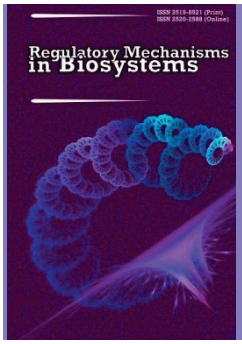

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Prospects for the use of sulfur-containing pteridines in toxic liver damage

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The presented material describes the results of the purposeful search for the novel hepatoprotective agents among synthetic sulfur-containing pteridines. The studied compounds were obtained using previously described synthetic procedures. SwissADME and ProTox-II services, as well as a docking study were used to predict the compounds` pharmacokinetics, drug-likeness and toxicity. The effects of sulfur-containing pteridines on lipid peroxidation in vitro and GSH/GSSG levels in vivo were studied for evaluation of their hepatoprotective potential. In silico procedures allowed us to exclude the compounds with inappropriate ADME parameters and high predicted toxicity. Molecular docking of the obtained compounds towards the active site of CYP-enzyme allowed us to clarify the specifics of ligand-enzyme interactions and predict cytochrome-inhibiting activity of the studied agents. The antioxidant activity of the studied compounds was evaluated in vitro using the linoleic acid peroxidation model. It has been found that sulfur-containing pteridines inhibit the peroxidation of linoleic acid. The structure – antioxidant activity relationships were evaluated and discussed as well. Additionally, it has been estimated that antioxidant properties of the studied pteridines directly correlate with their hydrophilicity and number of functional groups with exchangeable proton in molecules. In vivo studies showed that some of the studied sulfur-containing pteridines reduced severity of the hepatotoxic effects caused by the administration of carbon tetrachloride. (3-(7-Hydroxy-4-oxo-2-thioxo-1,2,3,4-tetrahydropteridin-6-yl)propanoyl)alanine has been identified as the most active hepatoprotective agent. The above mentioned compound significantly increases the content of GSH and decreases the level of GSSG compared with non-treated experimental pathology, which reliably affirms its hepatoprotective activity. The hepatoprotective activity of the studied compounds is likely associated with their ability to increase the level of GSH, inhibit lipid peroxidation and decrease prooxidant compounds levels. The obtained results attest the reasonability of further search for hepatoprotective agents among sulfur-containing pteridine derivatives.

Keywords: pteridines; inhibition of linoleic acid peroxidation; determination of glutathione in liver homogenate; hepatoprotective activity; free-radical scavenging activity; tetrachloromethane hepatitis in rats.

Introduction

Liver diseases are among the major threats to public health and a serious problem for national health systems around the world (Cheemerla & Balakrishnan, 2021). Infectious hepatitis, inherited metabolic disorders, exposure to various chemicals, and excessive alcohol consumption are the causes of the increasing incidence of acute and chronic liver failure (Mosca et al., 2024). The vascular, toxic, immune and hormonal mechanisms of liver damage have been well described (Edwards & Wanless, 2013). Recently it has been shown that oxidative and nitrosative stress play a key role in development of liver`s pathological states (Allameh et al., 2023). Additionally, the high importance of the liver in detoxification of xenobiotics and protection against toxic radicals has been stated (Görg et al., 2013; Cichoż-Lach & Michalak, 2014; Ramachandran & Jaeschke, 2018). During ischemia, liver cells undergo apoptosis and necrosis, which lead to platelet and neutrophil adhesion, releasing of bioactive substances (nitric oxide (NO), reactive oxygen species (ROS), cell adhesion molecules (ICAM-1), cytokines (IL), lysosomal proteases, calpains (CANP1), interferon-γ, etc.). The abovementioned processes result in activation of other cells, including Kupffer cells and T-lymphocytes (Edwards & Wanless, 2013). ROS and NO play a significant role in Kupffer cell activation and TNF-α release by many cell types. TNF-α induces the release of cytokines and chemokines, expression of endothelial adhesion molecules, which causes the accumulation and activation of neutrophils, increasing local damage to hepatocytes. Despite the high diversity of factors that initiate the above listed processes, there are a few final results including hepatocyte death, which in severe cases leads to liver cirrhosis.

Hepatotropic therapy is increasingly prescribed for treatment of liver diseases (Osodlo et al., 2022). The choice of hepatopro-tective medicines on the pharmaceutical market is rather wide and includes both natural and synthetic agents (Ilyas et al., 2016; Neha et al., 2019; Saurabh & Shubham, 2019). The most used active ingredients are bioflavonoids, phosphatidylcholines, thiol donors, natural amino acids, ursodeoxycholic acid, synthetic hepatoprotectors, inhibitors of the functional activity of Kupffer cells, and drugs with an indirect hepatoprotective effect (Osodlo & Fedorova, 2016). The most popular drugs in clinical practice are silymarin,

phosphatidylcholine, ursodeoxycholic acid, and natural amino acids. Pharmacological properties of abovementioned drugs are associated with enhancing the neutralizing function of hepatocytes via increasing the glutathione, taurine, and sulfates reserves. Activation of enzymes involved in the oxidation of xenobiotics, inhibiting of lipids peroxidation, scavenging of lipids peroxidation products, stabilization and repairing of membranes could be considered as additional mechanisms of the hepatoprotective drugs effects.

Sulfur-containing compounds namely glutathione (GSH), S-adenosylmethionine (SAM), acetylcysteine, methionine, and others are important among hepatotropic agents (Honda et al., 2017; Colovic et al., 2018; Vairetti et al., 2021). These compounds are detoxifiers of endogenous metabolites in the liver, inhibitors of organic substances free radical oxidation. Also, effects include lowering of blood ALT and triglycerides, increasing of membrane polarization, improvement of the of membrane-bound transport systems functioning, etc. Their use is reasonable for treatment of chronic alcoholic liver disease, chronic drug-induced and viral hepatitis, especially in the presence of cholestasis syndrome. It should be noted that despite the widespread use of the hepatoprotective agents for the treatment of liver diseases, the results obtained in experimental animal models are not always consistent with the results of clinical trials. Thereby, this group of drugs does not solve the problem of liver function stimulation, total protection of the organ and promotion of hepatocytes regeneration. The choice of pteridines as objects of the studies aimed at the search of a novel hepatoprotective agents is reasonable due to their previously described biological effects (Oettl & Reibnegger, 2002; Pontiki et al., 2015). Pteridines are involved in processes of hepatocytes redox state maintained through the proviision of homocysteine metabolism for glutathione synthesis (GSH) (Blom & Smulders, 2011; Lan et al., 2018).

5-Methyltetrahydrofolate (5-MTHF) and homocysteine are substrates of methionine synthase for biosynthesis of endogenic methionine which is S-adenosyl methionine (SAM). Since folic acid maintains normal concentrations of homocysteine, methionine, and SAM its deficiency disrupts the methionine metabolism and leads to hyperhomocysteinemia and SAM depletion (Blom & Smulders, 2011). Biological roles of pteridine derivatives actualize the studies aimed at the structural modification of the mentioned above heterocyclic scaffold as a route to novel hepatoprotective and antioxidant agents.

