=0.528 and Pi=0.031, with aromatic hydroxylation C28 – Pa=0.554 and Pi=0.066, Pa=0.508, with N-hydroxylation of N10 Pi=0.032.

For compound **3** in aliphatic hydroxylation C12 Pa=0.689 and Pi=0.044, in O-glucuronidation O10 – Pa=0.582 and Pi=0.056, in C-oxidation C6 – Pa=0.542 and Pi=0.064.

For compound **4** at C-oxidation C6 Pa=0.763 and Pi=0.027, at aliphatic hydroxylation C17 Pa=0.671 and Pi=0.049.

For compound **5** under hydrogenation C8 Pa=0.650 and Pi=0.024.

For compound **6** in O-glucuronidation O13 and O16 Pa=0.717 and Pi=0.031, in aliphatic hydroxylation of C15 Pa=0.613 and Pi=0.067, in C-oxidation C7 Pa=0.581 and Pi=0.054.

For compound **7** with N-glucuronidation N14 Pa=0.654 and Pi=0.012.

The introduction of methyl, propyl and fatty acid radicals in these sites will greatly slow down the biotransformation and excretion of test substances.

3.3. ADVERPred: *in silico* predicting side effects of drugs

Predictions of drug side effects such as arrhythmia, heart failure, hepatotoxicity, myocardial infarction, nephrotoxicity, and myocardial infarction were performed on the ADVERPred server.

For compound **1** possible heart failure (Pa=0.631 and Pi=0.014), myocardial infarction (Pa=0.598 and Pi=0.029), for compound **2** – hepatotoxicity (Pa=0.634 and Pi=0.127), heart failure (Pa=0.583 and Pi=0.025), myocardial infarction (Pa=0.575 and Pi=0.032), for compound **3** – myocardial infarction (Pa=0.934 and Pi=0.004), for compound **4** – myocardial infarction (Pa=0.932 and Pi=0.004, for compound **5** – hepatotoxicity (Pa=0.889 and Pi=0.031), for compound **6** – myocardial infarction (Pa=0.796 and Pi=0.007), for compound **7** – hepatotoxicity (Pa=0.687 and Pi=0.105).

As you could see, the obtained substances have a medium level of hepato- and cardiotoxicity, which should be considered in further clinical studies [18].

3.4. Forecast values of LD_{50} *in silico*

Prediction of LD_{50} (semi-lethal dose) *in silico* values for rats with four types of administration (Oral, intravenous IV, intraperitoneal IP, subcutaneous SC) was performed using GUSAR software (Tab. 3). The training kits were created based on data from the SYMYX MDL toxicity database.

They include information on ~ 10,000 chemical structures with acute toxicity data for rats presented by LD_{50} values [19].

Table 3

Prediction of LD_{50} values (semi-lethal dose) in silico for rats

No.	Rat IP LD_{50} (mg/kg)	Rat IV LD_{50} (mg/kg)	Rat Oral LD_{50} (mg/kg)	Rat SC LD_{50} (mg/kg)
1	644.800	152.200	814.000	1991.000
2	538.400	102.700	937.800	1669.000
3	339.700	486.800	1591.000	656.100
4	433.300	520.800	1251.000	1406.000
5	326.900	100.500	2180.000	660.000
6	270.600	513.200	1641.000	1343.000
7	633.600	127.100	606.300	441.900

The obtained data indicate that the least toxic effect is achieved by intravenous administration of test compounds, and the greatest toxicity is achieved by oral administration for compounds **3, 4, 5** and **6**.

3.5. Predicting carcinogenicity

ROSC-Pred is a free web service for predicting the carcinogenicity of individual rodent organs based on the structural formula of organic compounds. The forecast is based on PASS technology (forecast of activity spectra for substances) and training kits based on carcinogenic potency database (CPDB) [20].

