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Hypolipidemic activity in a series of new hybrids of orotic acid and 1,2,4-triazole

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Cardiovascular diseases associated with hyperlipidemia remain one of the leading causes of morbidity and mortality worldwide. Therefore, the search for new hypolipidemic agents with improved efficacy and safety profiles remains an important task of modern pharmacology. The aim of this study was to evaluate the hypolipidemic and antiatherosclerotic activity of sodium 5-(2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-4-ethyl-4H-1,2,4-triazole-3-thiolate (KP-372), a new hybrid derivative of orotic acid and 1,2,4-triazole, in a rat model of alimentary hyperlipidemia. Hyperlipidemia was induced in male rats by dietary administration of 4% cholesterol, 0.12% 6-methyluracil, and 25% sunflower oil for 14 days. Four groups of 10 animals each were formed: KP-372-treated, atorvastatin-treated, placebo, and intact. In the placebo-treated rats, the serum lipid profile showed clear dyslipidemia, with cholesterol 1.84 ± 0.08 mmol/L, triglycerides 0.81 ± 0.03 mmol/L, low-density lipoproteins 0.52 ± 0.03 mmol/L, and very low-density lipoproteins 0.36 ± 0.06 mmol/L versus 1.29 ± 0.09, 0.44 ± 0.04, 0.40 ± 0.03, and 0.20 ± 0.02 mmol/L, respectively, in intact animals. Administration of KP-372 improved these indices to 1.49 ± 0.09 mmol/L for cholesterol, 0.53 ± 0.05 mmol/L for triglycerides, 0.39 ± 0.08 mmol/L for low-density lipoproteins, and 0.23 ± 0.02 mmol/L for very low-density lipoproteins. In the atorvastatin group, the corresponding values were 1.50 ± 0.12, 0.44 ± 0.03, 0.52 ± 0.03, and 0.20 ± 0.02 mmol/L. Thus, KP-372 showed activity comparable to atorvastatin and a more favorable effect on low-density lipoproteins. Morphologically, placebo-treated animals demonstrated the most severe hepatic lesions with vacuolar fatty degeneration, necrobiotic changes, vascular congestion, and cellular infiltration, whereas KP-372 treatment was associated with milder changes, mainly granular and ballooning degeneration. Molecular docking showed a higher predicted affinity of KP-372 for pancreatic lipase than for 3-hydroxy-3-methylglutaryl-coenzyme A reductase, with binding energies of –7.260 and –5.136 kcal/mol, respectively, supporting a mechanism more closely related to modulation of dietary lipid digestion and absorption than to a classical statin-like pathway. These findings indicate that KP-372 is a promising hypolipidemic agent.

Keywords: hyperlipidemia; lipid metabolism disorders; experimental rats; lipid profile; pancreatic lipase inhibition; molecular docking; histopathology.

Introduction

Disorders of lipid metabolism are among the key pathogenetic links in the development of atherosclerotic lesions, metabolic liver abnormalities, cardiovascular pathology, and a range of related diseases (Martin et al., 2022). Control of total cholesterol, triacylglycerols, atherogenic lipoprotein fractions, and associated metabolic disturbances is therefore regarded as an important objective of modern pharmacology and veterinary medicine. In this context, the search for new biologically active compounds with hypolipidemic and potentially antiatherosclerotic properties, capable of affecting endogenous lipid synthesis as well as lipid metabolism, transport, or absorption, remains highly relevant.

Studies on the biological activity of 1,2,4-triazole derivatives indicate a broad spectrum of potential applications for this class of compounds (Aggarwal & Sumran, 2020). The 1,2,4-triazole ring is widely recognized as a privileged scaffold in medicinal chemistry, and numerous derivatives containing this heterocycle have been reported to exhibit antibacterial, antifungal, antiviral, anti-inflammatory, analgesic, antiparasitic, anticonvulsant, and other types of pharmacological activity. Owing to this multifunctionality and its favorable physicochemical and pharmacophoric features, the 1,2,4-triazole nucleus is considered a promising structural fragment for the development of new metabolically active agents.

At the same time, considerable attention has been paid to orotic acid derivatives and structurally related 2,6-dioxopyrimidine systems (Harden & Robinson, 1984). The orotate-related fragment is of particular interest as a structural element biogenetically related to natural pyrimidine metabolites and capable of influencing intracellular biochemical processes. Earlier studies showed that dietary orotic acid can alter lipid and lipoprotein metabolism, producing hypocholesterolemic effects in some experimental settings while also affecting hepatic lipid handling. These findings support the view that the orotic acid / pyrimidinedione motif is relevant to the search for new lipid-modulating agents.

The combination of such a fragment with a 1,2,4-triazole core appears to be a rational approach to the design of new hybrid molecules in which pronounced heterocyclic pharmacophoric properties may be combined with the potential to modulate metabolic processes, including those associated with lipid metabolism. Available literature indicates that both 1,2,4-triazole-based systems and orotate- or pyrimidinedione-related heterocycles may serve as promising platforms for the development of novel hypolipidemic agents (Idrees et al., 2009; Chhabria et al., 2011; Ma et al., 2012; Irshad et al., 2021). Representative compounds from these structural directions, for which lipid-lowering or lipid-modulating effects have been described, are shown in Figure 1. Thus, the available literature data support further exploration of new hybrid derivatives in which the combination of 1,2,4-triazole and orotate-/pyrimidinedione-related fragments may give rise to a new class (Karpenko et al., 2025a, 2025b) of biologically active compounds with potential hypolipidemic activity.

In veterinary practice, 1,2,4-triazole-containing drugs have already found practical application, and this scaffold is regarded as promising for further pharmacological investigation and implementation in veterinary medicine, including in Ukraine (Parchenko et al., 2024). At the same time, in a number of animal pathologies, particularly liver diseases, cardiovascular disorders, and metabolic disturbances, the use of agents capable of correcting cholesterol and lipoprotein levels

is justified. The dedicated veterinary arsenal for such indications remains limited, and in many cases approaches from human medicine are adapted. Therefore, expanding the spectrum of pharmacological activity of 1,2,4-triazole derivatives and creating accessible domestic agents that could compete with existing products remains an important and timely task (Ohloblina et al., 2022).

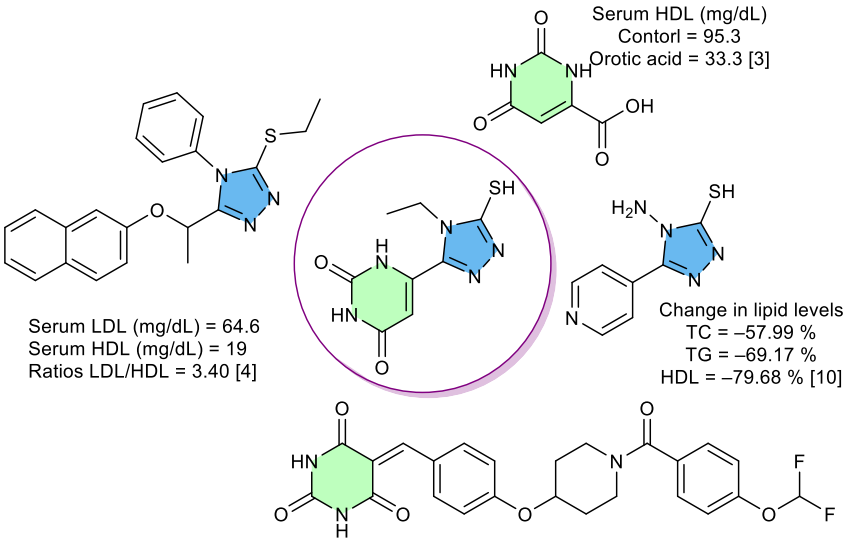

Fig. 1. Literature examples of compounds containing orotic acid, 1,2,4-triazole, or related heterocyclic fragments that exhibit hypolipidemic or lipid-modulating activity, together with the structure of the studied compound

Therefore, the search for new hybrids of orotic acid and 1,2,4-triazole as potential correctors of lipid metabolism is scientifically justified and promising. The combination of two pharmacologically significant heterocyclic fragments within a single molecule may create the prerequisites for a complex biological effect, including hypolipidemic and antiatherosclerotic activity (Cao et al., 2025; Siegel et al., 2025).

