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Hypolipidemic Activity of Sesquiterpene Lactones and their Derivatives

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Abstract

BACKGROUND: Despite the available range of lipid-lowering drugs, mainly of synthetic origin, the problem of atherosclerosis therapy and its manifestations remain unresolved. Bioinformatics methods, in particular molecular docking, are considered as a promising direction in terms of developing effective original lipid-lowering drugs. Today, as a promising source of natural lipid-lowering agents, it is advisable to consider natural terpenoids, namely sesquiterpene lactones, which are distinguished by a wide range of pharmacological activity. This article presents the results of a virtual and biological screening of the lipid-lowering activity of sesquiterpene γ-lactones and their chemically modified derivatives.

AIM: The aim is to evaluate the lipid-lowering properties of samples of sesquiterpene γ-lactones and their derivatives by virtual and biological screening methods.

METHODS: Molecular modeling of the binding energy of the "ligand-target" complex (docking). Molecular docking of the lipid-lowering activity of sesquiterpene γ-lactones and their derivatives was performed using the Glide program from the developer of the Schrodinger Small-Molecule Drug Discovery package using the Extra Precision algorithm (https://www.schrodinger.com/glide). The effect of sesquiterpene lactones on the expression of genes for key enzymes of lipid metabolism in the liver was studied in an *in vivo* model of hyperlipidemia caused by an atherogenic diet.

CONCLUSION: Virtual screening of the lipid-lowering activity of sesquiterpene γ-lactones and their derivatives by molecular docking revealed a number of promising compounds (matricin, matricarin, grossmisin oxime, austricin oxime, 5β (H)-austricin) receptor interactions on the enzyme system cholesterol 7α-hydroxylase (CYP7A1). New mechanisms of lipid-lowering activity for sesquiterpene γ-lactones were proposed, which were established in the study of gene expression of key enzymes of lipid metabolism in the liver in a model of hyperlipidemia caused by an atherogenic diet in rats under *in vivo* conditions.

Introduction

Cardiovascular diseases caused by atherosclerosis are one of the leading causes of death worldwide. The lipid-lowering drugs used in the treatment of atherosclerosis are mainly represented by synthetic statins, such as simvastatin, lovastatin, atorvastatin, and rosuvastatin; the problem of treating hyperlipidemia and its manifestations have not been completely resolved [1]. As promising sources of new effective anti-atherosclerotic agents, substances of plant origin are considered, which are characterized by a rather high pharmacotherapeutic potential, a low level of side effects, and good tolerance [2]. Among the potential sources of lipid-lowering agents, natural terpenoids are of interest, namely sesquiterpene lactones, which have a wide spectrum of pharmacological activity and, according to the structure of molecules, contain a lactone ring, similar to statins [3], [4], [5], and [6]. Today, thanks to the widespread introduction of high-performance computers into bioorganic chemistry, pharmacology, and molecular biology, it is possible to conduct a targeted search for biologically active sesquiterpene lactones, including those with hypolipidemic properties [7], [8], [9].

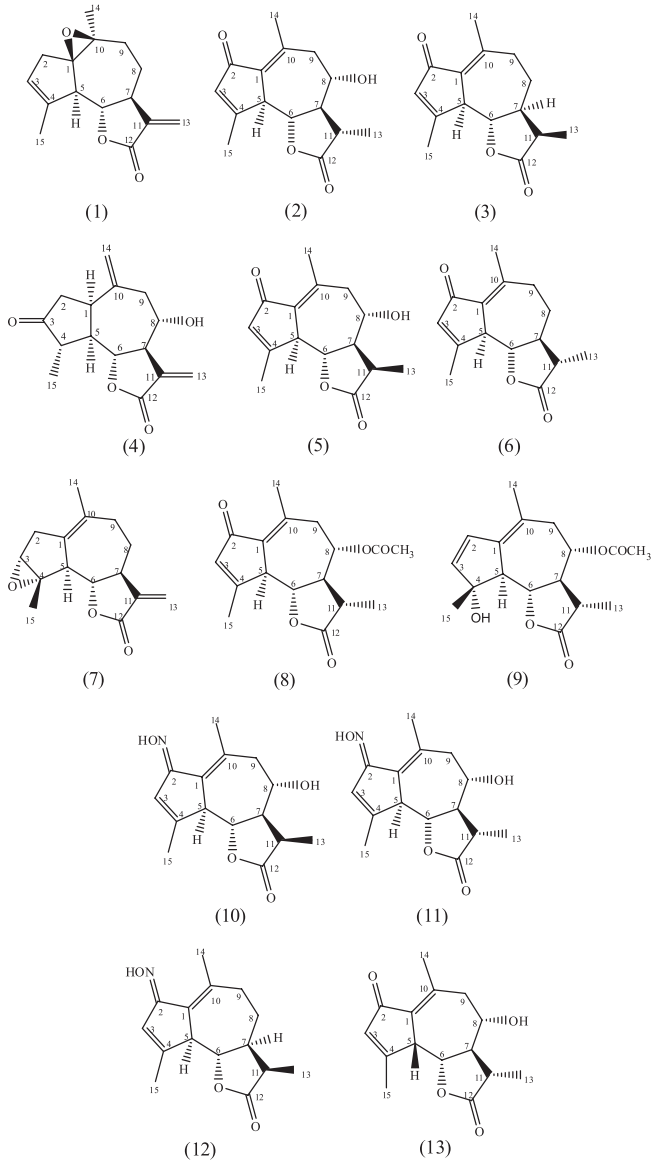
The aim of the work is to evaluate the lipid-lowering properties of samples of sesquiterpene γ-lactones and their derivatives by virtual and biological screening methods.

Research objectives

1. To carry out computer prediction of hypolipidemic activity for 10 molecules of sesquiterpene γ-lactones and their derivatives: arglabin (1), austricin (2), achillin (3), grossheimin (4), grossmisin (5), leukomycin (6), ludartin (7), matricarin (8), matricin (9), grossmisin oxime (10), austricin

oxime (11), achillin oxime (12), and 5β(H)-austricin (13) using the method of computer modeling of the binding energy of the complex "target ligand" (docking) on key enzyme systems of lipid metabolism under in silico conditions.

2. To study the effect of sesquiterpene γ-lactones arglabin (1), achillin (3), grossheimin (4), grossmisin (5), leukomycin (6), and ludartin (7) on the expression of genes of key enzymes of lipid metabolism in the liver in a model of hyperlipidemia, caused by an atherogenic diet in *rats in vivo.*



Materials and Methods

Virtual screening

Molecular modeling of the binding energy of the "ligand-target" complex (docking). Molecular docking of the lipid-lowering activity of sesquiterpene γ-lactones and their derivatives was performed using the Glide program from the developer of the Schrodinger Small-Molecule Drug Discovery package using the ExtraPrecision algorithm (https://www.schrodinger.com/glide). Virtual spatial structures were obtained from the open database of protein molecules, PDB (Protein Data Bank). For each compound, the most probable binding positions of the molecule to the receptor were predicted, and the values of the binding energy (E_{CB}) (G-score) of the ligand-target complex were calculated. Low (negative) E_{CB} values indicated a strong bond between the molecule and the receptor [10].