The results obtained for the compounds in female and male rats and mice were as follows:

1. has no carcinogenic effect;

2. Pa=0.520, Pi=0.191 system of hematopoiesis of female mice;

3. has no carcinogenic effect

4. Pa=0.778, Pi=0.048 thyroid gland of female mice

5. Pa=0.518, Pi=0.119 bladder of male rats, Pa=0.689, Pi=0.057 bladder of female rats, Pa=0.623, Pi=0.132 thyroid gland of male mice, Pa=0.500 and Pi=0.162 stomach of female mice.

6. Pa=0.752, Pi=0.058 thyroid gland of female mice

7. Pa=0.592, Pi=0.131 hematopoietic system of female mice.

Compounds **1** and **3** are completely non-carcinogenic, the rest have a moderate effect on the thyroid gland and hematopoietic system. This result is not a precaution to use but requires further research when put into practice.

3.6. Computational research (DFT research)

The investigated compounds were first constructed, optimized, and then analyzed using the Avogadro program. The force field MMFF94 (Merck Molecular Force Field [MMFF]) to create a three-dimensional structure was used. Thus, the ligand optimizer was implemented by minimizing the geometry using the force field MMFF94. The most reliable theoretical method, i.e., the calculation of functional density (DFT) was performed using B3LYP and the exchange correlation functional with a basic set of 6-31 G (d, p) for carbon, nitrogen atoms, oxygen, hydrogen [21].

DFT calculations allow us to interpret the atomic location of the test compound (Fig. 8) and were optimized using the basis B3LYP / 6–31 + G (d, p). The values of the dipole moment (D) and the energy of one point of the compound are checked by applying DFT / B3LYP 6–31 + G (d, p) base sets, which shows that compound **7** retains more energy of one point compared to other compounds. Compound **5** has less energy and higher dipole moment, which means greater stability compared to other compounds.

3.7. Investigation of the first molecular orbitals (FMO)

From FMO studies, one could understand the reaction of a compound and predict the active site located in the conjugate of the system, while EHOMO and ELUMO specify global descriptors of the reactivity of test compounds, namely chemical hardness, chemical potential and electrophilicity.

FMO studies explain the chemical reactivity and choice of active sites in the molecular system. Energy zones and energy differences (LUMO-HOMO) describe the interaction with charge transfer (CT). However, significant chemical parameters of reactivity, such as electronegativity (χ), chemical potential (η), global hardness (μ), global softness (S), and global electrophilicity index (ω) (Pearson, 1989; Padmanabhan et al., 2007) are given in Table 4 (Fig. 8).

Table 4

Global descriptors of reactivity

	HOMO (eV)	LUMO (eV)	dE (eV)	χ (Pauling)	η (eV)	σ	μ (eV)	S	ω (eV)	Single point energy (kcal/mol)	Dipole moment (D)
1	–0.213	–0.071	0.142	–0.142	0.0711	14.07	0.142	7.036	0.142	–2.3339	2.562
2	–0.207	–0.061	0.146	–0.134	0.0729	13.72	0.134	6.859	0.122	–2.0002	2.208
3	–0.241	–0.085	0.156	–0.163	0.0779	12.84	0.163	6.418	0.169	–1.127	5.099
4	–0.244	–0.092	0.152	–0.168	0.0762	13.12	0.168	6.562	0.184	–1.947	4.769
5	–0.215	–0.079	0.136	–0.147	0.068	14.72	0.147	7.358	0.158	–1.083	10.902
6	–0.228	–0.091	0.137	–0.159	0.0684	14.63	0.159	7.315	0.185	–1.562	4.402
7	–0.208	–0.058	0.15	–0.133	0.0752	13.3	0.133	6.649	0.117	–4.606	5.497