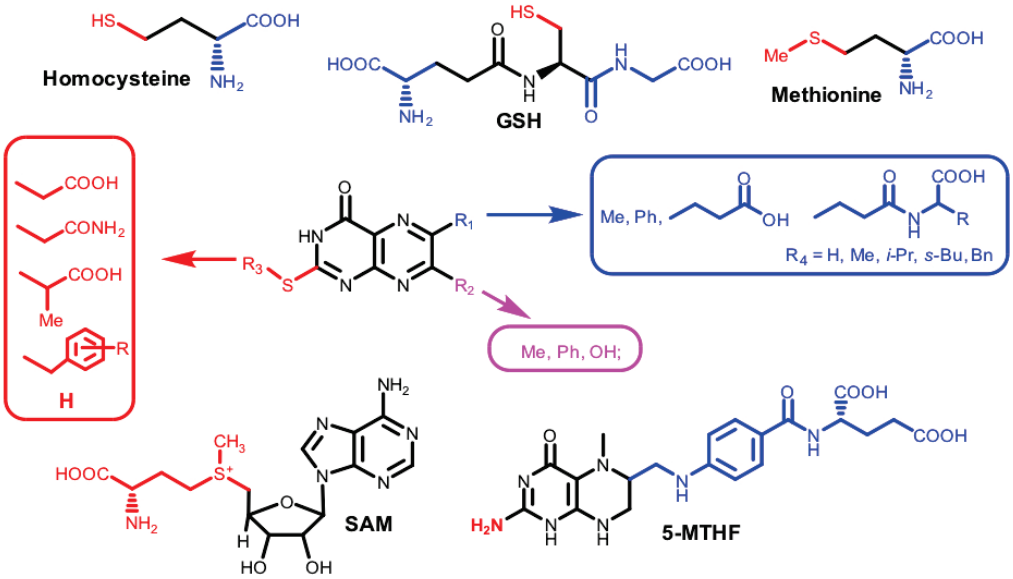

Fig. 1. Design of pteridine-containing hepatoprotective agents

Among promising directions of pteridine modification studies should be noted introduction of sulfur-containing moieties and fragments similar to SAM-cycle substrates as carriers of hepatoprotective and antioxidant properties (Fig. 1).

Thus, the present work is devoted to the evaluation of the prospects for application of S-substituted 6-R_1-7-R_2-2-mercapto-2,3-dihydropteridine-4(1*H*)-ones for the treatment of toxic liver damage using *in silico*, *in vitro* and *in vivo* methods.

Materials and methods

Synthesis and physicochemical properties. The synthesis and physicochemical properties which were described earlier (Kazunin et al., 2022) are shown in Figure 2.

Toxicity studies. The ProTox-II site (https://tox-new.charite.de/ protox_II/ index.php?site=compound_input) was used to predict the toxicity criteria of the molecules (Banerjee et al., 2018). It incorporates molecular similarity, fragment propensities and machine-learning, based on a total of 33 models for the prediction of various toxicity endpoints such as acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcomes (Tox21) pathways and toxicity targets.

SwissADME-analysis. The SwissADME site was used to calculate physicochemical descriptors, as well as to predict ADME parameters, pharmacokinetic properties, and drug similarity. The basic approaches and basic methodology of SwissADME, as a free web-based tool for evaluating pharmacokinetics and drug-likeness, are described in recent publications (Daina et al., 2014; Daina & Zoete, 2016; Daina et al., 2017).

Molecular docking studies. Validation of docking methodology was conducted by re-docking of native N-[4-(3-chloranyl-4-cyanophenoxy)cyclohexyl]-1,1,1-tris(fluoranyl)methane-sulfonamide (PDB ID 5A5I) to CYP2C9 (Skerratt et al., 2016). Root-mean-square deviation values (RMSD) for native and reference conformation were calculated with ProFit Results online resource. Estimated RMSD value was 1.967 Å, what proved reproducibility of experimental data.

The structures of studied compound were drawn with BIOVIADraw 2021 software and saved in mol format. The structure was optimized with Chem3D software using MM2 molecular mechanics algorithm. Optimized structures were saved as pdb files. The later were converted to pdbqt format using AutoDockTools-1.5.6 software with default torsions (Trott & Olson, 2010).

The molecules of water and ligands were deleted from macromolecule model using Discovery Studio Visualizer 2021. The protein structure was saved in pdbqt formate (Discovery Studio Visualizer v19.1.018287. Accelrys Software Inc., www.3dsbiovia.com). Polar hydrogens were added using AutoDockTools-1.5.6 and modes were saved in pdbqt format. The size of the Grid box and its center were set according to data about the location of the native ligand. The size of the Grid box: CYP 2C9 (PDB ID 5A5I) x = 71.14, y = 1.59, z = –1.77; size x = 18, y = 22, z = 16. Vina was used to carry docking (Trott & Olson, 2010). For visualization Discovery Studio 2021 was used.

Inhibition of linoleic acid peroxidation. 30 µL of the 10 mM solution of studied compound in DMSO and 30 µL of the 16 mM sodium linoleate in 0.05 M phosphate buffer (pH 7.4) were added to the quartz cuvette containing 2.79 mL of 0.05 M phosphate buffer (pH 7.4) prethermostated

at 37 °C. The oxidation reaction was initiated at 37 °C under air by the addition of 150 μL of 40 mM 2,2'-azo-bis(2-methylpropionamidine)dihydrochloride (AAPH) solution (Vlachou et al., 2023). The formed mixture was held at 37 °C for 1 hour. The optical density was recorded at λ = 234 nm using ULab 108 UV spectrophotometer. Trolox (CAS Number: 53188-07-1, Merck) was used as reference compound. The antioxidant activity was calculated using formula:

$$AOA\% = ((OD_{control} - OD_{experiment})/OD_{control})*100\%.$$

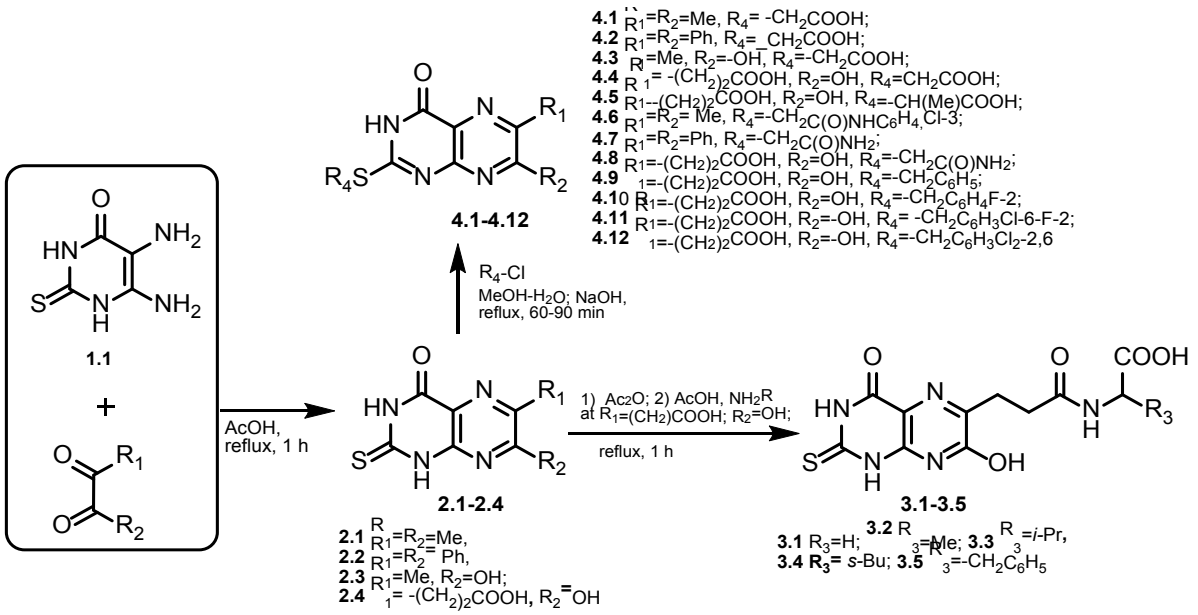

Fig. 2. Synthesis of S-substituted 6-R_1-7-R_2-2-thioxo-2,3-dihydropteridin-4(1*H*)-ones

In vivo study of hepatoprotective activity. Effects of the studied compounds on glutathione content in liver homogenate under tetrachloromethane-induced hepatitis conditions were evaluated on adult male rats (6–8 months old) of Wistar line weighing 220–350 grams. The rats were kept under standard vivarium conditions (temperature 20 ± 5 °C, humidity 65 ± 5%). The rats were kept on a standard diet with free access to water and food, under conditions of natural day and night change. Animal care and experimental protocols were carried out in accor-dance with the requirements of the Directive of the European Council of November 24, 1986 for the care and use of laboratory animals (86/609/EEC), the ethical principles of animal experiments adopted by the First National Congress of Ukraine on Bioethics (2001), international agreements and legislation of Ukraine in this area, were approved by the ethics committee, as well as in accordance with Directive 2010/63/EU of the European Parliament (European Convention, 1986). The study involved 36 rats divided into six equal groups, previously adapted to the experimental conditions. Each group contained six laboratory rats in polyacrylic cages. The experimental procedures were performed in the laboratory of the Faculty of Biology of Zaporizhzhia National University.