The aim of this study was to investigate the hypolipidemic and antiatherosclerotic activity of a new original hybrid derivative of orotic acid and 1,2,4-triazole under conditions of experimental alimentary hyperlipidemia.

Materials and methods

Chemical part. The object of the study was the original compound KP-0372, sodium 5-(2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-4-ethyl-4H-1,2,4-triazole-3-thiolate, which is the sodium salt of the corresponding 3-thiol derivative of 1,2,4-triazole. By its chemical nature, KP-0372 belongs to hybrid heterocyclic systems combining a 1,2,4-triazole fragment with a 2,6 dioxopyrimidine ring structurally related to orotic acid derivatives. For the anhydrous form of the compound, the molecular formula is $C_8H_8N_5NaO_2S$, and the relative molecular mass is 261.24.

Compound KP-0372 was obtained from the corresponding 3-thiol precursor by reaction with an equimolar amount of sodium hydroxide in an aqueous or aqueous-alcoholic medium, followed by isolation and drying of the target product to constant weight. For the pharmacological study, a freshly prepared 1% aqueous solution of the substance was used.

The individuality and purity of the compound were controlled by generally accepted physicochemical analytical methods. The structure of the substance was confirmed by elemental analysis, 1H NMR spectroscopy, and mass spectrometry (Karpenko et al., 2025c). The obtained substance was a crystalline powder soluble in water, which made it possible to use it in the form of an aqueous solution for intragastric administration to experimental animals.

Biological part. The study was carried out at the Educational and Scientific Laboratory of Poltava State Agrarian University and at the Centralized Biochemical Laboratory of the 4th City Clinical Hospital of Poltava.

The experiment was conducted in accordance with the methodological recommendations for preclinical studies of medicinal products.

Laboratory animals, namely white male rats weighing 200–240 g, were used in the experimental study. Four experimental groups were formed, with 10 animals in each group. Hyperlipidemia was induced in animals of the first, second, and third experimental groups. For this purpose, their basic diet was supplemented with cholesterol (4%), 6-methyluracil (0.12%), and sunflower oil (25%) for 14 days. Rats of the fourth experimental group remained intact. At the same time, animals of the first experimental group received a 1% aqueous solution of KP-0372 orally (by intragastric administration) at a dose of 0.5 mL at 48-hour intervals. Animals of the second experimental group were administered intragastrically the reference drug, namely a 1% aqueous solution of atorvastatin, at a dose of 0.5 mL at 48-hour intervals. Animals of the third experimental group were given physiological saline intragastrically at a dose of 0.5 mL at 48-hour intervals.

The experimental animals were kept under clinical observation. On day 15 of the experiment, after 18 hours of fasting, the animals were withdrawn from the study and blood samples were collected. In blood serum, the levels of cholesterol, triglycerides, high-density lipoproteins (α-lipoproteins), low-density lipoproteins (β-lipoproteins), very low-density lipoproteins, and the atherogenic index were determined.

Lipid profile parameters were measured using a Sapphire-400 biochemical analyzer (Japan) with reagent kits manufactured by Human (Germany).

Necropsy of the rats was also performed, and liver samples were collected for histological examination carried out according to generally accepted methods. Fragments of the sampled organs were fixed in 10% neutral formalin followed by washing. The material was then dehydrated in ethyl alcohols of increasing concentration, impregnated with embedding agents, embedded in paraffin, and paraffin blocks were prepared. Sections 3–10 μm thick were cut using an MS-2 sledge microtome. Histological sections were stained with Ehrlich's hematoxylin and eosin. The sections were deparaffinized in xylene for 15–20 min, transferred for 2 min into alcohols of decreasing concentration (96°, 70°), and then placed in distilled water for 2–3 min. After that, the sections were transferred to Ehrlich's hematoxylin for 15 min, then rinsed in running tap water for 5–10 min. If necessary, the sections were differentiated for 3–5 s. Subsequently, the sections were placed in a 0.1% aqueous eosin solution for 3–5 min and briefly rinsed in running water. The sections were dehydrated in alcohols of increasing concentration (70°, 96°), remaining in each portion for 1–2 min. They were then cleared in xylene for 2–3 min and mounted in

Canada balsam. Cell nuclei were stained blue, whereas the cytoplasm acquired a pink-red color.

Microscopic examination of histological sections was performed using a Micromed XS-4130 microscope. Images were captured with a Micromed CCD camera using TSView 6.2.4.5 software. The experimental part of the work was carried out in accordance with the General Ethical Principles for Animal Experiments, approved at the National Congress on Bioethics (Kyiv, 2001), and with the international requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

Molecular docking studies. Molecular docking was performed to explore the possible interactions of the test compound, sodium 5-(2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-4-ethyl-4H-1,2,4-triazole-3-thiolate, with protein targets relevant to hypolipidemic activity. Two enzymes were selected as biologically justified targets: 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), a key rate-limiting enzyme of cholesterol biosynthesis (Ballantyne & Norata, 2025; Cui et al., 2025), and pancreatic lipase, a major enzyme involved in the hydrolysis and intestinal absorption of dietary triglycerides. For docking calculations, the X-ray crystal structures deposited in the Protein Data Bank were used: 1HWK for human HMG-CoA reductase co-crystallized with atorvastatin and 1LPB for the pancreatic lipase–colipase complex inhibited by a C11 alkyl phosphonate (Cao et al., 2025; Jagtap & Paul, 2025; Subramaniyan & Hanim, 2025).

Protein structures were prepared by removing co-crystallized ligands, solvent molecules, and other non-essential heteroatoms, followed by the addition of polar hydrogen atoms and assignment of Kollman charges. The ligand structure was geometry-optimized and converted into the appropriate docking format. Docking simulations were carried out using AutoDock Vina (Eberhardt et al., 2021) within the binding regions defined according to the positions of the co-crystallized inhibitors. For HMG-CoA reductase (PDB: 1HWK, chain A), the grid box center was set to x = 18.31, y = 8.38, z = 15.17 Å, with a box size of 24 × 24 × 22 Å. For pancreatic lipase (PDB: 1LPB, chain B), the grid box center was set to x = 9.82, y = 23.49, z = 50.87 Å, with a box size of 22 × 24 × 24 Å. These search regions were chosen to cover the active sites containing the native ligands observed in the crystal structures. The docking poses were ranked according to binding affinity values predicted by AutoDock Vina, and the best-ranked conformations were further analyzed for hydrogen bonding, hydrophobic contacts, and other non-covalent interactions with catalytically important amino acid residues.

