Identifying of selective agonists targeting LXR β from terpene compounds of Alismatis Rhizoma

Chuanjiong Lin

Sichuan University

Jianzong Li (✉ lijianzong@hotmail.com)

Sichuan University

Chuanfang Wu

Sichuan University

Jinku Bao

Sichuan University

Original paper

Keywords: Hyperlipidemia, LXR α , LXR β , molecular docking, molecular dynamics simulations, agonist, Alismatis Rhizoma

Posted Date: February 1st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-164666/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Version of Record: A version of this preprint was published at Journal of Molecular Modeling on February 22nd, 2021. See the published version at <https://doi.org/10.1007/s00894-021-04699-z>.

Abstract

Hyperlipidemia is thought as an important contributor to coronary disease, diabetes and fatty liver. Liver X receptors β (LXR β) was considered as a validated target for hyperlipidemia therapy due to its role in regulating cholesterol homeostasis and immunity. However, many current drugs applied in clinic are not selectively targeting LXR β and they can also activate LXR α which activate SREBP-1c worked as activator of lipogenic genes. Therefore, exploiting agonists selectively targeting LXR β are urgent. Here, computational tools were used to screen potential agonists selectively targeting LXR β from 112 terpenes of *Alismatis Rhizoma*. Firstly, structural analysis between selective and nonselective agonists were used to explore key residues of selective binding with LXR β . Our data indicated that Phe²⁷¹, Ser²⁷⁸, Met³¹², His⁴³⁵, Trp⁴⁵⁷ were important to compounds binding with LXR β , suggesting that engaging ligand interaction with these residues may provide directions for development of ligands with improved selective profiles. Then, ADMET analysis, molecular docking, MD simulations and calculation of binding free energy and its decomposition were executed to screen the agonists whose bioactivity were favorable from 112 terpenes of *Alismatis Rhizoma*. We found that two triterpenes 16-hydroxy-alisol B 23-acetate and Alisol M 23-acetate showed favorable ADMET properties and high binding affinity against LXR β . These compounds could be considered as promising selective agonists targeting LXR β . Our work provides an alternative strategy for screening agonists selectively targeting LXR β from *Alismatis Rhizoma* for hyperlipidemia disease treatment.

Introduction:

Hyperlipidemia is characterized by increased triglyceride (TG), total cholesterol (TC) and lower-density lipoprotein cholesterol (LDL-C) level and decreased high-density lipoprotein cholesterol (HDL-C) level in the blood[1]. Hyperlipidemia is considered an essential contributor to not only coronary arteries and coronary heart disease, also hypertension and diabetes. 1% increase in plasma cholesterol level will enhance risk of coronary events almost 3%[2]. In addition, excessive accumulation of TG caused by hypercholesterolemia in the liver is responsible for nonalcoholic fatty liver disease[3]. According to the data model predicted by the World Health Organization (WHO), it will be up to 40% of all deaths that are related to cardiovascular disease by 2030[4]. Hyperlipidemias are divided into six types by WHO based on whether level of lipids and lipoproteins are increased[5]. The most common frequent hyperlipidemias are combined family hyperlipidemia and polygenic hypercholesterolemia[6]. Causes of hyperlipidemia are very complicated, which are relevant to environmental factors and genetic factors[5]. In recent years, due to people's diet and unhealthy lifestyle, risk of hyperlipidemia has greatly increased[7].

One of the causes of hyperlipidemia is high level of TC, which is tightly regulated by liver X receptor (LXR) [1, 8]. LXR is a nuclear receptor with two isoforms, LXR α and LXR β . LXR α is mainly distributed in liver, intestines, adipose tissue and macrophages while LXR β can be found in various systemic tissues[9, 10]. In vivo, LXR α and LXR β all have the ability to promote cholesterol efflux by modulating transcriptional activation of genes[11]. LXR α is the upstream activating gene of sterol response element binding protein-1c (SREBP-1c). SREBP-1c is a transcription factor to regulate expression of various lipogenic genes, such

as acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS), sequentially causing an undesirable increase of TC levels in plasma and liver[12, 13]. However, different from LXR α , LXR β is considered to have less effect on the lipid synthesis pathway[14]. After forming permissive heterodimers with retinoid X receptor (RXR), LXR functions as lowering blood lipid by binding to LXR response element (LRE), which has been found in the target gene promoter region[15]. Moreover, LXR ligands induce the expression of the members of the ATP binding cassette family of lipid transporter genes, such as ABCA1, ABCG and ABCA2. In addition, LXR ligands can also promote transcription of lipid synthesis genes, which are represented of SCD, ACC and FASN[16].