Experimental groups. To study the hepatoprotective activity, the rats were divided into the following groups of 6 animals each. Group I (intact animals) was injected intraperitoneally with saline (0.9% NaCl) at a single dose of 0.83 ± 0.05 mL for 14 days. Group II–VI (experimental groups) – experimental hepatitis was modelled by subcutaneous injection of tetrachloromethane (TCM) at a dose of 0.8 mL/100 g of body weight. TCM was administered as a 50% oil solution once daily for 2 days. The dosage of TCM, which was administered in the course of the experiment, was determined on the basis of the results obtained in previously conducted research (Groma et al., 2023). Group II (control pathology) – experimental TCM-hepatitis. Groups III–VI (experimental groups) – received orally once a day for 14 days an aqueous suspension of the synthesized compounds 3.1, 3.2, 4.3 and 4.5 at a dose of 1/50 LD_{50} (of the predicted value) in a 1% aqueous solution of TWIN-80 against the backdrop (of background) of experimental TCM hepatitis.

Sample preparation for biochemical analysis. Decapitation was performed on day 15 using ether anaesthesia from 9:00 to 11:00 am, at the end of the experiment. The liver homogenate was obtained by grinding 500 mg of liver tissue (homogenization) in 5 mL of 3 M perchloric acid solution in a glass Potter-Elwehm homogenizer (GPE Scientific,United Kingdom) under constant cooling in an ice bath (4 °C). The liver homogenate (10% w/v) was centrifuged at 5000 rpm for 15 min in a centrifuge angle rotor at 4 °C to remove unhomogenised and dead cells. The resulting protein-free supernatant was neutralised with 2 M potassium carbonate and centrifuged at 10000 rpm for 30 min at 4 °C.

The concentration of total glutathione (tGSH) and glutathione disulfide (GSSG) was measured according to the method previously described by Rahman et al. (2006). The experimental mixture for the determination of total glutathione contained: 20 μL of liver homogenate (diluted), 60 μL of DTNB (2 mg/3 mL) (5,5`-dithiobis-(2-nitrobenzoic acid), 99% (Acros Organics, Geel, Belgium), 60 μL of GR (10 U/3 mL) (Glutathione reductase (GR) from baker's yeast (Sigma-Aldrich, St. Louis, MO, USA); and 60 μL of NADPH (2 mg/3 mL). The decrease in absorbance was recorded spectrophotometrically at 412 nm. The concentration of glutathione (GSH) was calculated by subtracting the values from total glutathione and glutathione disulfide (GSSG) and expressed as nM/mg of proteins.

The concentration of glutathione disulfide (GSSG) was measured according to the method previously described by Rahman et al. (2006). The reaction assay included: 100 μl of liver homogenate (diluted) mixed with 2 μL of 2-vinylpyridine. The sample was incubated with stirring for 60 min, and then 6 μl of triethanolamine, 60 μL of DTNB (2 mg/3 mL), 60 μL of GR (10 U/3 mL) and 60 μL of NADPH (2 mg/3 mL) were added. The changes in absorbance were recorded at 412 nm. The concentration of oxidised glutathione was expressed in nM/mg protein and was obtained from the standard GSSG curve.

Statistical analysis. The results were statistically analyzed using the Tukey Test. The data are presented as mean value ± standard deviation.

Results

Effect of the synthesized compound on liver functionality. Recently it has been shown that water-soluble disodium 3-(2-((carboxylatomethyl) thio)-4,7-dioxo-3,4,7,8-tetrahydropteridin-6-yl)propanoate reveals a hepatoprotective activity. According to experimental data, the abovementioned compound inhibits cytolysis processes, maintains protein synthesis and improves the detoxification function of the liver under conditions of tetrachloromethane-induced hepatitis in animals. It has been assumed that hepatoprotective activity of the studied compound is associated with its antioxidant properties. According to this hypothesis, the studied compound protects hepatocytes in conditions of severe oxidative stress that are initiated by tetrachloromethane.

Considering the preliminary studies results and continuing the purposeful search for a hepatotropic agent, we evaluated the hepatoprotective potential of the wider range of S-substituted 6-R_1-7-R_2-2-mercapto-2,3-dihydropteridin-4(1*H*)-ones using *in silico*, *in vitro* and *in vivo* methods (Figs. 1, 2). It`s known that requirements for a promising hepatoprotective agent include sufficient absorption, the presence of a "first pass" effect through the liver, a pronounced ability to bind or prevent the formation of highly active radicals, lack of toxicity, etc. Thus, the efficiency and safety of hepatotropic agents depend on structural (physicochemical), pharmaceutical and biological factors that determine their pharmacodynamic and pharmacokinetic properties.

Toxicity studies. At the first stage of the study, we used the ProTox-II online website to predict toxicity parameters (Table 1). The toxicity indexes were determined according to the Globally Harmonised System of Labelling of Chemicals (GHS). Highly active substrates (GSH, SAM, F, and 5-MTHF) were included to the study to improve the prediction procedure. It was estimated that most of the compounds belong to the III–V class of toxicity when administered orally. It should be mentioned that hepatotoxic properties were predicted for some of the obtained compounds (2.1–2.4, 4.1, 4.2, 4.5, 4.7–4.11). However, the abovementioned structures have not been identified as immunotoxins, mutagens, or cytotoxic agents. It has been noticed that GSH has a mutagenic effect according to the prognosis results. Compounds (2.1–2.3, 4.1, 4.6, 4.7, 4.10) were excluded from experiments on animals due to the predicted cancero-genic properties.

SwissADME-analysis. "Drug-like" criteria are important in the drug discovery process. The abovementioned characteristics affect the pharmacokinetics and consequently pharmacological activity and efficacy of the investigational drugs. Inappropriate values of "drug-like" cause a risk of low bioavailability of possible active pharmaceutical ingredient (API). "Drug-like" criteria of the 21 of studied compounds presented in Table 2. Virtual screening results revealed that compounds 2–4 meet the Lipinski rules. SAM, F and 5-MTHF due to the n-ROTB (>10) and n-HBD (>5) criteria don't meet the Lipinsky rules. Compounds 2–4 have satisfactory parameters of the predicted n-octanol-water distribution (LogP ≤ 5). Most of the studied compounds don't meet the Veber and Egan rules according to the TPSA (> 140 Å²) value. The abovementioned criteria indicate a low ability to penetrate the blood-brain barrier and flexibly interact with the macromolecular target. However, compounds 2–4 have a significant n-HBA, which indicates the possibility of formation of hydrogen bonds with the amino acid residues in the active site of the target protein.