Results

The results of the biochemical analysis of the serum lipid profile in rats are presented in Table 1. The data presented in Table 1 show that animals receiving placebo exhibited significantly increased levels of cholesterol, triglycerides, low-density lipoproteins, and very low-density lipoproteins.

Table 1
Serum lipid profile parameters in experimental rats (x ± SD)

Parameters	First experimental group, n = 10	Second experimental group, n = 10	Third experimental group, n = 10	Fourth experimental group, n = 10
Cholesterol, mmol/L	1.49 ± 0.09^{b}	1.50 ± 0.12^{b}	1.84 ± 0.08c	1.29 ± 0.09^{a}
Triglycerides, mmol/L	0.53 ± 0.05^{b}	0.44 ± 0.03^{a}	0.81 ± 0.03^{c}	0.44 ± 0.04^{a}
HDL, mmol/L	1.32 ± 0.06^{b}	1.28 ± 0.08^{a}	1.32 ± 0.07^{b}	1.27 ± 0.12^{a}
LDL, mmol/L	0.39 ± 0.08^{a}	0.52 ± 0.03^{b}	0.52 ± 0.03^{b}	0.40 ± 0.03^{a}
VLDL, mmol/L	0.23 ± 0.02^{ab}	0.20 ± 0.02^{a}	0.36 ± 0.06^{b}	0.20 ± 0.02^{a}

Notes: different letters within a column indicate statistically significant differences between data sets ($P < 0.05$) according to Tukey's test.

An elevated level of low-density lipoproteins, as compared with intact animals, was also recorded in rats treated with atorvastatin. The obtained lipid profile data suggest that compound sodium 5-(2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-4-ethyl-4H-1,2,4-triazole-3-thiolate exhibits hypolipidemic activity comparable to that of atorvastatin and, in the case of low-density lipoproteins, may even exceed it.

Pathoanatomical examination of rats in the first group (Fig. 2) revealed that the liver was light brown in color and soft in consistency; the cut surface was dry and dull, and small hemorrhages were observed on the lobes. The liver of rats in the second group was unevenly dark brown in color, with hemorrhages on the lobes, had a flabby consistency, protruded beyond the capsule on sectioning, and showed a dull cut surface (Fig. 3).

The liver of rats in the third group (Fig. 4) was unevenly brown in color, flabby, swollen, and showed petechial hemorrhages. In rats of group IV (control group, Fig. 5), the liver was brown, unevenly colored, elastic in consistency, and red on the cut surface.

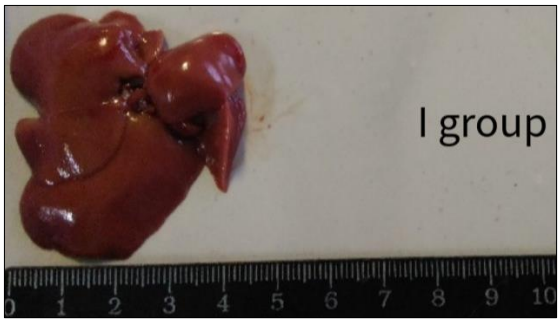

Fig. 2. Liver of a rat from the first group

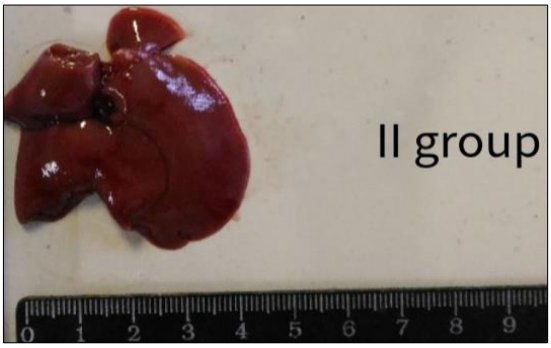

Fig. 3. Liver of a rat from the second group

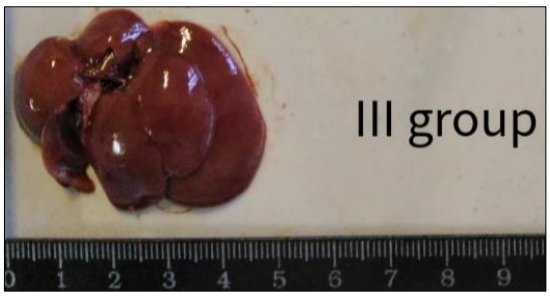

Fig. 4. Liver of a rat from the third group

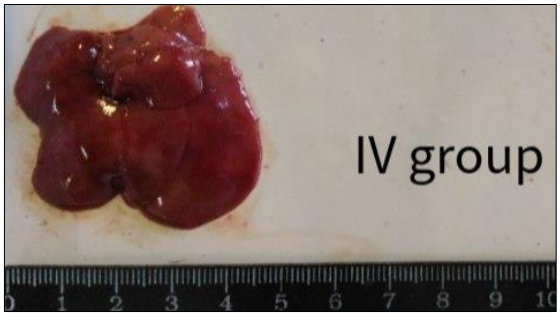

Fig. 5. Liver of a rat from the fourth group

The presented macroscopic photographs of rat livers from all experimental groups demonstrate differences in color, shape, degree of blood filling, and the severity of surface changes. In animals of gro-

ups I and IV, the liver retained a relatively more preserved lobular configuration, smoother contours, and less pronounced signs of circulatory disturbances, whereas in groups II and III more noticeable gross alterations were observed, including uneven coloration, a more intense dark-red or brownish-red color, visible swelling, and an overall impression of tissue congestion. In general, these photographs indicate variability in the macroscopic condition of the liver among the groups and confirm the presence of more pronounced pathological changes in some of the experimental animals, which is consistent with the need for further histological evaluation.

Histological examination of the liver in rats of the first group revealed granular degeneration, characterized by uneven staining of the organ, cytoplasmic cloudiness, the presence of protein granules in the cytoplasm, and enlargement and swelling of hepatocytes. The nuclei were enlarged, and chromatin structures were poorly differentiated. In cases of ballooning degeneration, hepatocytes were enlarged and rounded, lost their polygonal shape, and had a rarefied cytoplasm with heterogeneous granularity. The cells had clearly defined contours, while the nuclei were centrally located and showed signs of pyknosis and karyolysis; binucleated cells were also observed. Remnants of granular cytoplasm were detected around the nuclei or at the periphery of the cell wall. The central veins and portal triads of the liver were congested with blood and showed signs of erythrocyte stasis; cellular infiltrates were observed around the vessels. Dilatation of the lumens of sinusoidal capillaries and their blood filling were also noted (Fig. 6).

In the liver of rats of the second group, signs of granular degeneration were observed. Hepatocytes were swollen, enlarged in size, and contained protein granules in the cytoplasm, while the boundaries between cells were indistinct. The chromatin structure in the cell nuclei was poorly distinguishable, and in some areas only nuclear remnants were observed. Binucleated hepatocytes were also encountered. The lumens of the sinusoidal capillaries were markedly dilated and contained macrophages. The central veins and sinusoidal capillaries were congested with blood, and erythrocytes adhered together into a homogeneous mass (Fig. 7).