Docking of lipid-lowering activity was carried out on six structures obtained experimentally and used as models for predicting this pharmacological activity. Models used: rat carnitine palmitoyltransferase 2 (CPT2) in complex with 1-[(R)-2-(3,4-dihydro-1H-isoquinoline-2-carbonyl)-piperidin-1-yl]-2-phenoxy-ethanone (PDB-ID: 4EYW) [11]; human cholesterol 7α-hydroxylase (CYP7A1) in complex with 7-ketocholesterol (PDB-ID: 3V8D) [12]; human 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) (PDB-ID: 3CCT) [13]; rat regulatory domain carnitine palmitoyltransferase 1 (CPT1A) (PDB-ID: 2M76) and human acetyl-CoA carboxylase (ACACA), for which the SiteMap algorithm was used to bind to the identification site (PDB-ID: 5I87) [14], [15], and also a model of human acyl-CoA cholesterol acyltransferase (SOAT1) in complex with the Fab fragment of an agonistic antibody (PDB-ID: 4XWG) [16].

In vivo experiments

Investigation of gene expression of key enzymes of lipid metabolism in vivo

The effect of sesquiterpene lactones on the expression of genes for key enzymes of lipid metabolism in the liver was studied in an *in vivo* model of hyperlipidemia caused by an atherogenic diet.

The experiments were performed on 80 outbred Wistar rats with an average weight of 260–300 g. The animals were kept under standard vivarium conditions with free access to water and food. The studies were performed in accordance with the recommendations of the "Guidelines for Conducting Preclinical Studies of Drugs" [17]. After quarantine for two weeks, rats were selected for the experiment and formed into 10 groups of 8 animals each by randomization (Table 1).

One of the widely used methods for inducing hyperlipidemia is the long-term (2–3 weeks) use of a diet containing cholesterol (2.5%), methylthiouracil (0.12%), and 30% vegetable oil [17]. This model of hyperlipidemia varies both in the ratio of ingredients in the diet and in the duration of its use.

Animals received a high-fat diet (45% energy from animal fat) for 4 weeks containing 2.5% cholesterol (Sigma), 0.5% cholic acid (Sigma) (to improve absorption of cholesterol in the gastrointestinal tract), and 0.1% 2-thiouracil (Sigma) (to suppress thyroid function).

Table 1: Distribution of animals by experimental groups

Serial number	Group	Number of animals	Diet
1	2	3	4
1	Intact animals	8	Standard laboratory diet for 42 days, from 29 to 42 days 0.5% solution of starch mucus intragastrically 1 time/day
2	Atherogenic diet model (control)	8	Diet with a high content of animal fat and cholesterol for 42 days, from 29 to 42 days 0.5% solution of starch mucus intragastrically 1 time/day
3	Arglabin (1) (10 mg/kg) + atherogenic diet	8	Diet with a high content of animal fat and cholesterol for 42 days, from 29 to 42 days arglabin (1) at a dose of 10 mg/kg intragastrically 1 time/day
4	Achillin (3) (10 mg/kg) + atherogenic diet	8	Diet with a high content of animal fat and cholesterol for 42 days, from 29 to 42 days Achillin (3) at a dose of 10 mg/kg intragastrically 1 time/day
5	Grossheimin (4) (10 mg/kg) + atherogenic diet	8	Diet with a high content of animal fat and cholesterol for 42 days, from 29 to 42 days grossheimin (4) at a dose of 10 mg/kg intragastrically 1 time/day
6	Grossmisin (5) (10 mg/kg) + atherogenic diet	8	Diet with a high content of animal fat and cholesterol for 42 days, from 29 to 42 days grossmisin (5) at a dose of 10 mg/kg intragastrically 1 time/day
7	Leucomisin (6) (10 mg/kg) + atherogenic diet	8	Diet with a high content of animal fat and cholesterol for 42 days, from 29 to 42 days leukomizin (6) at a dose of 10 mg/kg intragastrically 1 time/day
8	Ludartin (7) (10 mg/kg) + atherogenic diet	8	Diet with a high content of animal fat and cholesterol for 42 days, from 29 to 42 days Ludartin (7) at a dose of 10 mg/kg intragastrically 1 time/day
9	Reference drug "Fenofibrate" (14) (100 mg/kg) + atherogenic diet	8	Diet with a high content of animal fat and cholesterol for 42 days, from 29 to 42 days "Fenofibrate" (14) at a dose of 100 mg/kg intragastrically 1 time/day
10	Reference drug Rosuvastatin calcium (15) (10 mg/kg) + atherogenic diet	8	Diet with a high content of animal fat and cholesterol for 42 days, from 29 to 42 days "Rosuvastatin calcium" (15) at a dose of 10 mg/kg intragastrically 1 time/day

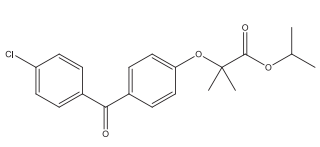
The standard laboratory diet was represented by special granules with mineral and fortified additives "ProKorm" for laboratory rats manufactured by CJSC "BioPro" (Novosibirsk), containing the following component composition (Table 2):

Table 2: Component composition of the standard laboratory diet for rats

Components	Share by weight (%)	Share by kcal (%)	Calorie content kcal/100 g
Proteins	22.0	28.0	90.2
Carbohydrates	47.7	60.8	195.8
Fat	4.0	11.2	36.0
Total	73.7	100.0	322.0

Algorithm for the preparation of food providing 45% energy from animal fat and containing 2.5% cholesterol, 0.5% cholic acid, and 0.1% 2-thiouracil (atherogenic diet): dissolved in 20 g of ghee (98% animal fat) 2.5 g of cholesterol (Sigma), 0.5 g of cholic acid (Sigma), and 0.1 g of 2-thiouracil (Sigma) mixed in 77 g of the standard food of the above composition. The calorie content of the received feed was 545 kcal per 100 g.

Experimental animals received an atherogenic diet for 4 weeks. After that, experimental groups of animals that continued to receive the same diet were intragastrically injected with samples of sesquiterpene lactones at a dose of 10 mg/kg for two weeks and reference drugs with different mechanisms of hypolipidemic action: fenofibrate [14] (100 mg/kg) ("Sigma Aldrich", USA) [18] and calcium rosuvastatin [15] ("Sigma Aldrich", USA) [19]. The preparations were administered to animals intragastrically in the form of a solution of 0.5% starch mucus.



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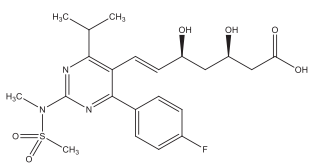


(15)

Animals in the control group on the background of an atherogenic diet were injected with equivalent amounts of a 0.5% solution of starch mucus for two weeks. The intact group of animals on a standard diet for two weeks also received equivalent amounts of a 0.5% solution of starch mucus.

After 14 days of daily administration of the studied samples of sesquiterpene lactones and reference drugs after fasting for 12 h, the animals were decapetized. Liver samples (25 mg) in RNAlater solution ("Ambion", USA) were placed in a refrigerator at +4°C for 24 h and then stored in a freezer at –80°C until RNA was isolated.

Estimation of mRNA expression of lipid metabolism genes

Total RNA was isolated from liver tissue using the Illustra RNAspin Mini RNA Isolation Kit ("GE Healthcare", UK) according to the protocol. The concentration and purity of the isolated RNA were assessed on a NanoDrop 2000 spectrophotometer ("Thermo Scientific", USA). RNA integrity was assessed using the R6K ScreenTape kit ("Agilent Technologies", USA) by capillary electrophoresis on a TapeStation instrument ("Agilent Technologies", USA).