Table 1
The prediction of substances' toxicity in program ProTox-II

Compounds*	Oral toxicity		Prediction: active, probability from##				
	Toxicity Index**	LD_{50}, mg/kg#	HT	CG	IT	MG	CT
GSH	5	5000	no	no	no	yes	no
SAM	5	3320	no	no	no	no	no
F	5	10000	no	no	no	no	no
5-MTHF	5	5000	no	no	no	no	no
2.1	2	19	yes	yes	no	no	yes
2.2	4	1800	yes	yes	no	no	no
2.3	4	837	yes	yes	no	no	no
2.4	4	383	yes	no	no	no	no
3.1	3	135	no	no	no	no	no
3.2	3	135	no	no	no	no	no
3.3	5	3000	no	no	no	no	no
3.4	5	3000	no	no	no	no	no
3.5	4	1600	no	no	no	no	no
4.1	2	19	yes	yes	no	no	no
4.2	3	1800	yes	no	no	no	no
4.3	4	1000	no	no	no	no	no
4.4	3	300	no	no	no	no	no
4.5	3	300	yes	no	no	no	no
4.6	4	1171	no	yes	no	no	no
4.7	3	1880	yes	yes	no	no	no
4.8	3	300	yes	no	no	no	no
4.9	4	1000	yes	no	no	no	no
4.10	4	1000	yes	yes	no	no	no
4.11	4	1000	yes	no	no	no	no
4.12	4	1000	no	no	no	no	no

Notes: * – GSH – Glutathione, SAM – S-adenosylmethionine, F – Folic acid, 5-MTHF – Methylenetetrahydrofolate; ** – Class I: fatal if swallowed ($LD_{50} \leq 5$); Class II: fatal if swallowed ($5 < LD_{50} \leq 50$); Class III: toxic if swallowed ($50 < LD_{50} \leq 300$); Class IV: harmful if swallowed ($300 < LD_{50} \leq 2000$); Class V: may be harmful if swallowed ($2000 < LD_{50} \leq 5000$); Class VI: non-toxic ($LD_{50} > 5000$); # – experimental toxicity (LD_{50}) for compounds 4.4 and 4.5 is 2830 and 1420 mg/kg; ## – Toxicity Model Report illustrates the confidence of positive toxicity results compared to the average of its class in HT (Hepatotoxicity), CG (Carcinogenicity), IT (Immunotoxicity), MG (Mutagenicity) and, CT (Cytotoxicity).

Table 2
Determination of the "Drug-like" criteria of the studied compounds

Compounds*	MW (Da)	n-ROTB	n-HBA	n-HBD	TPSA	logP	Drug-likeness		
							Lipinski	Veber	Egan
rule	< 500	< 10	< 10	≤ 5	< 140. Å²	≤ 5	< 2	< 2	< 2
GSH	307.32	11	6	4	202.07	–3.02	0	2	1
SAM	399.45	7	8	4	212.38	–3.21	1	1	1
F	441.40	10	9	6	213.28	–0.50	2	1	1
5-MTHF	459.46	10	7	7	202.77	–0.48	1	1	1
2.1	208.24	0	4	1	110.33	0.88	0	0	0
2.2	332.38	2	4	1	110.33	2.98	0	0	0
2.3	210.21	0	5	2	130.56	0.31	0	0	0
2.4	268.25	3	7	3	167.86	0.01	0	1	1
3.1	325.30	6	8	4	196.96	–0.55	0	1	1
3.2	337.35	6	7	3	176.73	0.34	0	1	1
3.3	367.38	7	8	4	196.96	0.31	0	1	1
3.4	381.41	8	8	4	196.96	0.62	0	1	1
3.5	415.42	8	8	4	196.96	0.86	0	1	1
4.1	266.28	3	6	2	134.13	0.76	0	0	1
4.2	390.42	5	6	2	134.13	2.84	0	0	1
4.3	268.25	3	7	3	154.36	0.00	0	1	1
4.4	326.29	6	9	4	191.66	–0.32	0	1	1
4.5	340.31	6	9	4	191.66	0.00	0	1	1
4.6	375.83	5	5	2	125.93	2.45	0	0	0
4.7	389.43	5	5	2	139.92	2.35	0	0	1
4.8	325.30	6	8	4	197.45	–0.65	0	1	1
4.9	358.37	6	7	3	154.36	1.59	0	1	1
4.10	376.36	6	8	3	154.36	1.86	0	1	1
4.11	410.81	6	8	3	154.36	2.26	0	1	1
4.12	484.31	9	8	4	183.46	1.96	0	1	1

Notes: * – MW – molecular weight, n-ROTB – number of rotatable bonds, n-HBA – number of hydrogen bond acceptors, n-HBD – number of hydrogen bonds donors, TPSA – topological polar surface area, * – GSH – Glutathione; SAM – S-adenosylmethionine; F – Folic acid; 5-MTHF – Methylenetetrahydrofolate.

Table 3
Pharmacokinetics drug-likeness violations

Compounds*	GI absorption	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	BS*	Lead-likeness
GSH	Low	no	no	no	no	no	0.11	no
SAM	Low	no	no	no	no	no	0.55	no
F	Low	no	no	no	no	no	0.11	no
5-MTHF	Low	no	no	no	no	no	0.11	no
2.1	High	yes	no	no	no	no	0.55	no
2.2	High	yes	no	yes	yes	yes	0.55	yes
2.3	High	no	no	no	no	no	0.55	no
2.4	Low	no	no	no	no	no	0.11	yes
3.1	Low	no	no	no	no	no	0.11	yes
3.2	Low	no	no	no	no	no	0.11	yes
3.3	Low	no	no	no	no	no	0.11	no
3.4	Low	no	no	no	no	no	0.11	no
3.5	Low	no	no	no	no	no	0.11	no
4.1	High	no	no	no	no	no	0.56	yes
4.2	Low	no	yes	yes	no	no	0.56	no
4.3	Low	no	no	no	no	no	0.11	yes
4.4	Low	no	no	no	no	no	0.11	yes
4.5	Low	no	no	no	no	no	0.11	yes
4.6	High	yes	no	yes	no	yes	0.55	no
4.7	Low	no	yes	yes	no	yes	0.55	no
4.8	Low	no	no	no	no	no	0.11	yes
4.9	Low	no	no	no	no	no	0.11	no
4.10	Low	no	no	no	no	no	0.11	no
4.11	Low	no	no	no	no	no	0.11	no
4.12	Low	no	no	no	no	yes	0.11	no

Note: *BS – bioavailability score (rule, F > 0.1).

According to the prediction results, most of the studied substances have the BS on the level of 0.11 units. The exceptions are compounds 2.1–2.3, 4.1, 4.2, 4.6 and 4.7, for which this value is 0.55 units (Table 3). It is surprising that low BS is also predicted for GSH, F and 5-MTHF. The results indicate that the compounds will be sufficiently absorbed orally or have moderate Caco-2 per-meability, at least 10% bioavailability.

Molecular docking study. According to the affinity index, most of the studied compounds were inferior to the index of the reference ligand (>-8.1 kcal/mol) (Table 4). Better indicators (from –8.2 to –8.9 kcal/mol) were predicted for phenyl-, benzyl-, and aryl-substituted derivatives – 2.2, 3.5, 4.2, 4.6, 4.7, 4.10–4.12. It has been estimated that presence of carboxylic group in R1/R4 moiety (2.4, 4.1, 4.3-4.6, 4.8) dramatically decrease the affinity.

The analysis of the nature of the interactions with the amino acids of the active site revealed that the auspicious placing is predicted only for ligands 4.2, 4.7, which matches the SwissADME prediction. These compounds contain phenyl substituents in R1/R2 and – CH_2COOH/ $CH_2C(O)NH_2$ in R4 (Fig. 3a, 3b).