Natural products are potential resources for discovery of small molecules modulating target activity. *Alismatis Rhizoma*, the dried tuber of *Alisma orientale* (Sam.) Juz. (Alismataceae), is a kind of traditional Chinese medicine (TCM) widely distributed in China, Russia, Japan, Mongolia and North India[17]. In East Asia, *Alismatis Rhizoma* has been used to treat many diseases, such as hyperlipidemia, diabetes, hypertension and urological diseases[18]. It's worth noting that the most effective constituents of *Alismatis Rhizoma* are attributed to terpenes[19]. Terpenes isolated from *Alismatis Rhizoma* have been proved to enhance transactivation for farnesoid X receptors (FXR), which belongs to nuclear receptors superfamily along with LXR[20]. These terpenes are mainly composed of sesquiterpenes, diterpenes and triterpenes[17]. Among them, protostane-type triterpenes are considered a major contributor to pharmacodynamic and bioactive compounds of *Alismatis Rhizoma*. Moreover, protostane-type triterpenes are also thought to be chemotaxonomic markers of *Alismatis Rhizoma*[21]. Therefore protostane-type triterpenes have drawn increasingly attention in recent years because of their unique biological functions in treating chronic diseases, such as hyperlipidemia, diabetes, hypercholesterol and cancer[22]. Alisol A, alisol B and their acetylation and alisol C 23-acetate are major components of protostane-type triterpenes[23]. In addition, alisol B 23-acetate, alisol C 23-acetate, alisol A and its acetylation have shown superb effect to reduce lipid, and alisol B and alisol B 23-acetate are involved in autophagy, apoptosis, endoplasmic reticulum stress in cancer cells[21].

The study aims to screen selective agonists targeting LXR β from terpenes of *Alismatis Rhizoma*. Firstly, structures of LXR α and LXR β were analyzed, and differences of protein sequences and residues which highly stabilized in site-directed mutation were evaluated. Then, we constructed a verification standard to verify agonists whether possessed LXR β selectivity by analyzing the differences of binding mode of developed agonists between LXR α and LXR β . A dataset of 112 terpenes from *Alismatis Rhizoma* was constructed as the raw material of the study. SwissADME and ADMETlab were used to predict ADMET properties in order to eliminate compounds whose pharmacokinetic parameters were unfavorable. Then, molecular docking was executed by Autodock Vina for the sake of preliminarily evaluating binding affinities of ligands with LXR α and LXR β . In order to verify reliability of docking model, Decoyfinder 2.0 was applied to construct a set of decoy molecules for a group of ligands which were binding closely with LXR β . The receiver operating characteristic (ROC) curves of docking score of decoy molecules were plotted and area under curves (AUC) values were calculated. Moreover, molecular dynamic simulation was conducted using Gromacs to test the dynamic binding mode of ligands and receptors during a specified simulation time. Furthermore, binding free energy and its decomposition were calculated by

g_mmpbsa package to predict binding affinity between ligands and proteins. In addition, the binding model analysis were executed by Ligplot⁺ and PyMOL. This study serves as an alternative strategy for screening selective agonists targeting hypolipidemic target from traditional Chinese medicine.

Methods:

Structure collection and preparation

Alismatis Rhizoma was proved effective herb that was responsible for treating hyperlipidemia, and terpenes were main active components of the herb. Therefore, 112 triterpenes and sesquiterpenes from *Alismatis Rhizoma* were collected from databases and literature[24]. In order to identify key residues that play selective role in ligand and LXR β binding, 22 known selective or nonselective agonists were collected from different databases. The structures of LXR α (PDB code:5HJS, X-ray diffraction resolution with 1.72 Å), LXR β (PDB code:6S4T, X-ray diffraction resolution with 2.00 Å) were retrieved from RCSB protein data bank[25].

Pairwise sequence alignment

In order to identify regions of similarity of LXR α and LXR β , the Application Programming Interfaces (APIs) tool in EMBL-EBI (European Molecular Biology Laboratory-European Bioinformatics Institute)[26] was used. Firstly, sequences of LXR α (Entry:Q13133) and LXR β (Entry:P55055) were obtained from Uniprot database[27], which was an essential database of protein sequences. Then, sequences of proteins were imported into the APIs tool and the parameters of tool were set as following: output format: pair. matrix: blosum62. gap open = 10. gap extend = 0.5. end gap penalty: false. end gap open = 10. end gap extend = 1. Click "Submit" to run the alignment and Smith-Waterman algorithm was applied during the process. The result was output in several seconds and the length, similarity, gaps and identity were shown as a list.

Alanine scanning

In order to estimate the stability effect of all possible mutations of each residue of LXR, FoldX 4.0[28] was introduced to calculate change of binding free energy of LXR between wild type and mutations. Each residue in mutations was changed into standard α -amino acids using the BuildModel function after applying the automatic RepairPDB function. The temperature was set to 298 K and other options were set to default. The result for respective value was shown as Δ Stability, which means binding free energy of the alanine mutations minus that of wild type (Δ Stability = $\Delta G_{mut} - \Delta G_{wt}$).

ADMET analysis

Suitable ADMET properties was the first hurdle for most drugs, pharmacokinetic parameters (absorption, distribution, metabolism, excretion and toxicity, ADMET) were significant contributors to discovery of drug candidates[29]. Therefore, SwissADME[30] and ADMETlab[31] were applied to predict ADMET properties. Here, compounds with high absorption and low toxicity and that met Lipinski's rule of five (RO5) were the

study considered for further. The details of RO5 are as follows: molecular weights (MW) ≤ 500 ; the calculated lipophilicity ($\log P$) ≤ 5 ; the number of hydrogen bond acceptors (HBA) ≤ 10 ; the number of hydrogen bond donors (HBD) ≤ 5 ; number of rotatable bonds (RB) < 5 [32, 33].