To synthesize cDNA from the RNA template, a reverse transcription reaction was performed using the RevertAid First Strand cDNA Synthesis Kit (Fermentas, Lithuania) according to the attached protocol. The resulting solution was placed in a Tertsik amplifier (DNA Technology, Russia) and incubated according to the program: 5 min at 25°C, then 60 min at 42°C. The reaction was stopped by heating at 70°C for 5 min. cDNA was stored at −20°C for further analysis.

Expression of lipid metabolism genes was assessed using quantitative real-time reverse transcription PCR using TaqMan technology on a Rotor-Gene 6000 cycler (Corbett Research, Australia).

Primers and probes (FAM-BHQ1) were selected using the Vector NTI Advance 11.5, Oligo 7.5 software and the NCBI Nucleotide Database (http://www.ncbi.nlm.nih.gov/nuccore) (Table 3).

Table 3: The sequence of primers and probes used in the study of gene expression

Amplicon	Primer type	Primer
1	2	3
CPT2	Direct primer	5'-GCTGTTCACGATGACTGGATAG-3'
NM_012930.1	Reverse primer	5'-TCGAAAATGTCTTCCAAGCA-3'
Amplicon 104 bp	Probe	5'-ACGCAATGCCCGAGAGTTTC-3'
CYP7A1	Direct primer	5'-CTGATGCTCTCCTGCTTTGA-3'
NM_012942.2	Reverse primer	5'-CATGTAGTGGTGGCAAAATTC-3'
Amplicon 103 bp	Probe	5'-TGTGGAGAGCCAAGTCAAGTGTC-3'
HMGCR	Direct primer	5'-GCTTGAGATCATGTGCTGCTT-3'
NM_013134.2	Reverse primer	5'-CCGAGAAAGCTCTAGGACCA-3'
Amplicon 106 bp	Probe	5'-CTGTATGTCCGTGCTTGCCAACT-3'
CPT1A	Direct primer	5'-CATTGACCTCCGCCTGA-3'
NM_031559.2	Reverse primer	5'-TGATGCCATTCTTGAACCG-3'
Amplicon 98 bp	Probe	5'-CCACGAAGCCCTCAAACAGAT-3'
ACACA	Direct primer	5'-CGCAGGCATCAGAAGATCA-3'
NM_022193.1	Reverse primer	5'-TGGCAAGTTTTACAGCACACT-3'
Amplicon 94 bp	Probe	5'-ACCCCAGCAGTATTTGAACACATG-3'
SOAT1	Direct primer	5'-GTGCTCGTGTCCTGGTCC-3'
NM_031118.1	Reverse primer	5'-AAGGCAAAGAACGAAAGGAA-3'
Amplicon 79 bp	Probe	5'-AGCACACCTGGCAAGATGGAGTT-3'
ACTB	Direct primer	5'-GAAAAGATGACCCAGATCATGT-3'
NM_031144.3	Reverse primer	5'-AACACAGCCTGGATGGCTA-3'
Amplicon 71 bp	Probe	5'-AGACCTTCAACACCCCAGCCAT-3'
LDLR	Direct primer	5'-GCCATCTATGAGGACAAAGTGT-3'
NM_175762.2	Reverse primer	5'-GCCACCAAATTCACATCTGA-3'
Amplicon 95 bp	Probe	5'-AGGCGGTTGGCACTGAAAATG-3'

1 NM is the mRNA sequence number in the NCBI nucleotide database (http://www.ncbi.nlm.nih.gov/nuccore), 2 bp - base pairs (Base Pare).

PCR was performed in duplicates in a volume of 15 μl on a Rotor-Gene 6000 amplifier ("Corbett Research", Australia). The two-step amplification program included: 1 cycle pre-denaturation - 94°C, 2 min; 40 cycles - 1 step, 94°C, 6 s and 2 steps, 60.5°C, 10 s. The purity of PCR products was checked using electrophoresis on a 2.5% agarose gel with 0.2% ethidium bromide. The expression level of each target gene was expressed in terms of units relative to the control (intact group) and the reference gene of the ACTB enzyme (Rattus norvegicus actin, beta).

The relative expression of lipid metabolism genes was assessed by the Pfaffl method. The following formula was used to determine the expression ratio between sample and calibrator:

$$\text{Ratio} = \frac{(E_{\text{target}})\Delta\text{Cttarget}(\text{calibrator} - \text{test})}{(E_{\text{ref}})\Delta\text{Ctref}(\text{calibrator} - \text{test})}$$

where E is the reaction efficiency, Ct is the threshold cycle of target genes (target) and referee genes (ref). ΔCt, target (calibrator – test) = Ct of the target gene in the calibrator minus Ct of the target gene in the test sample; ΔCt, ref (calibrator – test) = Ct of the referee gene in the calibrator minutes Ct of the referee gene in the test sample [20]. RNA isolated from the liver tissue of intact rats was used as a calibrator.

Results and Discussion

Virtual screening data implemented by molecular docking

As a result of the docking of molecules of sesquiterpene γ-lactones and their derivatives on the first model, namely, on rat CPT2 in a complex with 1-[(R)-2-(3,4-dihydro-1H-isoquinoline-2-carbonyl)-piperidin-1-yl]-2-phenoxy-ethanone (PDB-ID: 4EYW), the following binding energies (E_{CB}) of the molecule with the receptor were calculated and presented in Table 4.

Table 4: E_{CB} values for the carnitine palmitoyltransferase 2 model

Rank	Compound name	Binding energy (G-score) kcal/mol
1	Arglabin (1)	−5.93
2	Austricin (2)	−5.20
3	Achillin (3)	−5.29
4	Grossheimin (4)	−6.90
5	Grossmisin (5)	−5.47
6	Leucomisin (6)	−5.47
7	Ludartin (7)	−6.00
8	Matricarin (8)	−6.06
9	Matricin (9)	−6.29
10	Grossmisin oxime (10)	−6.61
11	Austricin oxime (11)	−5.95
12	Achillin oxime (12)	−5.49
13	5β(H)-austricin (13)	−5.47

As can be seen from the table, the E_{CB} values are not large and may indicate a weak intermolecular bond.

The most probable binding positions were predicted, and E_{CB} values were calculated for the second model of human cholesterol 7α-hydroxylase (CYP7A1) in complex with 7-ketocholesterol (PDB-ID: 3V8D) (Table 5).

Table 5: E_{CB} values for the cholesterol 7α-hydroxylase model

Rank	Compound name	Binding energy (G-score) kcal/mol
1	Arglabin (1)	−7.56
2	Austricin (2)	−6.97
3	Achillin (3)	−6.21
4	Grossheimin (4)	−7.47
5	Grossmisin (5)	−6.96
6	Leucomisin (6)	−6.62
7	Ludartin (7)	−7.24
8	Matricarin (8)	−7.28
9	Matricin (9)	−8.04
10	Grossmisin oxime (10)	−7.10
11	Austricin oxime (11)	−7.07
12	Achillin oxime (12)	−6.10
13	5β(H)-austricin (13)	−6.97

Relatively high values of "ligand-receptor" interaction were obtained for the cholesterol 7α-hydroxylase (CYP7A1) model, where the list of five molecules with the highest E_{CB} values included: matricin (9), arglabin (1), grossheimin (4), matricarin (8), and ludartin (7). For example, the matricin molecule (9), due to the presence of a free hydroxyl group in the structure, forms strong bonds with the protein target on the amino acid residues of HIS-101 and LEU-361 (Figure 1).