The compatible conformation analysis of the studied and native ligands also confirms the previous conclusion (Fig. 4a). The ligand 4.7 demonstrates deep and spatially identical placement into the active site relatively to the reference drug, with fixation of all fragments of the molecule by amino acid residues that are essential for the affinity. Instead, for most derivatives even with a high affinity value, the inability for deep immersion with fragmentary placement on the surface of the enzyme has been predicted (Fig. 4b, see the ligand 3.5 example).

Table 4
The results of the docking studies of the ligand 2, 3, 4 and the native inhibitor to the active site of CYP450 2C9

Compounds*	Binding energy, kcal/mol	Amino acid residues interaction**
CCCFMS	-8.1	a: Arg108(3), Asn204; b: Phe476, Leu362, Ala297 (2), Val113, Leu366
2.1	-6.5	a: Arg97(2), Ser365; b: Ile112, Val113, Cys435, Val436, Leu366(2), Cys435; c: Cys435 (Pi-Sulfur)
2.2	-8.6	a: Arg108; b: Leu208, Phe114, Leu208, Ile205
2.3	-6.5	a: Ser365(2), Ser429(2); b: Phe428(2), Ser429(2); Arg433, His368, Leu362, Cys435, Leu366, Cys435
2.4	-6.4	a: Arg97(3); b: Val113, Ala297, Cys435(2), Val436
3.1	-7.4	a: Arg97(2), Thr301, Ser365, Leu366(2), Gly437(2), Thr301, Gly298, Thr302, Arg433, Cys435; b: Ala297(2); c: Cys435 (Pi-Sulfur)
3.2	-7.0	a: Thr304, Ala1, Glu300; b: Asn474, Ile205, Val479, Ile205, Ala477(2); c: Glu300 (Pi-Anion)
3.3	-7.3	a: Thr304(2), Thr301, Asn474; b: Ile205(2), Val479, Leu208, Ala477(2); c: Glu300
3.4	-7.0	a: Thr304, Asn474(2), Glu300, Ser209; b: Ile205, Val113, Leu366, Phe114, Ala477(2), Val479; c: Glu300 (Pi-Anion)
3.5	-8.8	a: Arg97, Arg108(2), Arg433; b: Gly296, Val436, Val292, Asp293, Gly296, Ala297, Val113(2), Ala297(2), Ile112; c: Cys435 (Pi-Sulfur)
4.1	-7.5	a: Arg97(4), Cys435, Ser365, Val113; b: Ala297(2), Ile112, Val113(3), Leu294, Val436(3), Cys435(2), Leu366, Ala297; c: Cys435
4.2	-8.7	a: Arg108, Ala297, Asp293(2); b: Ala297(3), Leu366(3), Val113(3), Leu362, Cys435
4.3	-7.4	a: Arg97(3), Ser365, Leu366, Val113; b: Ile112(2), Val113(3), Leu294, Val436(2), Ala297; c: Cys435(2)
4.4	-7.6	a: Arg108, Thr301, Asp293(2), Glu300, Thr304; b: Ile205, Ala297, Ile205, Ala477
4.5	-6.9	a: Arg108, Thr301, Asp293(2), Glu300, Thr304; b: Ile205 (2), Ala297, Ala477
4.6	-8.7	a: Arg97(2), Ser365, Cys435, Pro427, Val113, Ser429, cys435: b: Ala297(4), Ile112, Val113 (3), Leu294(2), Val436(3), Cys435; c: Thr301
4.7	-8.9	a: Arg108, Ala297; b: Leu366(2), Phe114, Phe476, Val113(2), Ala297; c: Arg108 (Pi-Cation)
4.8	-6.8	a: Arg108(2), Ala297, Thr304, Glu300; b: Ile205(2), Ala297
4.9	-7.9	a: Arg108(2), Asn204, Gly296; b: Phe476, Leu208
4.10	-8.2	a: Arg108, Asn204, Val292; b: Ile205, Ala477; c: Glu300 (Electrostatic)
4.11	-8.4	a: Asp293, Glu300; b: Ile205, Ala477, Leu361, Ala297, Ile205, Ala477, Val479
4.12	-8.4	a: Arg108, Gly296; b: Ile205(2), Ala477, Leu361, Ile205, Ala477; c: Glu300

Notes: * – CCCFMS - N-[4-(3-chloranyl-4-cyanophenoxy)–cyclohexyl]-1,1,1-tris-(fluoranyl)methanesulfonamide; ** – a) hydrogen bonds; b) hydrophobic interactions; c) other interactions; () – the amount of the bonds with amino acid residue.

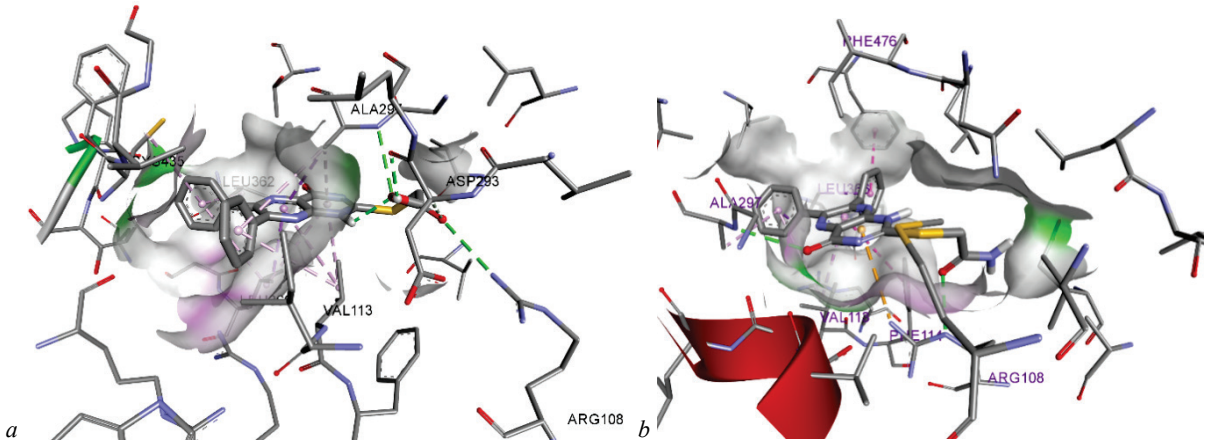

Fig. 3. Visualization of ligands 4.2a and 4.7b interaction with amino acid residues of the CYP 2C9 enzyme active site