Histological examination of the liver in rats of the third group revealed vacuolar degeneration, which was observed both in the peripheral and central parts of the lobules. Vacuoles of various sizes filled with cytoplasmic fluid were detected in the cytoplasm of hepatocytes. In some areas, cells without vacuolization were also present. In addition, hepatocytes with signs of necrosis were identified; their nuclei were reduced in size, unevenly stained, shrunken, and showed features of pyknosis, karyorrhexis, and karyolysis, while some cells contained two nuclei. Dilatation of the lumens of sinusoidal capillaries and venous vessels, as well as their blood filling, was observed. In the lumens, erythrocytes accumulated in clusters and formed columns. A small number of cellular infiltrates were noted around the triads and in perivascular areas (Fig. 8).

In rats of the control group (group IV, intact animals), the liver parenchyma was represented by hepatic lobules without distinct boundaries, separated by thin connective tissue septa. The interlobular connective tissue was poorly developed. Central veins were observed in the center of the lobules, from which hepatic cords radiated in the form of anastomosing strands of liver cells (Fig. 9A). Hepatocytes had a polygonal shape, homogeneous cytoplasm, and pale nuclei with nucleoli located in the center of the cells. Sinusoidal capillaries were present between the hepatic cords, and their lumens contained occasional macrophages (Fig. 9B).

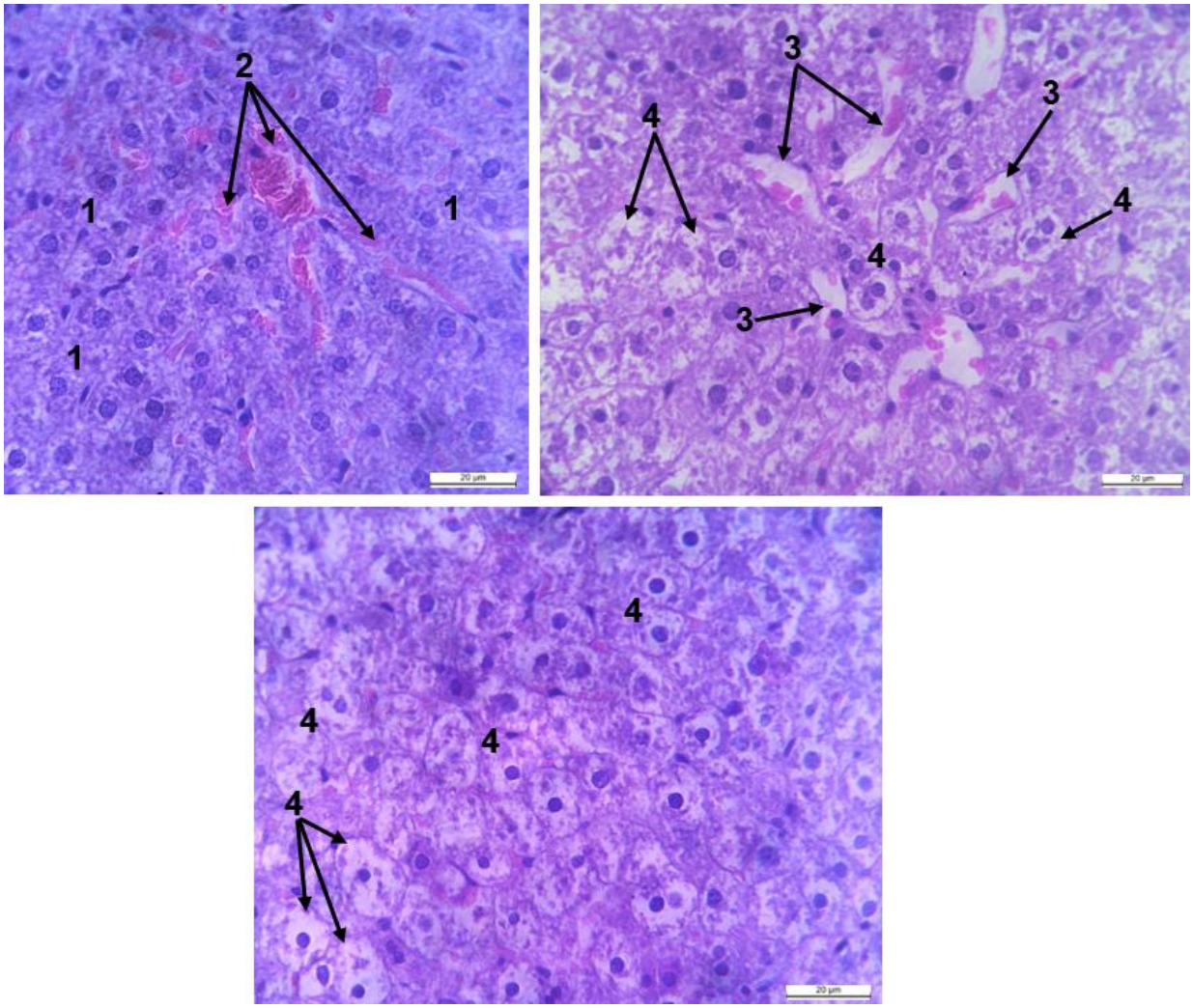

Fig. 6. Fragment of rat liver: *1* – granular degeneration of hepatocytes; *2* – blood filling of the central vein and sinusoidal capillaries; *3* – dilatation of the lumens of sinusoidal capillaries; *4* – ballooning degeneration of hepatocytes; hematoxylin and eosin staining

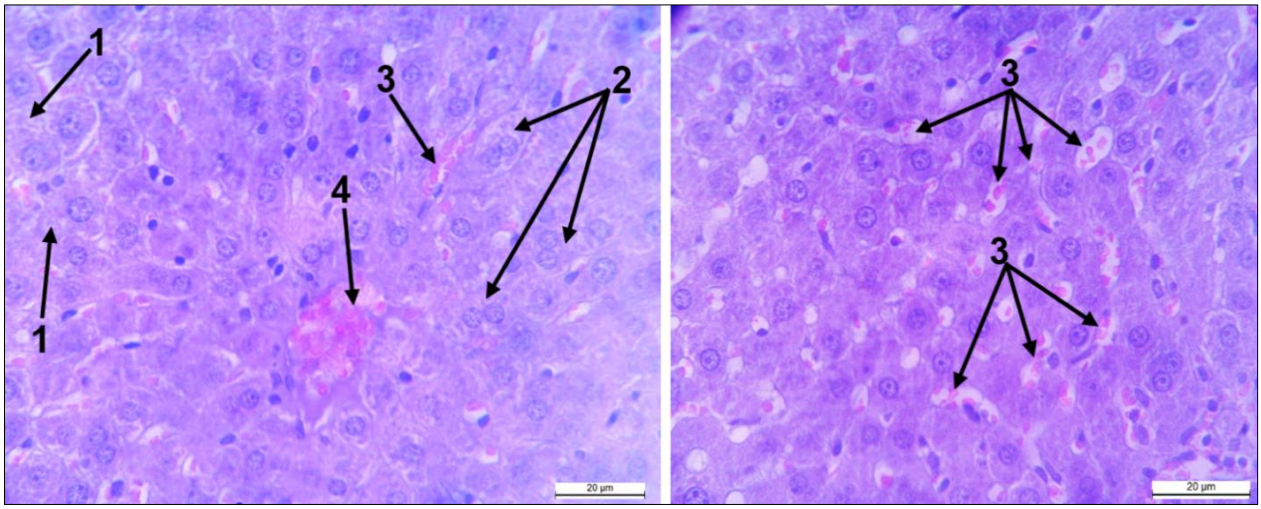

Fig. 7. Fragment of rat liver: *1* – granular degeneration of hepatocytes; *2* – binucleated cells; *3* – dilatation of the lumens of sinusoidal capillaries and their blood filling; *4* – blood filling of the central vein; hematoxylin and eosin staining