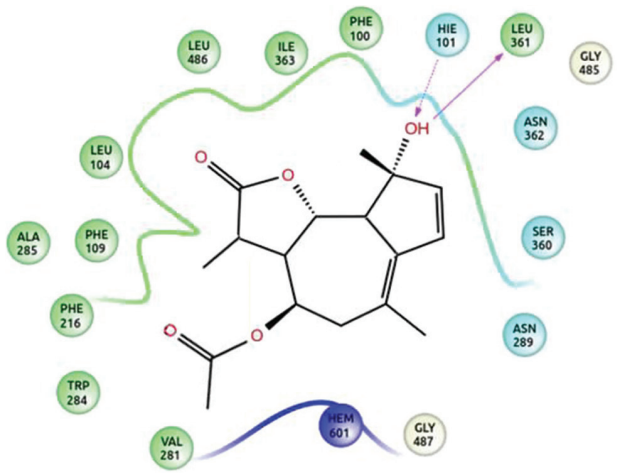


Figure 1: CYP7A1 in complex with matricin (9)

The maximum E_{CB} value for the grossheimin molecule (4) was calculated in combination with the B chain domain of the 7α-hydroxylase enzyme (–7.47 kcal/mol), which, due to the presence of a triple hydrogen bond in the structure, forms a strong bond with the receptor on the amino acid residues SER -360, LEU-361, and ASN-289 (Figure 2).

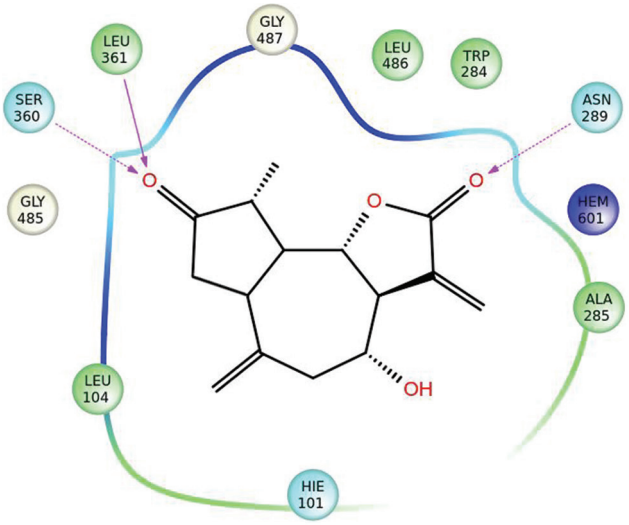


Figure 2: CYP7A1 in complex with grossheimin (4)

When the molecules of the studied compounds were docked to the target 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) (PDB-ID: 3CCT), the following values of the "ligand-receptor" interaction were calculated (Table 6).

Table 6: E_{CB} values for the 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) model

Rank	Compound Name	Binding energy (G-score) kcal/mol
1	Arglabin (1)	−4.29
2	Austricin (2)	−4.92
3	Achillin (3)	−3.26
4	Grossheimin (4)	−5.36
5	Grossmisin (5)	−4.78
6	Leucomisin (6)	−3.47
7	Ludartin (7)	−4.31
8	Matricarin (8)	−4.46
9	Matricin (9)	−5.02
10	Grossmisin oxime (10)	−4.39
11	Austricin oxime (11)	−4.12
12	Achillin oxime (12)	−3.58
13	5β(H)-austricin (13)	−5.02

It can be seen from the data in the table that the E_{CB} values of the molecules of the studied compounds with the HMGCR target are insignificant and may indicate a weak intermolecular bond.

Extremely low values of the "ligand-receptor" interaction (G-score) of the molecules of the studied compounds with the target were calculated for the fourth model, namely, the model of CPT1A (PDB-ID: 2M76) (Table 7).

Table 7: E_{CB} values for the carnitine palmitoyltransferase 1 model

Rank	Compound name	Binding energy (G-score) kcal/mol
1	Austricin (2)	−3.26
2	Achillin (3)	−2.48
3	Grossheimin (4)	−4.26
4	Grossmisin (5)	−3.01
5	Leucomisin (6)	−2.47
6	Matricarin (8)	−2.48
7	Matricin (9)	−2.92
8	Grossmisin oxime (10)	−3.04
9	Austricin oxime (11)	−3.33
10	Achillin oxime (12)	−2.88
11	5β(H)-austricin (13)	−2.85

Docking on a model of the human acetyl-CoA carboxylase (ACACA) enzyme system showed the following E_{CB} values (Table 8).

Table 8: E_{CB} values for the human acetyl-CoA carboxylase model

Rank	Compound name	Binding energy (G-score) kcal/mol
1	Arglabin (1)	−6.21
2	Austricin (2)	−3.87
3	Achillin (3)	−3.70
4	Grossheimin (4)	−7.38
5	Grossmisin (5)	−3.84
6	Leucomisin (6)	−3.18
7	Ludartin (7)	−5.89
8	Matricarin (8)	−2.90
9	Matricin (9)	−3.56
10	Grossmisin oxime (10)	−3.78
11	Austricin oxime (11)	−3.94
12	Achillin oxime (12)	−4.23
13	5β(H)-austricin (13)	−3.88

Compared to the Ecв values (G-score) calculated for CYP7A1, G-scores for the acetyl-CoA carboxylase (ACACA) model are generally low, which may indicate a weak intermolecular bond.

As a result of the docking of the molecules of the studied compounds to the target human acyl-CoA cholesterol acyltransferase (SOAT1) in complex with the Fab fragment of the agonistic antibody (PDB-ID: 4XWG), relatively high values of the "ligand-receptor" interaction (G-score) were calculated for molecules of grossmisin oxime (10), austricin oxime (11), and 5β(H)-austricin (13) (Table 9).

Table 9: E_{CB} values for the acyl-CoA-cholesterol acyltransferase model

Rank	Compound name	Binding energy (G-score) kcal/mol
1	Arglabin (1)	−5.99
2	Austricin (2)	−4.48
3	Achillin (3)	−5.18
4	Grossheimin (4)	−7.35
5	Grossmisin (5)	−2.90
6	Leukomisin (6)	−5.70
7	Ludartin (7)	−6.94
8	Matricarin (8)	−4.84
9	Matricin (9)	−4.94
10	Grossmisin oxime (10)	−7.27
11	Austricin oxime (11)	−7.07
12	Achillin oxime (12)	−5.85
13	5β(H)-austricin (13)	−6.64

It was found that due to the presence of two hydroxyl groups in the structure of the grossmisin oxime (10) molecule, the compound forms strong bonds with the enzyme target through the amino acid residues ARG-147 and VAL-81 (Figure 3).