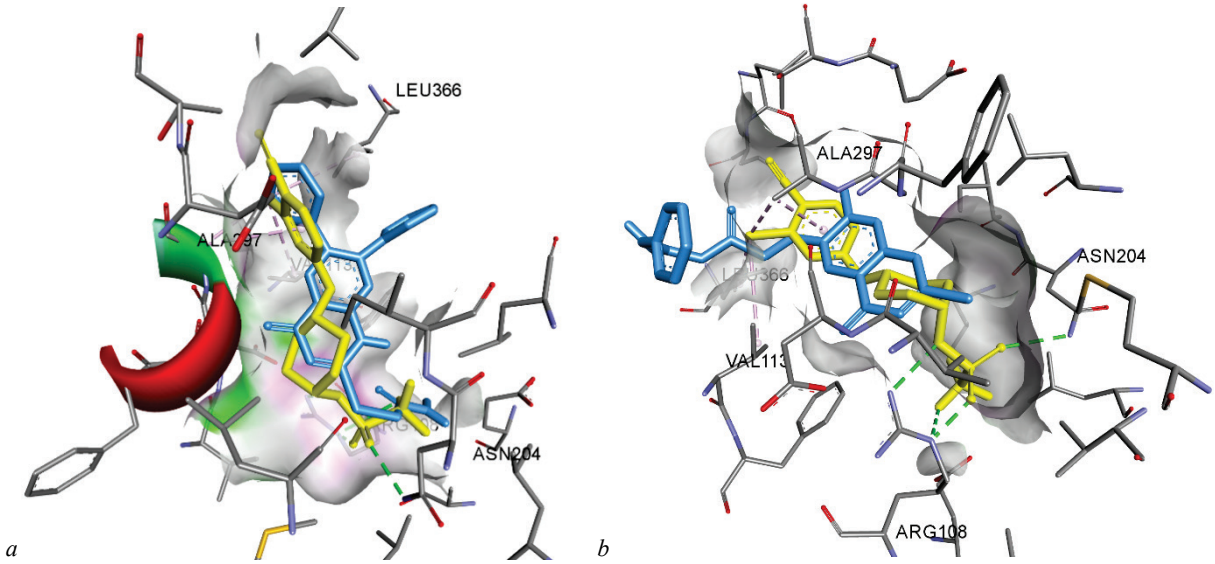

Fig. 4. Compatible conformation of the reference ligand (yellow molecule) and the studied ligands (blue molecules) 4.7a and 3.5b in the active site of CYP 2C9

Hence, according to the molecular docking results, only five ligands from the group 4, namely compounds 4.2, 4.7 and 4.10–4.12 (with R4 = $–CH_2ArHal$) have the probability of CYP 2C9 inhibiting activity. Thus, these compounds are less promising as potential hepatotropic agents and defines the prospects for study of all other synthesized compounds.

Inhibition of linoleic acid peroxidation. The antioxidant activity of obtained compounds has been studied *in vitro* on the linoleic acid peroxidation modes. The choice of the model was caused by the importance of radical scavenging activity or inhibition of radical formation for developing of hepatoprotective activity. It has been estimated that thio-containing pteridines inhibit the peroxidation of linoleic acid. It has been shown inhibitory activity level is determined by lipophilicity of studied compounds.

Thus, 6,7-dimethyl-2-mercapto-2,3-pteridin-4(1*H*)-ones (2.1) with logP = 0.88 reveals AOA on the level 9.5%. Replacement of the methyl group by more lipophilic phenyl fragments (2.2, logP = 2.98) decreases the activity to 5.26%. Structural modification of the molecule by the introduction of the hydroxy-(2.3) or ethylcarboxy-(2.4) groups to the pteridine cycle increases the hydrophilicity (logP = 0.31 (2.3); 0.01 (2.4)) and consequently improves the level of antioxidant activity (18.18% and 25.12% correspondingly.

The conversion of compound 2.4 into amides with aliphatic aminoacids` moieties (3.1–3.5), results in the significant increase of AOA (Table 5). Hydrophilicity – antioxidant activity direct correlation has been observed as well. The high hydrophilicity (logP = –0.55 (3.1); 0.34 (3.2); 0.31 (3.3); 0.62 (3.4); 0.86 (3.5)) provides the high antioxidant activity. Thus, among (3-(7-hydroxy-4-oxo-2-thioxo-1,2,3,4-tetrahydropteridin-6-yl)propanoyl)aminocarboxylic acids (3.1–3.5) AOA decreases in the following sequence: H (3.1) > Me (3.2) > i-Pr (3.3) > s-Bu (3.4) > Bn (3.5). The same patterns were observed for compounds 4.1–4.12 (Table 6). Particularly 6,7-dimethyl-4-oxo-3,4-dihydropteridin-2-yl)thio)acetic acid (4.1, logP = 0.76) and 6,7-diphenyl-4-oxo-3,4-dihydropteridin-2-yl)thio)acetic acid (4.2, logP = 2.84) exhibit antioxidant activity on the levels 12.2% and 14.5% correspondingly). Replacement of the methyl group in 7th position (4.1) by hydroxylic group (4.2) results high hydrophilicity (LgP = 0.00) and ability to inhibit linoleic acid peroxidation (AOA= 34.7%). The hydrophilicity increasing (LogP = –0.32) and the rising of AOA (AOA= 52.4%) have been observed as result of replacing of methyl group (4.3) in 6th position by carboxyethyl group (4.4) yields. The further structural modification of compound 4.4 via replacing of 2-carboxymethyl substituent by benzylthio-(4.9) or substituted benzylthio groups (4.10–4.12) results in the increase of predicted lipophilicity (logP = 1.59 (4.9), 1.86 (4.10), 2.26 (4.11), 1.96 (4.12)) and consequent decrease of AOA from 43.54% to 11.1%. The functionalization of the abovementioned fragment (4.4) to amide (4.8) or its replacement by α-propionic acid (4.5) moiety insignificantly changes lipophilicity and antioxidant activity. (Table 5). It should be noted that presence of one or several proton exchangeable functional groups (2.4, 3.1–3.4, 4.3–4.5, 4.7–4.9) causes high ability to inhibit linoleic acid peroxidation.

Generalization of *in silico* and *in vitro* studies results allows one to state that 8 of the 20 studied compounds (3.1–3.5, 4.3, 4.4, 4.12) keep satisfactory toxicometric characteristics, 7 compounds (2.2, 2,4, 3.1, 3.2, 4.1, 4.3–4.5, 4.8) meet "drug-like" criteria, 10 compounds (3.1–3.4, 4.3–4.5, 4.7–4.9) possess high linoleic acid peroxidation inhibiting activity. Besides all studied compounds except 4.7 have low affinity to CYP2C9

enzyme, which lessens the possibility of unpredictable or side effects and bioavailability lowering in case of peroral administration.

Table 5
Antioxidant activity S-substituted 6-R1-7-R2-2-thioxo-2,3-dihydropteridin-4(1*H*)-ones during the change of radicals

Compounds*	R_1	R_2	R_3	R_4	Antioxidant activity, %
2.1	Me	Me	–	–	9.47
2.2	Ph	Ph	–	–	5.26
2.3	Me	OH	–	–	18.18
2.4	$-(CH_2)_2COOH$	OH	–	–	25.12
3.1	–	–	H	–	49.04
3.2	–	–	Me	–	36.84
3.3	–	–	*i*-Pr	–	38.68
3.4	–	–	*s*-Bu	–	25.00
3.5	–	–	Bn	–	7.89
4.1	Me	Me	–	$-CH_2COOH$	12.20
4.2	Ph	Ph	–	$-CH_2COOH$	14.54
4.3	Me	OH	–	$-CH_2COOH$	34.69
4.4	$-(CH_2)_2COOH$	OH	–	$-CH_2COOH$	52.39
4.5	$-(CH_2)_2COOH$	OH	–	-CH(Me)COOH	50.00
4.6	Me	Me	–	$-CH_2C(O)NHC_6H_4Cl$-3	1.05
4.7	Ph	Ph	–	$-CH_2C(O)NH_2$	31.82
4.8	$-(CH_2)_2COOH$	OH	–	$-CH_2C(O)NH_2$	41.84
4.9	$-(CH_2)_2COOH$	OH	–	Bn	43.54
4.10	$-(CH_2)_2COOH$	OH	–	$-CH_2C_6H_4F$-2	25.12
4.11	$-(CH_2)_2COOH$	OH	–	$-CH_2C_6H_4Cl$-6-F-2	24.47
4.12	$-(CH_2)_2COOH$	OH	–	$-CH_2C_6H_4Cl_2$-2,6	11.05
Trolox*	–	–	–	–	93.61

Note: *– 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

The abovementioned compounds are the most promising for *in vivo* studies on the model of experimental toxic liver damage. Considering the mentioned above facts, compounds 3.1, 3.2, 4.3 and 4.5 were randomly selected for further studies.