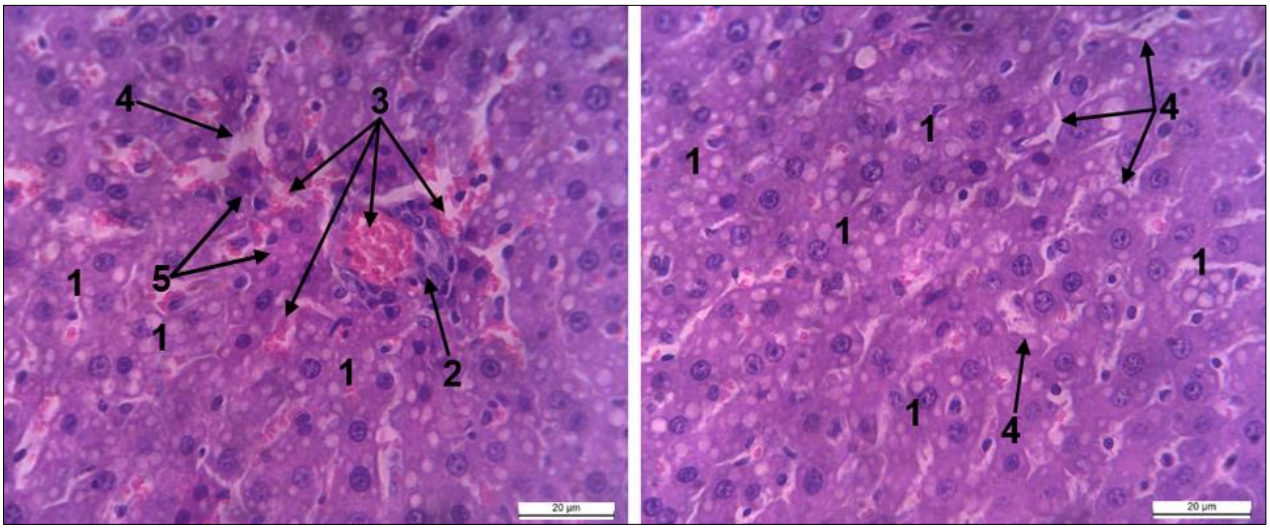

Fig. 8. Fragment of rat liver: *1* – vacuolar (fatty) degeneration of hepatocytes; *2* – cellular infiltrate; *3* – blood filling of the central vein and sinusoidal capillaries; *4* – dilatation of the lumens of sinusoidal capillaries; *5* – cell necrosis; hematoxylin and eosin staining

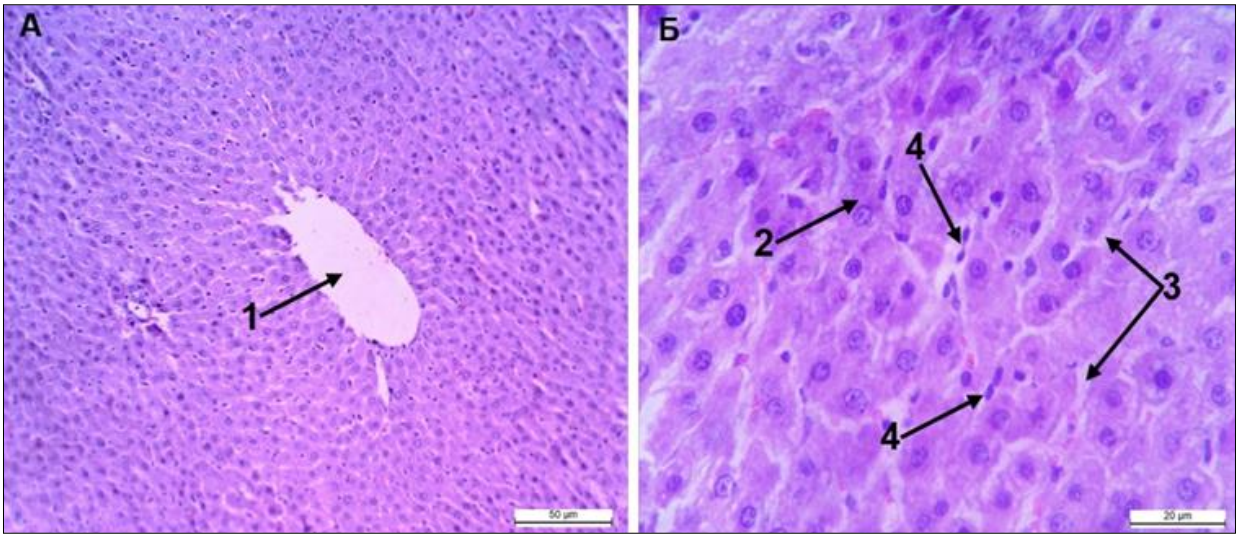

Fig. 9. Fragment of rat liver: *1* – central vein; *2* – hepatocytes; *3* – sinusoidal capillaries; *4* – macrophages; hematoxylin and eosin staining

Hepatic triads were observed in the connective tissue septa, including interlobular arteries, veins, and bile ducts. The walls of the veins were thin, with an indistinct muscular layer, and nuclei of endothelial cells were visible. The arteries had a well-developed muscular layer. The bile ducts were lined with simple cuboidal epithelium containing round nuclei (Fig. 10). To clarify the possible molecular basis of the observed hypolipidemic effect, molecular docking studies were additionally performed. Considering the experimental design and the ob-

tained biochemical profile, two targets relevant to lipid metabolism were selected: HMG-CoA reductase, which is a key enzyme of cholesterol biosynthesis, and pancreatic lipase, which plays a major role in the digestion and absorption of dietary lipids. Such an approach made it possible to compare the likelihood of a statin-like mechanism with an alternative mechanism associated with reduced lipid hydrolysis in the gastrointestinal tract.

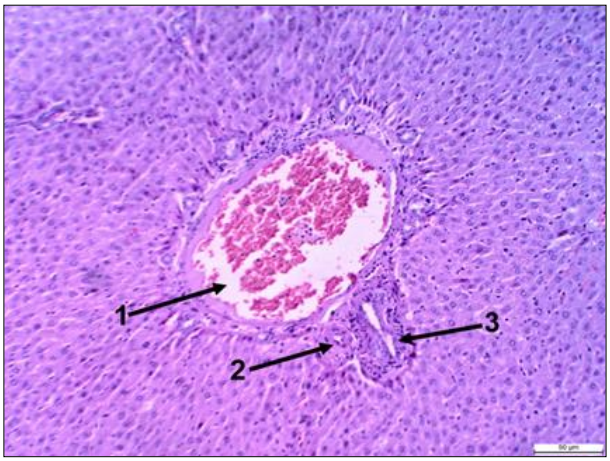

Fig. 10. Fragment of rat liver: *1* – interlobular vein; *2* – interlobular artery; *3* – interlobular bile duct; hematoxylin and eosin staining

The obtained biochemical, histological, and in silico data consistently indicate that the studied compound KP-372, sodium 5-(2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-4-ethyl-4H-1,2,4-triazole-3-thiolate, exhibits a biologically relevant hypolipidemic effect in the experimental model of alimentary hyperlipidemia in rats. Importantly, the interpretation of the results should be based not on a single parameter, but on the combined assessment of lipid profile indices, liver morphology, and molecular docking data, which together allow a more grounded discussion of the probable pharmacological action of this novel hybrid derivative of orotic acid and 1,2,4-triazole.