Molecular docking model validation

In order to identify reliability of docking model, the receiver operating characteristic (ROC) curves of docking functions were analyzed. Decoyfinder 2.0 was utilized to establish a set of decoy molecules for ligands which were obtained from the ChEMBL database according to affinities ($K_i \leq 3000$)[34]. The parameters of DecoyFinder 2.0 were set as follows[35]: Active ligand vs. decoy Tanimoto threshold ≤ 0.75 . Decoy vs. decoy Tanimoto threshold ≥ 0.90 . Hydrogen bond acceptors ± 2 . Hydrogen bond donors ± 1 . Molecular weight ± 25 Da. Rotational bonds ± 1 . Octanol-water partition coefficient ($\log P$) ± 1.00 . Maximum standard deviations away from active = 1.00. Maximum decoys per active ligand = 36. Minimum decoys per active ligand = 36. Then, the ligands and decoys were regarded as the input set of molecular docking, and docking results were shown as the ROC curves and the area under the curves (AUC) were calculated by IBM SPSS program. The parameters of molecular docking will be described in the next paragraph.

Molecular docking

After verifying the druggability of molecules and the reliability of the docking model, molecular docking was performed using Autodock Vina[36] as a tool of virtual screening. Firstly, the UCSF chimera molecular visualization application[37] was used to separate ligands and receptors and eliminate water on receptors. Then, Autodock Tools (ADT, version 1.5.6)[38] was applied to add hydrogen atoms, Gasteiger charges and atom types to receptors and the number of torsions for ligands. After that, ligands and receptors were output as pdbqt files which can be recognized by Autodock Vina program. A grid box was generated by ADT for AD4 atom types and centered on ligand. The size of box was set as follows: size_x = 15 Å, size_y = 18.75 Å, size_z = 15 Å, and the center of box was set as follows: center_x = 16.68, center_y = 10.492, center_z = 15.764. The Vina score was calculated by Autodock Vina.

Ledock score, Grid score and Amber score were also used to test binding affinity between ligands and receptors. Ledock score was obtained from Ledock[39]. Grid score and Amber score were calculated from UCSF Dock6[40]. First of all, preparation of docking models of the three functions were the same. Water removal of protein and energy minimization of ligands should be executed by UCSF chimera[37]. Then, operation steps were different for the three functions. For Ledock, GetBox plugin in PyMOL program was introduced to set an enclosing box which is centered on ligands. Then, proteins and ligands files were input into Ledock to dock. The protein files were set as the input of LePro and hydrogens were added automatically while the ligand files were input of LeDock. For Dock 6, docking process was executed according to the docking user manual, and the Grid score and Amber score were applied.

Molecular dynamics simulations

In order to probe the flexibility and structural stability of screened compound candidates, we chose the out files of molecular docking and the water-free receptors as raw materials of molecular dynamics (MD) simulations. MD simulations were executed by Gromacs 5.1.4[41] for a period of 20 ns. First of all, ligands and receptors were transformed into pdb2gmx format and mol2 format, respectively. To convert pdb files to pdb2gmx format, the AMBER99SB force field and the tip3p water model were applied to generate protein structure in the topology and gro files which contain protein sequences. The General Amber force field (GAFF)[42] was used for ligands, and partial charges of ligands were determined by Antechamber program[43]. To check missing force field parameters and amend the field files, the parmchk program was employed[43]. Antechamber Python parser interface (ACPYPE) tools were used to generate ligand structure in the topology and ligand sequences[44]. Then a box with distance of 1.0 nm from the protein to the edge of boundary was defined. Na⁺ and Cl⁻ were added at a concentration of 0.1 mol·L⁻¹ to ensure an electroneutral ionic environment, equivalent to one Na⁺ and one Cl⁻ for every 600 water molecules. In order to minimize total energy of the system, adjusting wrong bonds and angles of stretching or compression, energy minimization algorithm was introduced to stabilize the system[45]. After that, under guidance of index files and with limit of proteins and ligands, simulations of 100 ps NVT (constant number of particles, volume, and temperature) and 100 ps NPT (constant number of particles, pressure, and temperature) equilibrations were executed at constant temperature (300 K) and pressure (1 atm). 20 ns MD simulations were performed with a timestep of 2 fs at constant pressure and temperature. The Linear Constraint Solver (LINCS) algorithm was used to constrain all covalent bond lengths during the process[46] and particle mesh Ewald (PME) method was used to calculate the long-range electrostatic interactions[47], while the SETTLE algorithm is applied for water molecules[48]. Finally, gmx_rmsd and gmx_rmsf in Gromacs 5.1.4 were employed for root mean square deviation (RMSD) and root mean square fluctuation (RMSF) analysis on the entire 20 ns track[41]. Then, xmgrace was used to draw RMSD and RMSF results in order to visualize the output of RMSD and RMSF analysis.