Antioxidant activity in vivo. It has been shown that toxic damage to the liver of the rats of II group (control) was accompanied by the decrease of the GSH level by 2.3 times. At the same time, the GSSG – level increased by 2.4 times compared with the intact group. The administration of the studied compounds reliably prevents the depletion of GSH content and decrease of the GSSG level compared with the control group (Table 6). Compound 3.2 has been identified as the most active hepatoprotective agent. Thus, the above mentioned compound increases the GSH content by 81.9% and decreases the level of GSSG by 38.9% compared with non-treated experimental pathology. Hence, preservation of the thiosulfide balance in the cell probably contributes to the decrease of reactive radicals and synthesis of factors which improve the sustainability of hepatocytes. From this point of view antioxidant effect of metabotropic compounds is associated with their energy supply properties.

Discussion

Most of active pharmaceutical ingredients (API) getting into the human body are absorbed and metabolized, which is accompanied by modification of their pharmacological activity and hydrophilicity. Increase in hydrophilicity typically facilitates the excretion of API. API metabolism is mainly proceeds in the liver, where the cytochrome P450 oxygenase system (phase I) converts API into hydrophilic metabolites which determine the pharmacodynamics of the drug and promote their excretion in the urine (Hodgson, 2004). Activity of cytochrome P450 enzymes could be inhibited or induced by API. API structural modifications could result in unpredictable adverse reactions or therapeutic failures. The predicted effects of compounds 2–4 on cytochrome P450 isoenzymes are shown in Table 3. The cytochrome P450 inhibitory activity was not predicted for the studied compounds except 2.2, 4.2, 4.6, 4.7.

Table 6
Effect of CCl_4 and studied compounds on reduced glutathione (GSH) and oxidized glutathione (GSSG) in liver homogenate ($x \pm SD$, $n = 6$)

Marker	Experimental groups of animals					
	Group I (intact animals)	Group II (control pathology)	Group IV (control pathology compound 3.1)	Group VI (control pathology compound 3.2)	Group III (control pathology compound 4.3)	Group V (control pathology compound 4.5)
GSH, nM/mg Protein	58.2 ± 2.2^a	24.8 ± 1.0^b	30.5 ± 1.3^c	45.1 ± 1.7^d	27.9 ± 1.2^e	33.8 ± 1.5^f
GSSG, nM/mg Protein	2.68 ± 0.12^a	6.38 ± 0.22^b	4.90 ± 0.24^c	3.90 ± 0.16^d	5.54 ± 0.24^e	4.57 ± 0.19^c

Note: values with different superscripts in each lines are significantly different by Tukey's multiple comparison procedure ($P < 0.05$).

The molecular docking study of compounds 2–4 towards active site of the CYP2C9 was conducted due to the predicted cytochrome-inhibiting activity of the studied compounds.

CYP2C9 is a membrane-bound hemoprotein that catalyses the oxidative metabolism of structurally diverse molecules and belongs to six clinically important isoforms that metabolize 90% of all drugs. Binding to this enzyme can leads to significant restriction of their oral bioavailability (Kato, 2020). On the other hand, inhibition of the mentioned enzyme increases the possibility of serious side effects and early termination of drug candidate development (Ogu & Maxa, 2000). The binding energy of ligands 2, 3, 4 was evaluated in the site of the CYP2C9 inhibitor – N-[4-(3-chloranyl-4-cyano-phenoxy)cyclohexyl]-1,1,1-tris(fluoranyl)methane-sulfonamide (CCCFMS). It should be mentioned that the abovementioned fragment of macromolecule serves as a binding site for other experimentally determined inhibitors – flurbipofen, S-warfarin, fluvastatin, fluvoxamine, zafirlukast, antifungal imidazole compounds (miconazole, fluconazole) and others (McMasters et al., 2007; Skerratt et al., 2016). According to the affinity index, most of the studied compounds were inferior to the index of the reference ligand (>–8.1 kcal/mol, Table 4). Better indicators (from –8.2 to –8.9 kcal/mol) were predicted for phenyl-, benzyl-, and aryl-substituted derivatives – 2.2, 3.5, 4.2, 4.6, 4.7, 4.10–4.12. It has been estimated that the presence of carboxylic group in R1/R4 moiety (2.4, 4.1, 4.3–4.6, 4.8) dramatically decreases the affinity. The obtained data don't agree with the published results regarding the better binding of hydrophobic molecules containing acidic groups to SYP2C9 (Ogu & Maxa, 2000) and the effect of the molecule ionization degree on the possibility of bonding with key amino acid residues – polar arginine and asparagine (Arg108, Asn204) (Skerratt et al., 2016).

Further the content of glutathione (GSH) and glutathione disulfide (GSSG) in homogenate of the rat's liver was evaluated since the abovementioned compounds' ratio is marker of hepatocyte damage (Vairetti et al., 2021). Moreover, GSH is one of the most important cell redox buffers and an oxidative stress protector. The mechanisms of the antioxidative protection include scavenging of reactive oxygen species (ROS), nitrogen oxide and its derivatives, ensuring the electron transferring chains, neutralization of toxic compounds and safeguarding of DNA, lipids, and proteins. Research indicates that in the control group (Group II) of rats, toxic liver damage leads to a significant decrease in GSH levels, specifically to 2.3 times lower than those observed in the intact group. Concurrently, the GSSG levels increased by 2.4 times. The administration of the studied compounds effectively prevents this depletion of GSH and reduces the GSSG levels compared to the control group, as demonstrated in Table 6. Among the compounds tested, Compound 3.2 emerged as the most potent hepatoprotective agent (Groma et al., 2023). Consequently, it helps maintain the thiol-disulfide balance within cells, likely contributing to a reduction in reactive radicals and promoting the synthesis of factors that enhance hepatocyte resilience. From this perspective, the antioxidant effect of metabotropic compounds is linked to their energy-providing properties.

Conclusion

It has been shown that application of in silico and in vitro methods is reasonable for estimation of S-substituted 6-R1-7-R2-2-thioxo-2,3-dihydropteridin-4(1H)-ones hepatoprotective activity. The abovementioned methods allowed us to select compounds 3.1, 3.2, 4.3 and 4.5 with satisfying

parameters of toxicity, correspondence to the "drug-like" criteria, absence of CYP2C9 inhibiting effects and high antioxidant activity. The conducted *in vivo* study revealed that compounds 3.1, 3.2, 4.3 and 4.5 reduced severity of the hepatotoxic effects caused by the administration of carbon tetrachloride. The hepatoprotective effect of the obtained compounds is based on their ability to increase the level of GSH, inhibition of lipids peroxidation and decrease in prooxidant compounds levels. It has been shown that 3-(7-hydroxy-4-oxo-2-thioxo-1,2,3,4-tetrahydropteridin-6-yl)propanoic acid modified by glycine (3.1) and alanine moieties (3.2) are promising hepatoprotective agents which require in depth research.

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References

Allameh, A., Niayesh-Mehr, R., Aliarab, A., Sebastian, G., & Pantopoulos, K. (2023). Oxidative stress in liver pathophysiology and disease antioxidants. Antioxidants, 12(9), 1653.

Banerjee, P., Eckert, A., Schrey, A. K., & Preissner, R. (2018). ProTox-II: A webserver for the prediction of toxicity of chemicals. Nucleic Acids Research, 46(1), 257–263.

Blom, H. J., & Smulders, Y. (2011). Overview of homocysteine and folate metabolism. With special references to cardiovascular disease and neural tube defects. Journal of Inherited Metabolic Disease, 34(1), 75–81.

Cheemerla, S., & Balakrishnan, M. (2021). Global epidemiology of chronic liver disease. Clinical Liver Disease, 17(5), 365–370.