The experimental model used in the present study proved to be adequate for the induction of lipid metabolism disorders. In the animals of the placebo group receiving a cholesterol-enriched diet with 6-methyluracil and sunflower oil, a clear dyslipidemic shift was observed. In comparison with intact rats, these animals demonstrated an increased serum level of total cholesterol, triglycerides, low-density lipoproteins, and very low-density lipoproteins. Thus, the model reproduced the key biochemical manifestations of hyperlipidemia and created the appropriate background for evaluating the corrective effect of the test compound. The fact that the placebo group showed the highest values of atherogenic lipid fractions confirms that the chosen experimental conditions were sufficient to provoke a metabolically unfavorable state accompanied by hepatic damage.

Against this background, administration of KP-372 was associated with a distinct normalization tendency in serum lipid parameters. In the first experimental group, total cholesterol decreased to 1.49 ± 0.09 compared with 1.84 ± 0.08 mmol/L in the placebo group. A similar trend was observed for triglycerides, which were reduced to 0.53 ± 0.05 versus 0.81 ± 0.03 mmol/L in untreated hyperlipidemic rats. The level of very low-density lipoproteins also remained lower in the KP-372-treated group than in the placebo group. Particularly noteworthy is the LDL value: in rats receiving KP-372, LDL was 0.39 ± 0.08 mmol/L, which was not only lower than in the placebo group but also numerically lower than in animals treated with atorvastatin. This point is especially important for the interpretation of the results because LDL is one of the most clinically relevant fractions in the context of atherogenic risk.

The overall lipid profile changes suggest that KP-372 does not merely produce a random biochemical fluctuation but exerts a directed hypolipidemic action. At the same time, the pattern of this action deserves special attention. Although atorvastatin also reduced total cholesterol and triglycerides, the LDL level in the second experimental group remained elevated relative to intact animals. In contrast, KP-372 demonstrated a more favorable profile with respect to LDL. Therefore, the biological effect of the studied compound may not be reduced simply to the classical mechanism characteristic of statins. On the contrary, the structure of the observed response points to the possibility that KP-372 acts through a different or at least partially different pathway affecting lipid metabolism.

This interpretation becomes even more convincing when biochemical findings are considered together with the pathological and histological state of the liver. In the placebo group, the liver showed the most pronounced lesions. Macroscopically, the organ was flaccid, swollen, unevenly colored, with petechial hemorrhages, which is generally consistent with metabolic and circulatory disturbances occurring under persistent alimentary lipid overload. Histologically, the dominant lesion pattern in this group was vacuolar, that is, fatty degeneration of hepatocytes, accompanied by necrobiotic changes, vascular congestion, dilation of sinusoidal capillaries, and inflammatory cell infiltration. Such a complex of morphological alterations indicates a significant metabolic burden on the liver and confirms that the induced hyperlipidemia was associated not only with changes in serum lipid indices but also with structural hepatic injury.

In animals treated with KP-372, the morphological changes were less severe. Although granular and ballooning dystrophy of hepatocytes was still observed, the lesion pattern in this group differed qualitatively from that seen in untreated hyperlipidemic rats. The predominance of granular dystrophy rather than pronounced vacuolar fatty degeneration may be regarded as a sign of lower intensity of lipid-associated hepatocellular damage. Granular dystrophy usually reflects reversible intracellular metabolic disturbances, whereas severe vacuolar degeneration with necrotic changes indicates deeper structural damage and impaired cell viability. Therefore, the liver histology in the KP-372 group indirectly supports the biochemical evidence of metabolic correction and suggests that the compound attenuated the severity of alimentary hyperlipidemia-induced hepatic injury.

The group treated with atorvastatin also showed a partial protective effect, as evidenced by lower total cholesterol and triglycerides than in placebo-treated rats. However, the persistence of an elevated LDL level and the presence of granular dystrophy indicate that the protective effect was also incomplete under the given experimental conditions. In this context, the findings obtained for KP-372 are of particular interest because they suggest that the novel compound may be comparable to the reference drug in some aspects of hypolipidemic action and may even demonstrate a more favorable effect on selected lipid fractions, especially LDL.

To obtain a mechanistic interpretation of these *in vivo* observations, molecular docking studies were performed using two biologically relevant targets: HMG-CoA reductase (PDB: 1HWK) and pancreatic lipase (PDB: 1LPB). These proteins were selected because they represent two distinct but complementary pharmacological points of influence on lipid metabolism. HMG-CoA reductase is the key rate-limiting enzyme of endogenous cholesterol biosynthesis and is the canonical target of statins. Pancreatic lipase, in turn, plays a central role in the hydrolysis and gastrointestinal processing of dietary lipids, and therefore its inhibition may reduce lipid absorption and overall lipid burden. The simultaneous use of these two targets allowed the assessment of whether KP-372 is more likely to behave as a statin-like agent or whether its effect may be related to modulation of lipid digestion and absorption.

The docking results clearly demonstrated that KP-372 has a higher predicted affinity toward pancreatic lipase than toward HMG-CoA reductase. The calculated binding affinity for 1HWK was –5.136 kcal/mol, whereas for 1LPB it was -7,260 kcal/mol. Such a difference is meaningful for comparative in silico interpretation and indicates that the ligand forms a more energetically favorable complex with pancreatic lipase. At the same time, the moderate affinity toward HMG-CoA reductase suggests that interaction with this enzyme cannot be completely excluded; however, it is unlikely to be the dominant mechanism explaining the observed biological activity (Fig. 11).

The interaction pattern in the 1HWK complex supports this conclusion. According to the generated interaction map, KP-372 was lo-

cated in the environment of MET655, GLY656, MET657, ASN658, MET659, GLN766, ASP767, VAL805, GLY806, GLY807, and GLY808. This binding mode appears to be stabilized mainly by spatial complementarity and a limited number of nonspecific contacts. The pyrimidinedione fragment of the ligand is oriented toward polar residues, while the triazole-thiol moiety occupies a region formed predominantly by residues that provide van der Waals stabilization rather than a dense network of strongly directed interactions. Such a binding pattern is compatible with the relatively modest docking score and suggests that KP-372 does not reproduce the highly optimized interaction profile typical of classical HMG-CoA reductase inhibitors such as atorvastatin.