Calculation of MM/PBSA binding free energy

In order to explore binding affinity between ligands and proteins, the Molecular Mechanics Poisson-Boltzmann Surface Area (MM/PBSA)[49] was introduced to calculate binding free energy of ligand-receptor complexes by g_mmpbsa tool. The last 2 ns trajectory of MD results was extract as raw material of binding free energy. In this approach, binding free energy consists of the following components:

$$\Delta G_{\text{bind}} = \Delta H - T \Delta S \approx \Delta G_{\text{sol}} + \Delta G_{\text{MM}} - T \Delta S$$

$$\Delta G_{\text{MM}} = \Delta G_{\text{int}} + \Delta G_{\text{vdw}} + \Delta G_{\text{ele}}$$

$$\Delta G_{\text{sol}} = \Delta G_{\text{polar}} + \Delta G_{\text{apolar}}$$

Where G_{bind} represented binding free energy. G_{MM} was molecular mechanics energy of gas phase, and G_{sol} was solvation free energy. G_{int} was bonded interaction between molecules, including bond, angle and dihedral energy. G_{vdw} was Van der Waals' force and G_{ele} was electrostatic interaction. G_{sol} was composed of G_{polar} and G_{apolar} . The former represented polar solvation energy for unbound protein, unbound ligand

and protein-ligand complex while the latter was nonpolar solvation energy for them. The entropic contribution was not considered due to huge amount of computation and low accuracy. ΔG_{int} was taken as zero because receptor-ligand complex was usually considered as an invariant conformation in calculation. Consequently, binding free energy (G_{bind}) were composed of Van der Waals' force (G_{vdw}), electrostatic interaction (G_{ele}), polar solvation energy (G_{polar}) and nonpolar solvation energy (G_{apolar}). Thus

$$\Delta G_{\text{bind}} = \Delta G_{\text{MM}} + \Delta G_{\text{polar}} + \Delta G_{\text{apolar}}$$

$$\Delta G_{\text{MM}} = \Delta G_{\text{vdw}} + \Delta G_{\text{ele}}$$

Decomposition of MM/PBSA binding free energy

The binding free energy decomposition was performed to obtain contribution of key residues for the sake of analysing interactions between ligands and LXR through dynamic way. g_mmpbsa tool in Gromacs 5.1.4 was used to extract a section of 2 ns trajectory from MD results and turned the trajectory into a total of 100 snapshots. Subsequently, decomposition of residues was calculated. Finally, a script written in Python was applied to extract detailed information about binding free energy decomposition in the form of ΔG_{MM} , ΔG_{polar} , and ΔG_{apolar} .

Binding model analysis

The binding model analysis was performed to explore the key residues of LXR-ligand binding. The output files of molecular docking and pdb files of LXR β were combined as the raw material of binding model analysis. Then, Ligplot⁺ (version 2.2)[50] was introduced to analyse binding pattern in 2D. 3D LXR-ligand interaction diagram was analysed and illustrated by PyMOL (version 1.8).

Results:

Structural analysis of LXR and construction of validation standard

In order to explore structure of LXR α and LXR β and search residues with significant differences between them, alanine scanning and pairwise sequence alignment were used to evaluate stability effect of residues after site-directed mutation and differences of protein sequences respectively. We also selected selective and nonselective agonists from different database and discovered differences of the binding mode between them. We regarded residues which bind to all selective agonists but bind to nonselective agonists poorly as LXR β selective residues. The residues were used as standard to verify the selectivity of screened compounds.

Comparison of protein sequences of LXR α and LXR β

In order to analyze the differences of protein sequences between LXR α and LXR β , pairwise sequence alignment of LXR α and LXR β was executed by APIS tool using Smith-waterman algorithm. The pairwise sequence alignment results are illustrated in Figure 1. Full length of LXR attained 62.5% sequence identity and 72.7% sequence similarity. Ligand binding domain (LBD) shared 88.5% sequence similarity and 78.3% sequence identity, which was consistent with previous research[51]. In addition, full length of LXR attained 9.2% gaps and there were no gaps in LBD sequences, which means that the LBD possessed a higher degree of homology and gaps were focus on non-LBD regions.