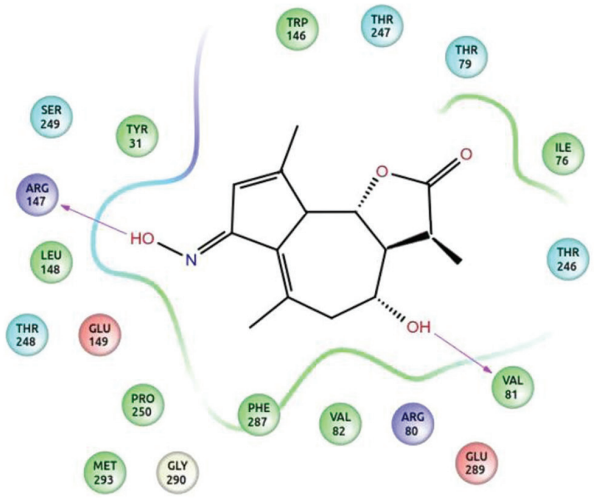


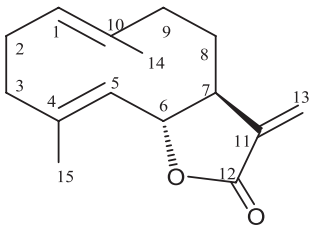
Figure 3: SOAT1 in complex with grossmisin oxime molecule (10)

Thus, as a result of computer modeling of the binding energy of the "ligand-target" complex (docking), it was found that for the molecules of sesquiterpene γ-lactones, matricin (9), arglabin (1), grossheimin (4), matricarin (8), grossmisin oxime (10), austricin oxime (11), and 5β(H)-austricin (13), relatively high and promising values of the energy of the "ligand-receptor" interaction (G-score) on the cholesterol 7α-hydroxylase (CYP7A1) enzyme system were calculated. In particular, relatively high values of Eb were calculated for the molecules matrixin (9), arglabin (1), and matricarin (8): −8.04, −7.56, and −7.28 kcal/mol, respectively. At the same time, the ability of grossheimin (4), due to the presence of a triple hydrogen bond in the structure, to form strong bonds with the receptor through the amino acid residues SER-360, LEU-361, and ASN-289 was found. Similarly, the matrixin molecule (9), due to the presence of a free hydroxyl group in the structure, forms strong bonds with the enzyme target through the amino acid residues HIS-101 and LEU-361. Also, promising $E_{св}$ values (G-score) were calculated for grossmisin oxime (10), austricin oxime (11), and 5*β*(H)-austricin (13) molecules using the acyl-CoA-cholesterol acyltransferase (SOAT1) model. Thus, due to the presence of two hydroxyl groups in the structure of the grossmisin oxime (10) molecule, a strong bond with the receptor is formed through the amino acid residues ARG-147 and VAL-81. Based on the data obtained, it is advisable to conduct biological screening of the lipid-lowering activity of these compounds *in vitro* and *in vivo*.

Results of biological screening for hypolipidemic activity in vivo

Metabolic syndrome causes an increase in the risk of diabetes mellitus II and cardiovascular diseases by 5 and 2 times, respectively. Long-term feeding of animals with a high fructose content causes a decrease in glucokinase activity and an increase in the activity of glucose-6-phosphatase in the liver, which leads to an increase in fructose gluconeogenesis. Fructose intake enhances ceramide biosynthesis and increases plasma, liver, and skeletal muscle concentrations of ceramides, which induce local insulin [21]. In addition, the levels of triacylglycerides, total cholesterol, and LDL increase significantly. Fructose enhances lipogenesis in the liver and reduces fatty acid oxidation in the liver, both of which can lead to increased accumulation of fat in the liver, which then causes liver inflammation and insulin resistance [22].

A study [23] showed that oral administration of the sesquiterpene lactone costunolide (16) significantly reduced AST, ALT, serum creatinine, and uric acid levels. Costunolid (16) restores normal concentrations of GSH, which can enhance cellular defense mechanisms, inhibit lipid peroxidation, and therefore protect body tissues from oxidative damage. These results indicated an effective mechanism of action for costunolide (16), reducing oxidative damage and increasing the cellular antioxidant pool.



(16)

1. Evaluation of the effect of the studied sesquiterpene γ-lactones and reference drugs on the expression of mRNA of the low-density lipoprotein receptor (LDLR) gene in the liver of rats with an atherogenic diet *in vivo*.

One of the processes that determine the level of intracellular cholesterol is its entry into the cell as part of low-density lipoprotein particles (LDLR) by receptor-mediated pinocytosis, which is carried out with the participation of LDLR receptors located on the plasma membrane of hepatocytes [24].

As a result of the experiment, it was found that an atherogenic diet led to a decrease in the expression of the mRNA of the LDLR receptor gene by 60% (0.40 ± 0.04 units relative to the control, $p < 0.05$) (Figure 4).

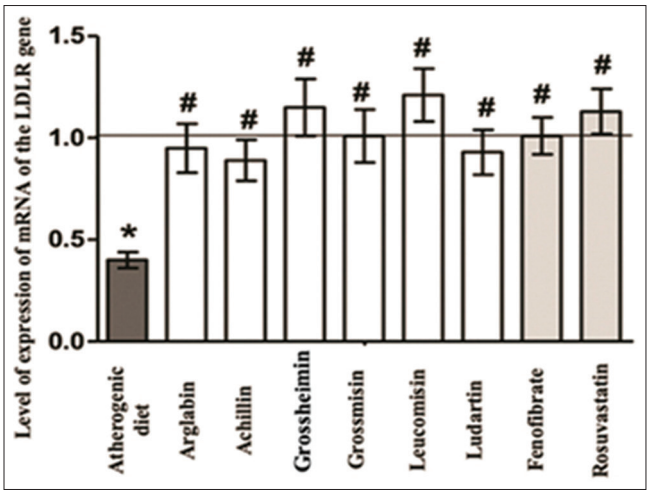
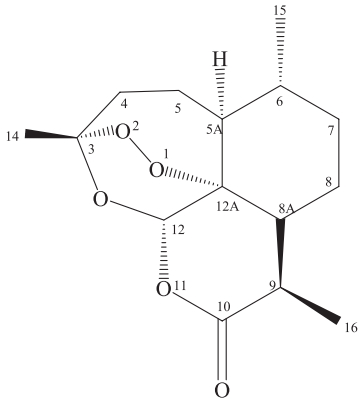


Figure 4: The effect of sesquiterpene lactones, fenofibrate (14) and rosuvastatin (15) on the expression of mRNA of the LDLR receptor gene in the liver of rats with an atherogenic diet, M ± m

Where, the value of mRNA expression of the LDLR gene is expressed in arb. units and normalized for the reference gene β-actin (ACTB) and control (standard diet). The relative expression of mRNA of the LDLR gene in the control was taken as one; * is the level of statistical significance compared with the control, $p < 0.05$; # is the level of statistical significance in comparison with the atherogenic diet, $p < 0.05$.

It is known that a decrease in the expression of the mRNA of the LDLR receptor gene under these conditions is one of the reasons for the increase in atherogenic LDLR circulating in the blood [24].

It was found that artemisinin (17) suppresses the expression of both mRNA and CD36 proteins and does not affect PPARα and SREBP1. As a very important molecule in the regulation of lipid metabolism, PPARα is a nuclear receptor expressed mainly in the liver, regulates gene transcription, and is involved in the uptake and oxidation of fatty acids by the liver. Whereas SREBP1 is the most important transcriptional factor regulation gene involved in de novo lipogenesis. According to the results of experiments, it was determined that artemisinin (17) does not affect hepatocellular lipid synthesis and fatty acid oxidation [25].