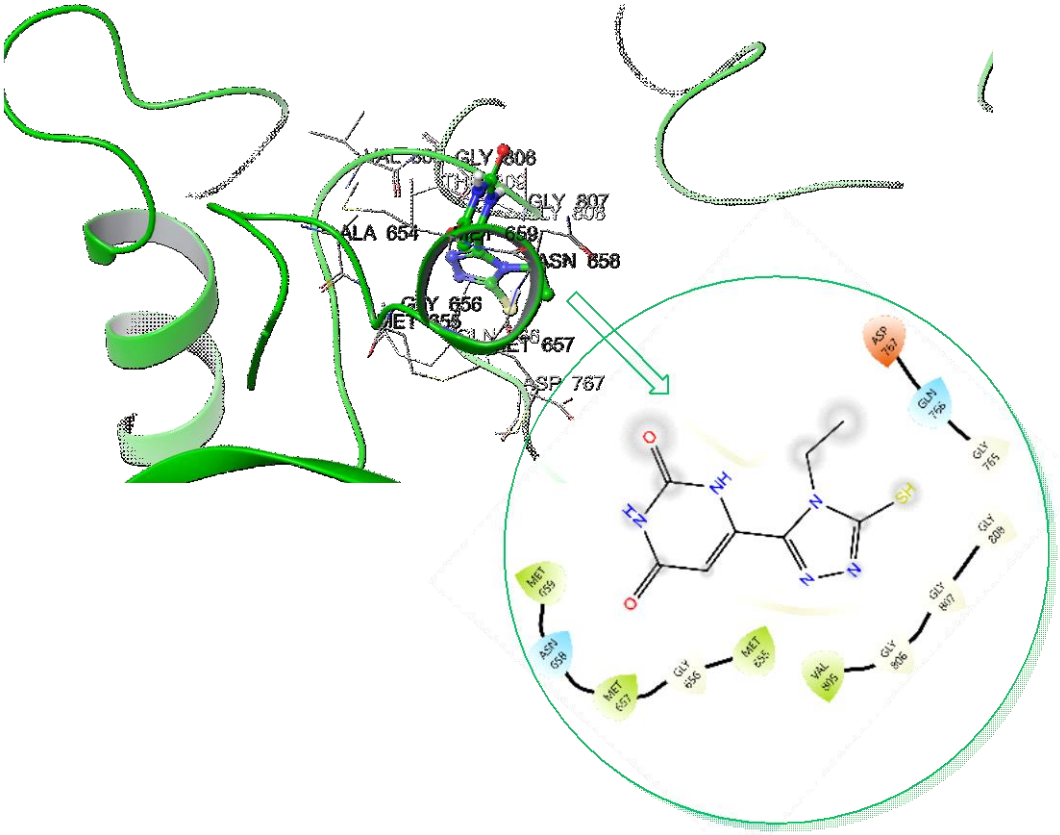

Fig. 11. Visualization of molecular docking results showing the interactions between compound and the HMG-CoA reductase (PDB: 1HWK, chain A) in both 3D and 2D views

In contrast, the docking pose of KP-372 in the 1LPB binding cavity (Fig. 12) was characterized by a more favorable arrangement and a more developed interaction network. The ligand was surrounded by ASP79, GLY76, HIS151, ILE78, PHE77, TRP85, SER152, LEU153, HIS263, LEU264, ARG256, and ALA259. The carbonyl oxygen atoms of the pyrimidinedione fragment appear to serve as important anchoring centers directed toward polar regions of the active site. At the same time, the heterocyclic triazole core and the sulfur-containing fragment are accommodated in a pocket formed by residues capable of hydrophobic and mixed polar-hydrophobic stabilization. Such a distribution of contacts creates a more balanced and structurally favorable binding environment, which is consistent with the lower calculated free energy of binding for pancreatic lipase.

Discussion

The results of the present study demonstrate that the hybrid compound KP-372, combining structural fragments of orotic acid and 1,2,4-triazole, exhibits pronounced hypolipidemic activity in rats with alimentary hyperlipidemia. The combined analysis of biochemical parameters, liver morphology, and molecular docking results provides consistent evidence that the studied compound influences lipid metabolism and reduces the severity of diet-induced metabolic disturbances.

The experimental model used in this study successfully reproduced key manifestations of dyslipidemia. In placebo-treated animals receiving a cholesterol-enriched diet supplemented with sunflower oil and 6-methyluracil, a marked increase in total cholesterol, triglycerides, LDL, and VLDL was observed. These changes correspond to typical biochemical features of experimental hyperlipidemia and confirm that excessive dietary lipid intake leads to significant disturbances of lipid homeostasis and hepatic metabolic stress. Previous studies have demonstrated that dyslipidemia induced by high-fat or cholesterol-enriched diets is frequently accompanied by structural alterations in liver tissue and plays an important role in the development of fatty liver disease and related metabolic disorders (Martin et al., 2022).

Administration of KP-372 led to a significant improvement in serum lipid profile parameters. In animals treated with the compound, total cholesterol, triglycerides, and VLDL levels were lower than in untreated hyperlipidemic rats. Particularly noteworthy was the decrease in LDL levels, which were not only lower than in the placebo group but also numerically lower than in animals receiving atorvastatin. Since LDL is considered one of the principal atherogenic lipoprotein fractions and an important indicator of cardiovascular risk, this observation suggests that the tested compound may possess a biologically relevant antiatherogenic potential.

The lipid-lowering effect observed in the present study is consistent with previous reports describing the pharmacological potential of heterocyclic compounds containing the 1,2,4-triazole ring. This heterocyclic scaffold is widely recognized in medicinal chemistry as a “privileged structure” capable of supporting a wide range of biological activities due to its favorable electronic and pharmacophoric properties (Aggarwal & Sumran, 2020). Earlier studies demonstrated that various triazole derivatives exhibit antihyperlipidemic activity and are capable of reducing serum cholesterol and triglyceride levels in experimental models (Chhabria et al., 2011). The results obtained in the present work further confirm that incorporation of a triazole fragment into hybrid molecular systems may contribute to the development of biologically active lipid-modulating agents.

Another important structural component of the studied compound is the pyrimidinedione fragment related to orotic acid derivatives.

Orotic acid and related heterocyclic systems have previously been reported to influence lipid metabolism and hepatic biochemical processes (Harden & Robinson, 1984). Moreover, pyrimidine-based derivatives have been investigated as potential therapeutic agents for metabolic disorders and fatty liver disease (Ma et al., 2012). In this context, the hybridization of the triazole nucleus with a pyrimidinedione fragment, as realized in KP-372, may create a synergistic pharmacological effect affecting multiple aspects of lipid metabolism.