Stability landscape of all possible mutations of LXR

In order to evaluate stability effect of all possible mutations of different residues, alanine scanning on LXR α and LXR β was executed by calculating the change of free energy of mutations. The results were shown as Figure 2. Here, 0.5 kcal·mol⁻¹ to -0.5 kcal·mol⁻¹ means effect of mutations was neutral. 0.5 kcal·mol⁻¹ to 1 kcal·mol⁻¹ means effect of mutations was slightly destabilizing while -0.5 kcal·mol⁻¹ to -1 kcal·mol⁻¹ means effect of mutations was slightly stabilizing. 1 kcal·mol⁻¹ to 2 kcal·mol⁻¹ means effect of mutations was destabilizing while -1 kcal·mol⁻¹ to -2 kcal·mol⁻¹ means effect of mutations was stabilizing. Over 2 kcal·mol⁻¹ means effect of mutations was highly destabilizing while below -2 kcal·mol⁻¹ means effect of mutations was highly stabilizing. In LXR α , 178 of the 208 residues became unstable, accounting for as much as 85.58%. 181 of the 217 mutational residues destabilized accounting for 83.41% in LXR β . It suggested that most residues on LXR α and LXR β attained high thermodynamic stability effects. As shown Figure 3, color of different residues from red to blue means values of Δ Stability changed from low to high. Residues whose Δ Stability values were below -2 kcal·mol⁻¹ were marked as spheres. The Δ Stability values that were less than -2 kcal·mol⁻¹ were exhibited as the Supplementary Table 1. The Lys²⁷³, Asp³¹⁸, Lys⁴¹⁰, Ser⁴¹⁸, Lys⁴³³ and Pro⁴³⁷ were shared by LXR α and LXR β , while the residue numbers in LXR β are Lys²⁸⁷, Asp³³², Lys⁴²⁴, Ser⁴³², Lys⁴⁴⁷ and Pro⁴⁵¹. The Δ Stability of the residues of LXR α was slightly higher than that of LXR β , where the average of Δ Stability of the former was -5.60 kcal·mol⁻¹ and the latter was -5.72 kcal·mol⁻¹. The Gly²¹², Asp²⁷⁰, Asp²⁸⁵, Asn³⁴⁷, Asp³⁷⁴, Asp⁴⁰⁰, Asp⁴⁴⁴ and His⁴⁴⁶ were the specific residues to LXR α . The minimum of Δ Stability of the residues was -8.50 kcal·mol⁻¹ and other residues also possessed considerably low values, which means that the residues possessed an unstable structure before mutating. The Lys²⁴⁰, Lys²⁴⁸, Gly²⁹⁶, Lys³³¹, Lys³³⁷, Asp³⁶⁶, Asn³⁷⁷, Pro³⁹⁸, Pro⁴¹² and Ser⁴⁵⁴ were specific mutational residues to LXR β . Average of Δ Stability of the residues was -3.29 kcal·mol⁻¹ and minimum was only -5.98 kcal·mol⁻¹. In addition, most of the residues except Lys²⁴⁸ were located on helix regions, which are agonist conformation found in some developed LXR β agonists especially helix 3 (residues from Arg²⁶¹ to Val²⁸⁹) and helix 12 (residues from Pro⁴⁵¹ to Trp⁴⁵⁷)[52,53]. Coincidentally, Ser⁴⁵⁴ was located on helix 12, indicating it attained favorable binding activity with some developed agonists such as T0901317 and GW3965. Based on this, Ser⁴⁵⁴ may become a potential residue for screening LXR β .

Exploring specific key residues binding with LXR β

In order to analyze the differences underlying the binding mode of agonists between LXR α and LXR β , 22 known selective or nonselective agonists were docked to LXR and the results were shown as Table 1. The binding affinity could be calculated by docking score, and we proposed that the selectivity of agonists between LXR could be reflected by Δ Score, the docking score of agonists against LXR β minus that of LXR α . The lower value of Δ Score, the higher selectivity against LXR β . As can be seen in Table 1, BDBM27173, BDBM27174 and BDBM50300572[54] showed favorable binding affinity against LXR β and poor binding affinity against LXR α , and Δ Score of them were $-8.0 \text{ kcal}\cdot\text{mol}^{-1}$, $-6.1 \text{ kcal}\cdot\text{mol}^{-1}$ and $-3.8 \text{ kcal}\cdot\text{mol}^{-1}$ respectively. These compounds are analogs of GW3965[55], a tertiary-amine LXR agonist. GW3965 used to be considered as a nonselective and nonsteroidal agonist for LXR. However, it has been demonstrated that GW3965 was a potent full agonist that possessed LXR β specificity (EC₅₀ values are 176 nM and 15 nM respectively)[56]. Therefore, GW3965 was classified as a selective agonist. RGX-104[57], a small molecule LXR agonist, obtained $-5.6 \text{ kcal}\cdot\text{mol}^{-1}$ of Δ Score. WYE672 as a LXR agonist showed potent binding affinity to LXR β (IC₅₀ = 53 nM). It had little binding affinity for LXR α (IC₅₀ > 1.0 μ M) [58] and its Δ Score was $-3.6 \text{ kcal}\cdot\text{mol}^{-1}$. ChEMBL4215604 is a 2-Thienyl analog exhibited potent LXR β selective agonistic activity (EC₅₀ = 0.559 μ M)[59] and its Δ Score was $-3.1 \text{ kcal}\cdot\text{mol}^{-1}$. N-acylthiadiazolines[60] is a selective LXR β agonist with poor pharmacokinetics property and its Δ Score calculated here was $-2.8 \text{ kcal}\cdot\text{mol}^{-1}$. 4-(3-biaryl) quinoline sulfones (BDBM50317731) is a high affinity LXR β agonist with modest binding selectivity over LXR α and its Δ Score calculated here was $-2.3 \text{ kcal}\cdot\text{mol}^{-1}$ [10].