(17)

Comparator drugs from the groups of fibrates (fenofibrate (14)) and statins (rosuvastatin (15)) prevented a decrease in the level of LDLR gene mRNA expression, which is associated with one of the mechanisms of their hypolipidemic action [26]. The value of mRNA expression of the LDLR receptor gene when using rosuvastatin (15) was 1.13 ± 0.11 arb. units, and fenofibrate (14) – 1.01 ± 0.09 arb. units, which did not differ from the value of LDLR gene mRNA expression in the liver of intact animals. The studied sesquiterpene γ-lactones, like the reference drugs, prevented a decrease in the LDLR gene mRNA expression in the liver of rats on an atherogenic diet. This parameter was 0.95 ± 0.12 arb. units (arglabin (1)), 0.89 ± 0.10 arb. units (achillin (3)), 1.15 ± 0.14 arb. units (grossheimin (4)), 1.01 ± 0.13 arb. units (grossmisin (5)), 1.21 ± 0.13 arb. units (leucomisin (6)), and 0.93 ± 0.11 arb. units (ludartin (7)), and did not differ from the level of LDLR gene mRNA expression in the liver of animals fed standard laboratory food.

2. Evaluation of the effect of sesquiterpene lactones, fenofibrate (14) and rosuvastatin (15), on the mRNA expression of the 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) gene in the liver of rats with an atherogenic diet *in vivo*.

The enzyme 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) is localized to the endoplasmic reticulum and peroxisomes and limits the rate of cholesterol biosynthesis. This enzyme is expressed in all tissues but in the greatest amount in the liver, which plays a central role in the regulation of cholesterol metabolism and its concentration in blood plasma [27].

It was experimentally established that the mRNA expression of the HMGCR gene during an atherogenic diet decreased by 29% ($p < 0.05$) and amounted to 0.71 ± 0.08 arb. Units. It is known that the end product of the metabolic pathway, cholesterol, regulates the rate of transcription of the HMGCR gene according to the principle of negative feedback. Therefore, a decrease in HMGCR gene expression in the liver with an atherogenic diet is due to a significant increase in the cholesterol level in hepatocytes. Fenofibrate (14) prevented the decrease in HMGCR mRNA expression in the liver of animals with an atherogenic diet and amounted to 1.21 ± 0.10 arb. units ($p < 0.05$). This value did not differ from the value of HMGCR mRNA expression in the liver of animals fed the standard diet. Rosuvastatin (15) increased HMGCR mRNA expression by 1.8 times (1.82 ± 0.17 c.u., $p < 0.05$) (Figure 5).

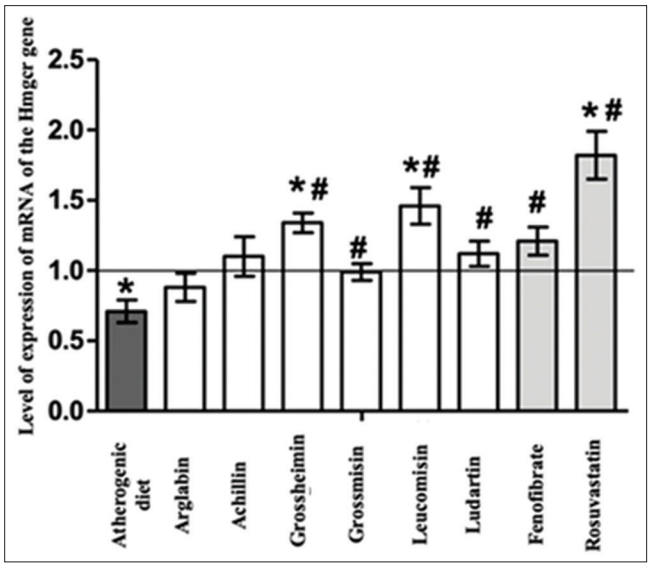


Figure 5: The effect of sesquiterpene lactones, fenofibrate (14) and rosuvastatin (15) on the expression of HMGCR gene mRNA in the liver of rats with an atherogenic diet, M ± m

It is known that inhibition of HMGCR *in vivo* by statins causes a complex response accompanied by an increase in the expression of HMGCR genes and LDLR receptors [24]. Grossheimin (4) and leukomisin (6) on the background of an atherogenic diet, like rosuvastatin (15), contributed to an increase in the expression of HMGCR gene mRNA by 1.3 times (1.34 ± 0.07 c.u., $p < 0.05$) and 1.5 times (1.46 ± 0.13 units, $p < 0.05$), which may be due to inhibition of the activity of this enzyme. An increase in HMGCR mRNA expression in the liver of animals under the action of leucomisin (6) confirms the previously shown ability of this lactone (6) to inhibit the activity of the key enzyme of cholesterol synthesis [28]. Sesquiterpene γ-lactones arglabin (1), achillin (3), grossmisin (5), and ludartin (7), as well as fenofibrate (14), prevented a decrease in the HMGCR gene mRNA expression in the liver of rats with an atherogenic diet and amounted to 0.88 ± 0.10 arb. units ($p > 0.05$), 1.10 ± 0.14 arb. units ($p > 0.05$), 0.99 ± 0.06 arb. units ($p > 0.05$), and 1.12 ± 0.09 arb. units ($p > 0.05$), respectively.

3. Evaluation of the effect of sesquiterpene lactones, fenofibrate (14), and rosuvastatin (15), on the mRNA expression of the acyl-CoA-cholesterol acyltransferase (SOAT1) gene in the liver of rats with an atherogenic diet *in vivo*.

Acyl-CoA cholesterol acyltransferase (SOAT1) is an enzyme catalyzing the formation of cholesterol esters from cholesterol [29]. It was found that the atherogenic diet in rats led to an increase in the expression of mRNA of the SOAT1 gene by 3.2 times (3.17 ± 0.31 units, $p < 0.05$) compared with the expression of the enzyme in the liver of animals treated with standard laboratory food (Figure 6).

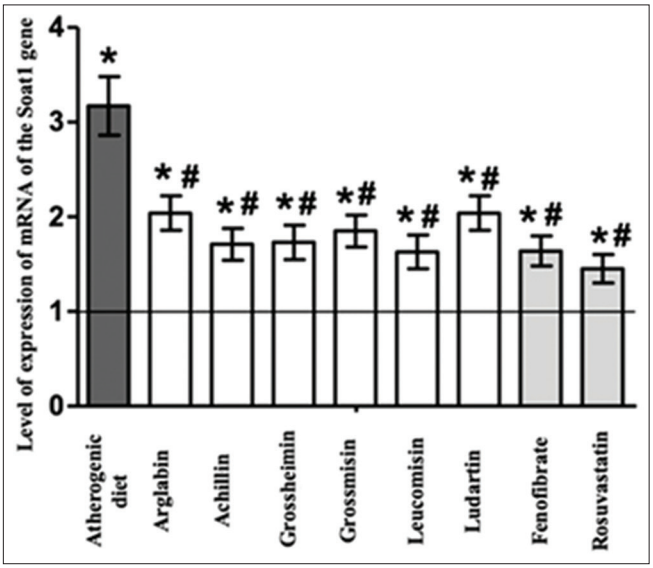


Figure 6: Influence of sesquiterpene lactones, fenofibrate (14) and rosuvastatin (15) on SOAT1 gene mRNA expression in rat liver with atherogenic diet, M ± m

The comparator drugs rosuvastatin (15) and fenofibrate (14) on the background of an atherogenic diet contributed to a decrease in the SOAT1 mRNA expression by 54% (1.45 ± 0.15 c.u., $p < 0.05$) and 49% (1.64 ± 0.16 units, $p < 0.05$), respectively. Achillin (3) and leukomycin (6) also reduced the SOAT1 mRNA expression increased on the background of an atherogenic diet by 46% (1.71 ± 0.17 c.u., $p < 0.05$) and by 49% (1.63 ± 0.18 units, $p < 0.05$), respectively. Arglabin (1) (2.04 ± 0.18 units, $p < 0.05$), grossheimin (4) (1.73 ± 0.18 units, $p < 0.05$), grossmisin (5) (1.85 ± 0.17 units, $p < 0.05$) and ludartin (7) (2.04 ± 0.18 units, $p < 0.05$) decreased the mRNA expression of the studied gene by 36%, 45%, 42% and 36% respectively.