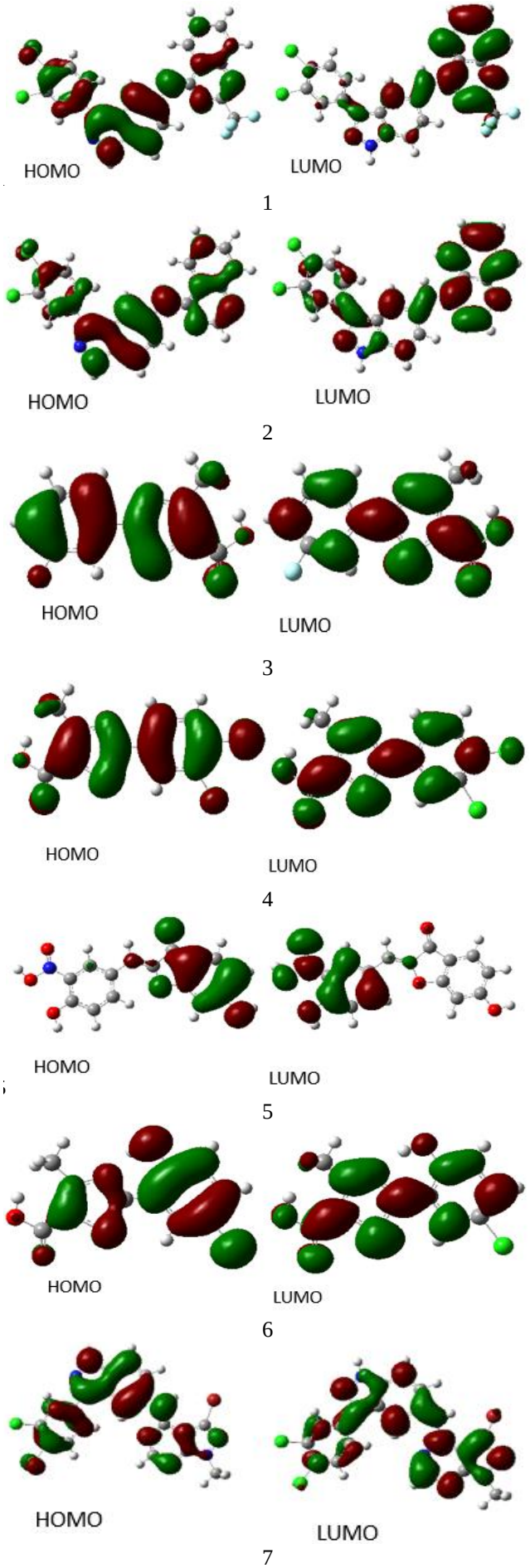


Fig. 8. Quantum chemical parameters and location of upper filled orbitals and lower unfilled molecular orbitals

In Fig. 8 shows that compounds **3, 4** and **6** have a uniform redistribution of energy over HOMO and LUMO, which means high value [HOMO – LUMO] and low reactivity. Compounds **1, 2** and **7** have a certain asymmetry and already greater reactivity and biological activity. The low value of [HOMO – LUMO] in compound **5** and the greatest asymmetry of HOMO and LUMO energy levels indicate a high reactivity among all studied compounds.

However, negative values of EHOMO and ELUMO confirmed that the test compounds were stable. The difference in binding energy clearly explains the chemical reactivity and chemical stability of active molecules.

Study limitations. The study was limited to the complexity of obtaining and synthesizing the studied compounds, so this paper presents only those that have been able to obtain stably.

Prospects for further research. In the future it is planned to use, and preclinical in vitro and in vivo study of the compounds presented in this work as inhibitors of proteinase CK2 – an important part of the tumour process

6. Conclusions

The methods used in our study allow to determine the dose parameters of the effects of compounds on the studied cell lines, but do not allow to make an accurate conclusion about the ways of cell death after exposure to the obtained substances. The obtained results are very important in several respects. First, differences in cell line sensitivity and dose-dependent effects detected during the study should be considered when calculating the optimal working concentrations of drugs. In addition, several dose-dependent effects are possible in the mechanism of action of nitrogen-containing heterocycles, such as effects on cell death pathways, signalling cascades, glycolytic activity, etc. Secondly, the results of the study are necessary to understand the patterns of toxic effects of the drug on cell lines B16, HEC293 and U251, to clarify their mechanisms of action and further use in preclinical studies.

Conflict of interests

The authors declare that they have no conflicts of interest.

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