Nonselective agonist, such as AZ876[61], LXR623[62] and T0901317[63] attained Δ Score higher than $-2 \text{ kcal}\cdot\text{mol}^{-1}$. It has been indicated that the experimental binding free energy ΔG_{exp} approach to $-RT \ln \text{IC}_{50}$, thus for every difference in binding free energy of $1 \text{ kcal}\cdot\text{mol}^{-1}$ the difference in activity is about 5.9 times. Thereby we suggested that values of $-2 \text{ kcal}\cdot\text{mol}^{-1}$ was the threshold of whether a compound possesses selectivity. An agonist would possess selectivity against LXR β if Δ Score lower than $-2 \text{ kcal}\cdot\text{mol}^{-1}$ and a compound with Δ Score over $-2 \text{ kcal}\cdot\text{mol}^{-1}$ is more likely to be a nonselective agonist. The physiological ligands for LXR are oxysterols, including 24(S)-hydroxyl cholesterol (cpd.9) and 24(S),25-epoxycholesterol (cpd.8), they did not show observed selectivity between LXR α and LXR β compared with other synthetic ligands. Meanwhile the affinity against LXR of physiological ligands calculated here were also more unfavorable than synthetic ligands. However, despite many efforts have been made to discover effective and selective LXR β agonists, there is rarely approved drug has been reported.

Table 1. Docking score ($\text{kcal}\cdot\text{mol}^{-1}$) of 22 LXR agonists

Name*	LXR α	LXR β	Δ Score	Descriptor
BDBM27173 ^a	-7.2	-15.2	-8.0	Selective
BDBM27174 ^a	-9.3	-15.4	-6.1	Selective
RGX-104	-9.7	-15.2	-5.6	Selective
BDBM50300572 ^a	-11.4	-15.2	-3.8	Selective
WYE672	-6.7	-10.3	-3.6	Selective
CHEMBL4215604 ^b	-7.9	-11.0	-3.1	Selective
N-Acylthiadiazolines	-9.1	-11.9	-2.8	Selective
BDBM50317731 ^a	-7.8	-10.2	-2.3	Selective
GW3965	-9.7	-14.4	-4.8	Selective
LXR623	-10.8	-11.6	-0.7	Nonselective
WAY-254011	-9.8	-11.8	-2.0	Nonselective
Quinoline 16	-10.4	-11.8	-1.4	Nonselective
AZ876	-9.3	-10.6	-1.3	Nonselective
BDBM35089 ^a	-11.6	-11.9	-0.2	Nonselective
cpd.8	-10.1	-10.1	0.0	Nonselective
T0901317	-10.7	-10.7	0.0	Nonselective
SCHEMBL14056665 ^c	-9.4	-9.3	0.1	Nonselective
cpd.9	-8.6	-8.0	0.6	Nonselective
SCHEMBL1272009 ^c	-11.3	-10.6	0.7	Nonselective
SCHEMBL12847006 ^c	-11.9	-10.7	1.2	Nonselective
SCHEMBL3419782 ^c	-10.2	-8.4	1.7	Nonselective
CHEMBL427914 ^b	-8.8	-7.5	1.3	Nonselective

* Compound with a unique database ID, which takes the form of a 'BDBM', 'CHEMBL' or 'SCHEMBL' prefix immediately by an integer. ^a The Binding Database. ^b ChEMBL database. ^c SureChEMBL database.

Furthermore, we investigated the differences of binding mode of selective and nonselective agonists targeting LXR using 2D and 3D ligand-receptor interaction diagram. Among 22 known agonists collected from literature, 9 agonists are selective agonists of LXR β and the rests are nonselective agonists. As shown in Table 2, 29 key residues from LBD participated in ligand binding. Residues Leu²⁷⁴, Thr³¹⁶ and Phe³²⁹ participated in LXR β binding for all selective and nonselective agonists, indicating these residues are very important for agonist binding. Residues Phe²⁷¹, Ser²⁷⁸, Met³¹² and His⁴³⁵ were involved in selective agonists binding, and they all participated in 9 selective agonists binding, but partially occurred in binding of nonselective agonists. It has been indicated that the hydrophobic part of agonist should be located in hydrophobic pocket constituted by Phe²⁷¹, Phe³⁴⁰ and Phe³⁴⁹ to simulate the activity of LXR[64]. Here, we suggest that Phe²⁷¹, Ser²⁷⁸, Met³¹² and His⁴³⁵ might be very important for selective agonist binding with LXR β .

Table 2. Frequency of occurrences of key residues in LBD of LXR β binding with agonists

Residues	Frequency (%)		Residues	Frequency (%)	
	Selective	Nonselective*		Selective	Nonselective*
Leu ²⁷⁴	100.00	100.00	Ile ³⁵³	55.56	71.43
Thr ³¹⁶	100.00	100.00	Thr ²⁷²	55.56	42.86
Phe ³²⁹	100.00	100.00	Leu ³¹³	55.56	14.28
Phe ²⁷¹	100.00	71.43	Phe ²⁶⁸	55.56	0.00
Ser ²⁷⁸	100.00	71.43	Glu ³¹⁵	55.56	0.00
His ⁴³⁵	100.00	71.43	Phe ³⁴⁹	44.44	85.71
Met ³¹²	100.00	42.86	Asn ²³⁹	44.44	0.00
Ala ²⁷⁵	88.89	57.14	Ile ³⁰⁹	44.44	0.00
Leu ³³⁰	88.89	42.86	Ile ³⁵⁰	44.44	0.00
Phe ³⁴⁰	77.78	100.00	Phe ³⁵⁴	33.33	0.00
Glu ²⁸¹	77.78	42.86	Leu ⁴⁴⁹	22.22	85.71
Arg ³¹⁹	77.78	42.86	Gln ⁴³⁸	11.11	28.57
Leu ³⁴⁵	66.67	42.86	Ser ²⁴²	11.11	0.00
Trp ⁴⁵⁷	66.67	28.57	Met ²¹²	0.00	14.28
Ile ²⁷⁷	66.67	14.29			