Cholesterol esterification mediated by SOAT1 is known to play an important role in the packaging, production, and secretion of VLDL by the liver [30]. The SOAT1 enzyme modulates the catabolism of cholesterol in hepatocytes and its concentration in plasma, reducing the level of free cholesterol in liver cells, contributing to a decrease in the expression and activity of 7α-hydroxylase (CYP7A1). With an atherogenic diet, an increase in SOAT1 activity increases the secretion of VLDL by hepatocytes. It has been shown that inhibitors of this enzyme increase in the synthesis of fatty acids through an increase in the expression of the CYP7A1 gene. Thus, an increase in SOAT1 expression may contribute to a decrease in the expression of the CYP7A1 enzyme and the development of hypercholesterolemia in an atherogenic diet [31].

4. Evaluation of the effect of sesquiterpene lactones, fenofibrate (14), and rosuvastatin (15) on the expression of mRNA of the cholesterol 7α-hydroxylase (CYP7A1) gene in the liver of rats with an atherogenic diet *in vivo*.

The liver eliminates excess cholesterol from the body by direct secretion into bile or after its conversion into bile acids through enzymatic pathways, which are influenced by the rate-limiting enzyme in bile acid synthesis, cholesterol 7α-hydroxylase (CYP7A1) [32], [33].

During the experiment, it was found that the atherogenic diet in rats led to a decrease in the expression of mRNA of the CYP7A1 gene by 25% and amounted to 0.75 ± 0.06 arb. units ($p < 0.05$). One of the reasons for the decrease in CYP7A1 expression in an atherogenic diet may be the increased expression of the SOAT1 gene found in these animals [31]. Rosuvastatin (15) increased the expression of CYP7A1 mRNA in the liver of animals with an atherogenic diet by 4.6 times (4.62 ± 0.46 c.u., $p < 0.05$) (Figure 7). As is known, statins increase the expression of mRNA of the key enzyme of fatty acid synthesis CYP7A1 [34].

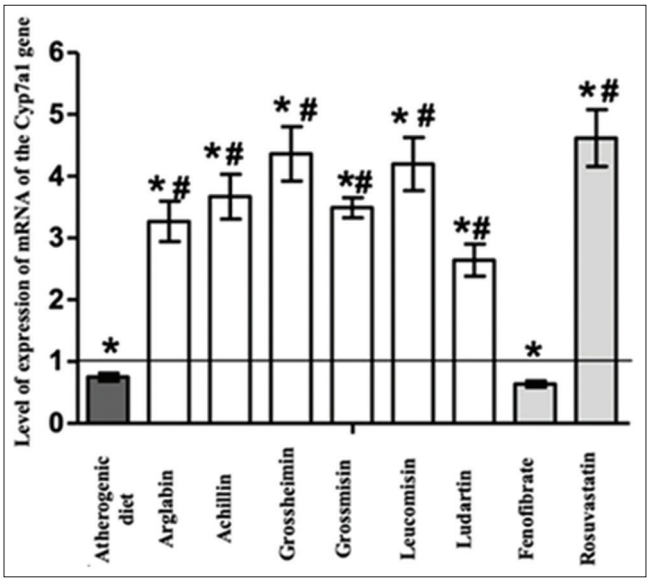


Figure 7: Effect of sesquiterpene lactones, fenofibrate (14) and rosuvastatin (15) on CYP7A1 mRNA expression in rat liver with an atherogenic diet, M ± m

Fenofibrate (14), on the contrary, did not change the value of CYP7A1 mRNA expression, which was reduced on the background of an atherogenic diet, while its value remained below the control and amounted to 0.64 ± 0.06 arb. units, $p < 0.05$. Fibrates, including fenofibrate (14), reduce CYP7A1 mRNA expression in patients with hyperlipidemia [35], [36] and in HepG2 hepatoma cell culture [37]. A decrease in the expression

of mRNA of the CYP7A1 gene contributes to a decrease in the synthesis and secretion of fatty acids, which leads to an increase in the lithogenicity of bile, that is, to the risk of gallstone formation [32]. Therefore, fibrates are contraindicated in patients with gallstone disease. The tested lactones, like rosuvastatin (15), increased the mRNA expression of the studied gene. Grossheimin (4) increased the expression of the CYP7A1 gene to the greatest extent (4.4 times) (4.36 ± 0.44 units, $p < 0.05$). Leukomisin (6) increased the expression of mRNA of the CYP7A1 gene by 4.2 times (4.2 ± 0.43 units, $p < 0.05$), grossmisin (5) by 3.5 times (3.49 ± 0, 16 units, $p < 0.05$), Achillin (3) by 3.7 times (3.67 ± 0.36 units, $p < 0.05$), arglabin (1) by 3.3 times (3.27 ± 0.33 units, $p < 0.05$), and Ludartin (7) 2.6 times (2.64 ± 0.26 units, $p < 0.05$).

5. Evaluation of the effect of sesquiterpene lactones, fenofibrate (14) and rosuvastatin (15) on the mRNA expression of the CPT1A and CPT2 genes in the liver of rats with an atherogenic diet *in vivo*.

Fatty acids with a long hydrocarbon chain are transported through the inner membrane of mitochondria with the help of carnitine. Therefore, CPT1A is an enzyme that regulates the rate of oxidation of long-chain fatty acids in mitochondria. This enzyme is located on the outer membrane of mitochondria and transports long-chain fatty acids in mitochondria, catalyzing the reaction with the formation of acylcarnitine. The resulting acylcarnitine passes through the intermembrane space to the outer side of the inner membrane and is transported by carnitine acylcarnitine translocase to the inner surface of the inner mitochondrial membrane, where the CPT2 enzyme catalyzes the transfer of acyl to the pool of intramitochondrial CoA [38]. In this regard, studies of mRNA expression of both genes of enzymes involved in fatty acid catabolism were carried out. As a result, it was found that with an atherogenic diet, the value of mRNA expression of the CPT1A and CPT2 genes decreased by 29% (0.71 ± 0.1 arb. units, $p < 0.05$) and 26% (0.74 ± 0.08 arb. units). units, $p < 0.05$), respectively (Figures 8 and 9).