Cichoż-Lach, H. (2014). Oxidative stress as a crucial factor in liver diseases. World Journal of Gastroenterology, 20(25), 8082.

Colovic, M. B., Vasic, V. M., Djuric, D. M., & Krstic, D. Z. (2018). Sulphur-containing amino acids: Protective role against free radicals and heavy metals. Current Medical Chemistry, 25(3), 324–335.

Daina, A., & Zoete, V. (2016). A BOILED-egg to predict gastrointestinal absorption and brain penetration of small molecules. ChemMedChem, 11(11), 1117–1121.

Daina, A., Michielin, O., & Zoete, V. (2014). iLOGP: A simple, robust, and efficient description of n-octanol/water partition coefficient for drug design using the GB/SA approach. Journal of Chemical Information and Modeling, 54(12), 3284–3301.

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Reports, 7(1), 42717.

Edwards, L., & Wanless, I. (2013). Mechanisms of liver involvement in systemic disease. Best Practice Research Clinical Gastroenterology, 27(4), 471–483.

Görg, B., Schliess, F., & Häussinger, D. (2013). Osmotic and oxidative/nitrosative stress in ammonia toxicity and hepatic encephalopathy. Archives of Biochemistry and Biophysics, 536(2), 158–163.

Groma, N., Berest, G., Antypenko, O., Voskoboinik, O., Kopiika, V., Kovalenko, S., & Shvets, V. (2023). Evaluation of the toxicity and hepatoprotective properties of new S-substituted pteridins. Current Issues in Pharmacy and Medical Sciences, 36(1), 21–26.

Hodgson, E. (2011). A textbook of modern toxicology. John Wiley & Sons, New Jersey.

Honda, Y., Kessoku, T., Sumida, Y., Kobayashi, T., Kato, T., Ogawa, Y., Tomeno, W., Imajo, K., Fujita, K., Yoneda, M., Kataoka, K., Taguri, M., Yamanaka, T., Seko, Y., Tanaka, S., Saito, S., Ono, M., Oeda, S., Eguchi, Y., Aoi, W., Sato, K., Itoh, Y., & Nakajima, A. (2017). Efficacy of glutathione for the treatment of nonalcoholic fatty liver disease: an open-label, single-arm, multicenter, pilot study. BMC Gastroenterology, 17(1), 96.

Ilyas, U., Katare, D. P., Aeri, V., & Naseef, P. P. (2016). A review on hepatoprotective and immunomodulatory herbal plants. Pharmacognosy Reviews, 10(19), 66–70.

Kato, H. (2020). Computational prediction of cytochrome P_{450} inhibition and induction. Drug Metabolism and Pharmacokinetics, 35(1), 30–44.

Kazunin, M., Groma, N., Nosulenko, I., Kinichenko, A., Antypenko, O., Shvets, V., Voskoboinik, O., & Kovalenko, S. (2022). Thio-containing pteridines: Synthesis, modification, and biological activity. Archiv der Pharmazie, 355(12), e2200252.

Lan, X., Field, M. S., & Stover, P. J. (2018). Cell cycle regulation of folate-mediated one-carbon metabolism. Wiley Interdisciplinary Reviews: Systems Biology and Medicine, 10(6), e1426.

Lin, Z., Cai, F., Lin, N., Ye, J., Zheng, Q., & Ding, G. (2013). Effects of glutamine on oxidative stress and nuclear factor-κB expression in the livers of rats with nonalcoholic fatty liver disease. Experimental and Therapeutic Medicine, 7(2), 365–370.

Mardinoglu, A., Bjornson, E., Zhang, C., Klevstig, M., Söderlund, S., Ståhlman, M., Adiels, M., Hakkarainen, A., Lundbom, N., Kilicarslan, M., Hallström, B. M., Lundbom, J., Vergès, B., Barrett, P. H. R., Watts, G. F., Serlie, M. J., Nielsen, J., Uhlén, M., Taskinen, M., & Boren, J. (2017). Personal model-assisted identification of NAD+ and glutathione metabolism as intervention target in NAFLD. Molecular Systems Biology, 13(3), 916.

McMasters, D. R., Torres, R. A., Crathern, S. J., Dooney, D. L., Nachbar, R. B., Sheridan, R. P., & Korzekwa, K. R. (2007). Inhibition of recombinant cytochrome P_{450} isoforms 2D6 and 2C9 by diverse drug-like molecules. Journal of Medicinal Chemistry, 50(14), 3205–3213.

Mosca, A., Manco, M., Braghini, M. R., Cianfarani, S., Maggiore, G., Alisi, A., & Vania, A. (2024). Environment, endocrine disruptors, and fatty liver disease associated with metabolic dysfunction (MASLD). Metabolites, 14(1), 71.

Neha, K., Haider, M. R., Pathak, A., & Shahar Yar, M. (2019). Medicinal prospects of antioxidants: A review. European Journal of Medicinal Chemistry, 178, 687–704.

Oettl, K., & Reibnegger, G. (2002). Pteridine derivatives as modulators of oxidative stress. Current Drug Metabolism, 3(2), 203–209.

Ogu, C. C., & Maxa, J. L. (2000). Drug interactions due to cytochrome P450. Baylor University Medical Center Proceedings, 13(4), 421–423.

Osodlo, H., & Fedorova, O. (2016). Combined liver protection as basis of modern hepatoprotection. Rational Pharmacotherapy, 39, 45–52.

Osyodlo, H., Boichak, M., & Fedorova, O. (2022). Rational choice of hepatoprotectors for drug-induced liver injury. Gastroenterology, 56(3), 179–189.

Pontiki, E., Hadjipavlou-Litina, D., Patsilinakos, A., Tran, T. M., & Marson, C. M. (2015). Pteridine-2,4-diamine derivatives as radical scavengers and inhibitors of lipoxygenase that can possess anti-inflammatory properties. Future Medicinal Chemistry, 7(14), 1937–1951.

Rahman, I., Kode, A., & Biswas, S. K. (2006). Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. Nature Protocols, 1(6), 3159–3165.

Ramachandran, A., & Jaeschke, H. (2018). Oxidative stress and acute hepatic injury. Current Opinion in Toxicology, 7, 17–21.

Saurabh, N., & Shubha, M. (2019). Pharmacological strategies on medicinal plants as hepatoprotective agents. Acta Scientific Pharmaceutical Sciences, 3(7), 9–14.

Skerratt, S. E., de Groot, M. J., & Phillips, C. (2016). Discovery of a novel binding pocket for CYP 2C9 inhibitors: Crystallography, pharmacophore modelling and inhibitor SAR. MedChemComm, 7(5), 813–819.

Thong-Ngam, D., Samuhasaneeto, S., Kulaputana, O., & Klaikeaw, N. (2007). N-acetylcysteine attenuates oxidative stress and liver pathology in rats with nonalcoholic steatohepatitis. World Journal of Gastroenterololy, 13(38), 5127–5132.

Trott, O., & Olson, A. J. (2009). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of Computational Chemistry, 31(2), 455–461.

Vairetti, M., Di Pasqua, L. G., Cagna, M., Richelmi, P., Ferrigno, A., & Berardo, C. (2021). Changes in glutathione content in liver diseases: An update. Antioxidants, 10(3), 364.

Vlachou, E., Pontiki, E., Hadjipavlou-Litina, D. J., & Litinas, K. E. (2023). Synthesis and biological evaluation of substituted fused dipyranoquinolinones. Organics, 4(3), 364–385.

Zhou, X., Han, D., Xu, R., Wu, H., Qu, C., Wang, F., Wang, X., & Zhao, Y. (2016). Glycine protects against high sucrose and high fat-induced non-alcoholic steatohepatitis in rats. Oncotarge, 6, 80223–80237.