* The Δ Score of collected nonselective agonists were all over -2 kcal·mol⁻¹ but not over 0 kcal·mol⁻¹ for results of LXR β binding patterns with agonists that possess LXR α specificity would not be considered in this study.

Binding pattern between agonists and LXR β was predicted by Ligplot⁺, the results of eight representative ligands were illustrated as Figure 4 and Figure 5. First of all, as mentioned above, Leu²⁷⁴, Thr³¹⁶ and Phe³²⁹ which were considered as important residues for agonists, were found in all agonists. Glu³¹⁵, a residue located on the helix region centered on Arg³¹⁹, was the specific residue binding with selective agonists. It was worth noting that only selective agonist s could bind with Trp⁴⁵⁷ while Trp⁴⁵⁷ was not a key residue in nonselective agonists-LXR β binding. Trp⁴⁵⁷ located on helix 12 which was agonist conformation found in some developed LXR β agonists. Trp⁴⁵⁷ had been also proved to form a close contact to His⁴³⁵ to help it form polar interaction with ligands easier[52].

Therefore, a standard that we verify whether compounds possesses LXR β selectivity were constructed. Firstly, Trp⁴⁵⁷ was selected to identify a compound whether possessed selectivity due to its unique properties. Then, His⁴³⁵ was indispensable for it was the bridge of interaction between Trp⁴⁵⁷ and ligands. In addition, Phe²⁷¹, Ser²⁷⁸, Met³¹² were involved in all selective agonists binding. Taken together, a compound which targets LXR β selectively should bind to all these residues in the protein: Phe²⁷¹, Ser²⁷⁸, Met³¹², His⁴³⁵, Trp⁴⁵⁷.

Identifying of selective agonists targeting LXR β

In order to evaluate pharmacokinetics properties and binding affinity of agonists from 112 terpenes of *Alismatis Rhizoma*, ADMET analysis and molecular docking were adopted to screen compounds. Binding free energy and MD simulations were introduced to further eliminate ligands whose binding affinity are unfavorable.

Pharmacokinetics and physicochemical analysis of collected terpenes

In order to understand physiochemical and pharmacokinetics properties of 112 collected terpenes, the ADMET (absorption, distribution, metabolism, excretion and toxicity) properties were predicted. RO5 which signifies the number beyond Lipinski's rule of five regions were calculated based on the result. Values of RO5 of all compounds were less than 3 and that of 97 terpenes were located on the recommend range. 52 compounds showed RO5 were 0, which means the compounds possessed potential druggability. Log S, log D and log K_p stand for aqueous solubility, distribution coefficient D at PH=7.4 and skin permeability, respectively. All of them are important properties to evaluate the physicochemical properties of drugs. Caco-2 permeability was represented by log Papp. In our results, 97 of 112 terpenes showed that values of log Papp were located on the recommend range. HIA means human intestinal absorption. Compounds whose HIA more than or equal 30% were marked as 1 while HIA less than 30% were marked as 0. 69 of 112 terpenes showed that values of HIA were 1, which means that the body may have a high absorption for these compounds. BBB stands for brain blood barrier and too polar compounds will not cross through brain blood barrier [65]. 0 means that a compound can't pass through blood brain barrier while 1 represents a compound can go through blood brain barrier. Results showed that none of compounds can pass through the barrier. However, LXR β was mainly distributed in livers, and therefore BBB has little effect on the treatment effect of the compounds we screened. DILI means drug induced liver injury, and 0 represents that the drug will cause little damage to liver function. Compounds we screened must make sure values of DILI were 0 for the drugs will mainly affect in the liver. CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4 inhibitor were the probability of a molecule being the inhibitor of Cytochrome P450 families. The ADMET properties of the compounds involved in this study are presented in the Table 3. The cpd.1 to 8 indicate 16-hydroxy-alisol B 23-acetate, 16-oxo-alisol A 23-acetate, 25-O-ethylaliso A, Alismanol C, Alisol G, Alisol M 23-acetate, Alisol N 23-acetate, 24(S),25-epoxy cholesterol, respectively.

Table 3. The ADMET properties of compounds involved in the study.