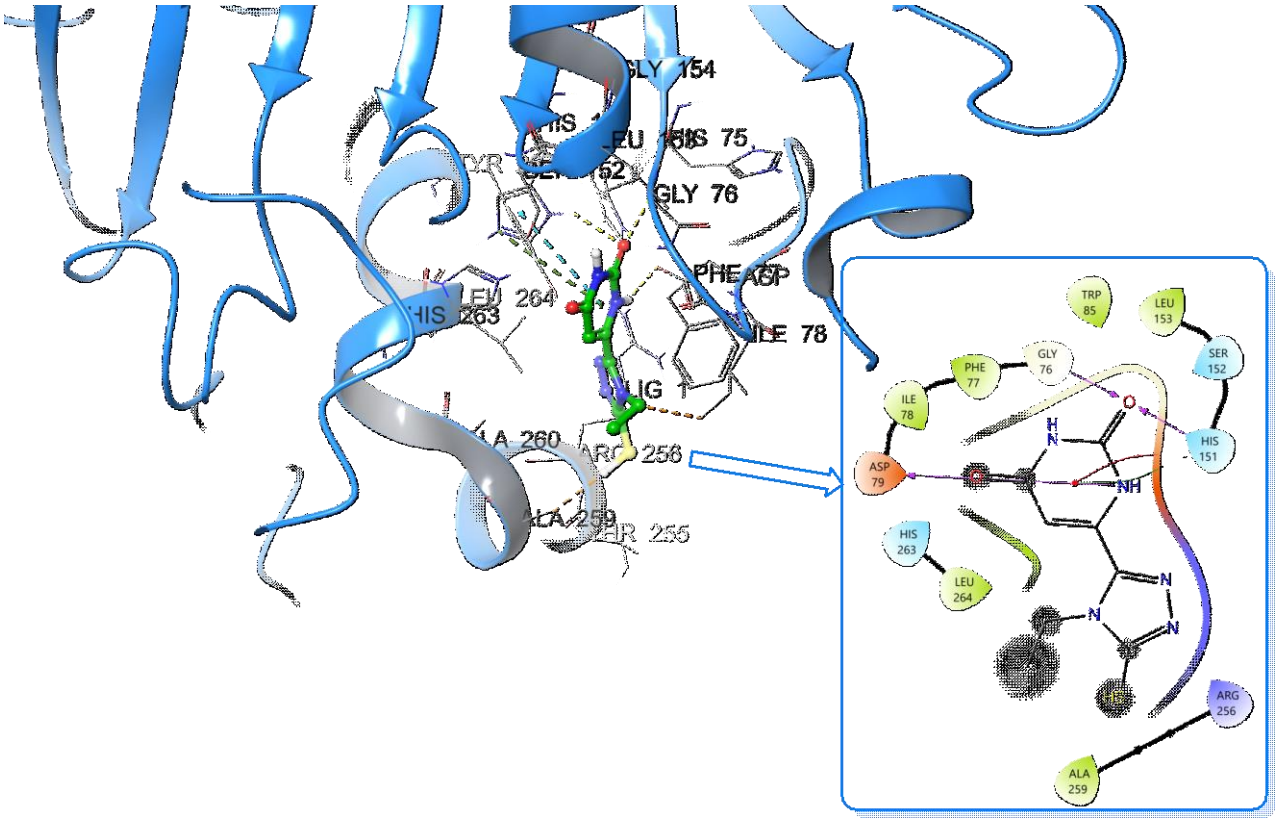

Fig. 12. Visualization of molecular docking results showing the interactions between compound and the pancreatic lipase (PDB: 1LPB, chain B in both 3D and 2D views

The histological findings obtained in this study further support the biochemical results. In untreated hyperlipidemic animals, liver tissue showed pronounced pathological changes, including vacuolar (fatty) degeneration of hepatocytes, necrobiotic alterations, vascular congestion, and inflammatory infiltration. These morphological features are characteristic of lipid-associated hepatic injury and reflect the metabolic burden imposed on the liver under conditions of excessive lipid intake. In contrast, rats treated with KP-372 demonstrated milder structural changes in hepatic tissue. The predominant alterations were represented by granular and ballooning degeneration of hepatocytes rather than the extensive vacuolar fatty degeneration observed in untreated animals. Granular degeneration is generally considered a reversible manifestation of metabolic disturbance, whereas severe vacuolar degeneration and necrosis indicate more profound cellular damage. Therefore, the observed histological pattern suggests that KP-372 attenuates the severity of lipid-induced hepatocellular injury and partially protects hepatic tissue under conditions of experimental hyperlipidemia. Comparison with the reference drug atorvastatin also provides important insights. As expected, atorvastatin improved certain lipid parameters, particularly total cholesterol and triglycerides. However, LDL levels in this group remained relatively elevated compared with intact animals. In contrast, KP-372 demonstrated a more favorable LDL profile. Although the overall hypolipidemic activity of KP-372 was comparable to that of atorvastatin, the pattern of lipid changes suggests that the mechanisms of action of these compounds may not be identical.

The results of molecular docking analysis support this assumption. The calculated binding affinity of KP-372 toward pancreatic lipase was higher than that toward HMG-CoA reductase. Since HMG-CoA reductase is the key enzyme of cholesterol biosynthesis and the primary target of statins, a stronger interaction with pancreatic lipase suggests that the compound may exert its hypolipidemic effect predominantly through modulation of dietary lipid digestion and absorption rather than through direct inhibition of endogenous cholesterol synthesis. Pancreatic lipase plays a central role in the hydrolysis of dietary triglycerides and represents an important pharmacological target for reducing lipid absorption and metabolic burden associated with high-fat diets (Subramaniyan & Hanim, 2025).

Such a mechanism is particularly plausible in the context of the present experimental model, where hyperlipidemia was induced by alimentary factors. By reducing the hydrolysis and intestinal uptake of dietary lipids, inhibition of pancreatic lipase may decrease the amount of lipid entering systemic metabolism and consequently reduce hepatic lipid accumulation. This interpretation is consistent with the observed improvement of liver morphology in the KP-372-treated group.

Despite these promising findings, several limitations should be considered. Molecular docking provides only a theoretical prediction of ligand-protein interactions and cannot fully confirm enzyme inhibition. Therefore, the proposed mechanism involving pancreatic lipase modulation should be verified in future studies using direct enzymatic assays and kinetic analyses. In addition, further investigations involving longer experimental periods, oxidative stress markers, and expanded biochemical profiling would be valuable to better understand the pharmacological properties of the studied compound.

Overall, the combined biochemical, histological, and in silico results obtained in this study suggest that KP-372 represents a promising candidate for further pharmacological investigation as a novel hypolipidemic and potentially antiatherogenic agent.

Conclusion

Experimental alimentary hyperlipidemia induced by cholesterol, 6-methyluracil, and sunflower oil caused marked disturbances of lipid metabolism in rats, including increased serum cholesterol, triglyceri-

des, LDL, and VLDL levels, and was accompanied by pronounced hepatic injury with predominantly vacuolar (fatty) degeneration of hepatocytes. These findings confirm that the selected model is suitable for reproducing dyslipidemia associated with liver damage.

Against this background, intragastric administration of KP-372 was associated with correction of the lipid profile, particularly with reductions in total cholesterol, triglycerides, LDL, and VLDL compared with untreated hyperlipidemic animals. Histologically, liver damage in this group was less severe and was characterized mainly by granular and ballooning degeneration rather than the pronounced vacuolar-fatty changes observed in the placebo group, indicating attenuation of lipid-associated hepatic injury.

Atorvastatin also improved some lipid parameters, mainly cholesterol and triglycerides, but elevated LDL levels persisted relative to intact animals, and granular dystrophy was still observed in the liver. In this regard, KP-372 demonstrated at least comparable hypolipidemic activity and a more favorable effect on LDL.

Molecular docking supported the biological findings by showing a higher predicted affinity of KP-372 for pancreatic lipase than for HMG-CoA reductase, suggesting that its hypolipidemic action may be mediated predominantly through modulation of dietary lipid digestion and absorption rather than through a classical statin-like mechanism. Taken together, the biochemical, histological, and in silico results indicate that KP-372 is a promising hypolipidemic and potentially antiatherosclerotic agent worthy of further investigation.

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