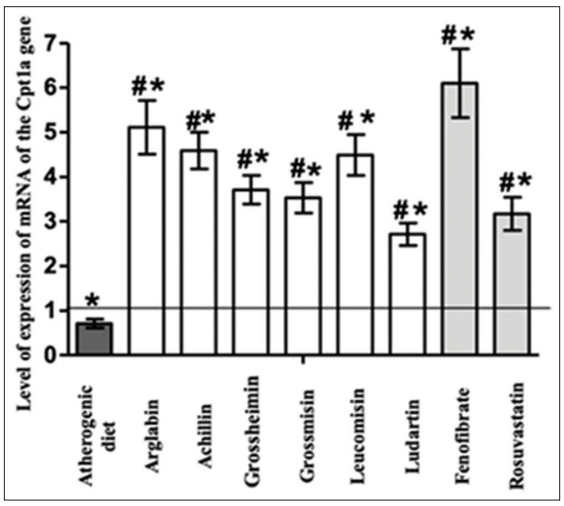


Figure 8: Effect of sesquiterpene lactones, fenofibrate (14) and rosuvastatin (15) on CPT1A mRNA expression in rat liver with atherogenic diet, M ± m

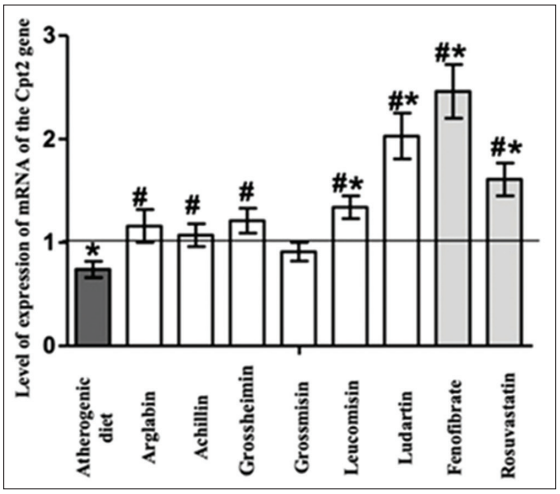


Figure 9: The effect of sesquiterpene lactones, fenofibrate (14) and rosuvastatin (15) on the expression of CPT2 gene mRNA in the liver of rats with an atherogenic diet, M ± m

It is known that as a result of activation of PPAR-α, there is an increase in the expression of genes for enzymes involved in the oxidation of long-chain fatty acids. On the contrary, in animals fed cholesterol (1%) with conventional food, a decrease in the amount of CPT1A and CPT2 mRNA is observed [27]. This indicates that when rats are fed cholesterol, fatty acid catabolism decreases, which may be the reason for the increase in triglyceride levels in the liver.

Thus, fenofibrate (14) significantly increased the mRNA expression of both genes by 6.1 times (CPT1A) and 2.5 times (CPT2), while the expression value was 6.1 ± 0.77 arb. units ($p < 0.05$) and 2.46 ± 0.26 arb. units ($p < 0.05$), respectively. Rosuvastatin (15) also increased the expression of mRNA of the CPT1A and CPT2 genes by 3.2 times (3.17 ± 0.37 units, $p < 0.05$) and 1.6 times (1.61 ± 0.16 units, $p < 0.05$).

Like the reference drugs, the studied lactones increased the value of CPT1A mRNA expression: arglabin (1) by 5.1 times (5.11 ± 0.60 units, $p < 0.05$), achillin (3) by 4.6 times (4.59 ± 0.41 units, $p < 0.05$), leucomisin (6) 4.5 times (4.49 ± 0.46 units, $p < 0.05$), grossheimin (4) by 3.7 times (3.71 ± 0.32 units, $p < 0.05$), grossmisin (5) by 3.5 times (3.53 ± 0.34 units), $p < 0.05$), ludartin (7) by 2.7 times (2.71 ± 0.25 c.u., $p < 0.05$).

The studied lactones had a less pronounced effect on the expression of the CPT2 gene mRNA. Thus, ludartin (7) and leucomisin (6) increased the expression of CPT2 mRNA by 2 times (2.03 ± 0.22 units, $p < 0.05$) and 1.3 times (1.34 ± 0.11 units, $p < 0.05$), respectively. Arglabin (1) (1.16 ± 0.16 units, $p > 0.05$), Achillin (3) (1.07 ± 0.11 units, $p > 0.05$), and Grossheimin (4) (1.21 ± 0.12 c.u., $p > 0.05$) prevented the decrease in CPT2 mRNA expression caused by the atherogenic diet, and their values did not differ from the control (standard laboratory diet). Grossmisin (5) had no effect on the level of CPT2 mRNA expression.

Conclusion

1. Virtual screening of the lipid-lowering activity of sesquiterpene γ-lactones and their derivatives by molecular docking revealed a number of promising compounds (matricin, matricarin, grossmisin oxime, austricin oxime, 5β(H)-austricin) receptor interactions on the enzyme system cholesterol 7α-hydroxylase (CYP7A1). Relatively high values of E_{CB} were calculated for the molecules of matricin and matrikarin: 8.04 and –7.28 kcal/mol-1, respectively. At the same time, the ability to form strong bonds with the amino acid residues HIS-101 and LEU-361 on the protein target of the matrixin molecule was found due to the presence of a free hydroxyl group in the structure of the molecule. Similarly, prospective Eb values were calculated for grossmisin oxime, austricin oxime, and 5β(H)-austricin molecules using the acyl-CoA cholesterol acyltransferase (SOAT1) model.
2. New mechanisms of lipid-lowering activity for sesquiterpene γ-lactones were proposed, which were established in the study of gene expression of key enzymes of lipid metabolism in the liver in a model of hyperlipidemia caused by an atherogenic diet in rats under *in vivo* conditions.

Thus, the sesquiterpene γ-lactone arglabin, on the background of an atherogenic diet in the liver of rats, increases the expression of mRNA of the cholesterol 7α-hydroxylase (CYP7A1) and CPT1A genes and reduces the expression of the acyl-CoA-cholesterol acyltransferase (SOAT1) gene.

Achillin increases the mRNA expression of the CPT1A gene, the cholesterol 7α-hydroxylase (CYP7A1) gene, and reduces the expression of the acyl-CoA-cholesterol acyltransferase (SOAT1) gene.

Sesquiterpene γ-lactone grossheimin on the background of an atherogenic diet in the liver of rats increases the amount of mRNA expression of the cholesterol 7α-hydroxylase gene (CYP7A1), the CPT1A gene, the 3-hydroxy-3-methylglutaryl-CoA reductase gene (HMGCR), the gene receptor for LDLR, and reduces the amount of expression of the gene acyl-CoA-cholesterol acyltransferase (SOAT1).

Grossmisin, on the background of an atherogenic diet in rat liver, increases the mRNA expression of the CPT1A gene and the cholesterol 7α-hydroxylase (CYP7A1) gene and also reduces the expression of the acyl-CoA cholesterol acyltransferase (SOAT1) gene. On the background of an atherogenic diet in the liver of rats, leucomisin increases the mRNA expression of the CPT1A, CPT2 gene, and the 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) gene and reduces the expression of the acyl gene, CoA-cholesterol acyltransferase (SOAT1).

Lactone ludartin against the background of an atherogenic diet in the liver of rats reduces the mRNA expression of the acyl-CoA-cholesterol acyltransferase (SOAT1) gene, increases the mRNA expression of the cholesterol 7α-hydroxylase (CYP7A1) gene, the CPT1A gene, and the carnitine gene-palmitoyltransferase 2 (CPT2).

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