ADMET properties	Molecules								Recommend range*
	cpd.1	cpd.2	cpd.3	cpd.4	cpd.5	cpd.6	cpd.7	cpd.8	
RO5	1	1	1	1	0	1	1	1	≤1
Molecular weight	528.8	546.7	518.8	530.7	472.7	542.8	528.8	400.6	150-500
Rotatable bonds	6	7	7	7	5	6	6	4	≤5
H-bond acceptors	5	7	5	6	4	6	5	2	0-10
H-bond donors	2	3	3	2	3	2	2	1	0-5
log P ^a	5.37	3.94	5.04	4.8	4.97	4.68	5.42	5.86	-2-5
log S ^b	-5.99	-4.97	-5.58	-5.6	-5.68	-5.61	-6.11	-6.22	-6.5-0.5
log D ^c	2.90	3.75	4.49	3.84	4.29	2.83	2.95	4.52	1-5
log Papp ^d	-5.07	-5.04	-4.97	-5.01	-5.03	-5.03	-5.06	-4.79	> -5.15
Pgp substrate ^e	0	1	0	1	0	1	0	0	0
HIA ^f	1	0	1	1	1	1	1	1	1
log K _p ^g	-5.84	-7.16	-6.1	-6.24	-5.53	-6.45	-5.71	-4.05	-8.0-1.0
BBB ^h	0	0	0	0	0	0	0	0	0
PPB ⁱ	86.52	90.02	84.62	92.44	90.01	86.77	86.31	81.12	90
CYP1A2 inhibitor ^j	0	0	0	0	0	0	0	0	0
CYP2C19 inhibitor ^j	0	0	0	0	0	0	0	0	0
CYP2C9 inhibitor ^j	0	0	0	0	0	0	0	0	0
CYP2D6 inhibitor ^j	0	0	0	0	0	0	0	0	0
CYP3A4 inhibitor ^j	0	1	1	1	1	0	0	0	0
hERG ^k	0	0	0	0	0	0	0	1	1
DILI ^l	0	0	0	0	0	0	0	0	0

* The Recommend range of properties referred from ADMETlab[31] and SwissADME[30]. ^a average of octanol/water partition coefficient by different prediction, which consists of iLOGP[66], XLOGP3[67], WLOGP[68], MLOGP and SILICOS-IT. ^b aqueous solubility, which was implemented from ESOL[69]. ^c Distribution Coefficient D at PH=7.4. ^d Caco-2 Permeability. ^e 0: Non-substrate; 1: Substrate. ^f Human Intestinal Absorption. ^g skin permeability, unit is cm/s. ^h brain-blood barrier. ⁱ Plasma Protein Binding, significant with drugs that are highly protein-bound and have a low therapeutic index. ^j 0: Non-inhibitor; 1: Inhibitor. ^k hERG (human ether-a-go-go related gene) Blockers, 0: Non-blockers; 1: Blockers. ^l Drug Induced Liver Injury, 0: DILI negative; 1: DILI positive.

In order to verify reliability of docking models, ROC curves were used to evaluate performance of docking functions that distinguish active compounds and inactive compounds from a given dataset. A set of decoy molecules for ligands which were obtained from the ChEMBL database were constructed while they were input to dock with LXRβ with different score functions. Then, the ROC curves of each function were plotted in Figure 6A and the AUC values of Ledock, Grid, Amber, Vina are 0.710, 0.729, 0.786, 0.887 respectively. The range of AUC values is from 0 to 1. In addition, 0.5 is deemed as the threshold of whether a function has ability to distinguish[70]. AUC<0.5 suggests that the test results are worse than random results while AUC>0.5 shows that test is effective[71]. Based on this, the study chose AUC value of 0.5 as reference line, which was named Random in Figure 6A. To compare the AUC values of different functions with reference line, all of them demonstrated acceptable discrimination and the Vina score function was the most outstanding of them. Then, redock with LXRβ was executed by Autodock Vina for the sake of identifying the accuracy of Vina function in the most straightforward way[72]. RMSD is thought to be an essential standard of evaluating how much structure is different from the original

structure[73]. Furthermore, if RMSD value between pre- and post-docking structure is below 2 Å, the docking model is considered to be great and can be applied for docking[74]. Herein, the RMSD value between redock structure and its initial structure of LXRβ using Vina function in the study was 1.9 Å, which met the requirement mentioned above. The result of redock and its initial structure is shown as Figure 6B and the binding pocket of LXRβ used in the study is displayed in Figure 6C.

Screening potential LXRβ ligands using molecular docking

Table 4. Structure and binding affinity of reference and screened compounds candidates.

Name	Structure	Score (kcal·mol ⁻¹)		ΔScore(kcal·mol ⁻¹)
		LXRβ	LXRα	
cpd.1		-11.8	-6.1	-5.8
cpd.2		-11.9	-5.3	-6.6
cpd.3		-11.7	-8.5	-3.2
cpd.4		-13.1	-10.9	-2.2
cpd.5		-12.0	-8.8	-3.2
cpd.6		-11.0	-7.2	-3.7
cpd.7		-11.2	-7.1	-4.2
cpd.8		-10.1	-10.1	0
cpd.9		-8.0	-8.6	0.6

The Vina scores were applied to compute binding affinity between ligands and LXR using Autodock Vina program. In order to screen ligands targeting LXR β selectively, Δ Score was calculated which means that values of Vina score of LXR β minus that of LXR α . As mentioned above, we thought that compounds whose Δ Score values below $-2 \text{ kcal}\cdot\text{mol}^{-1}$ possessed preferential binding affinity with LXR β . Seven screened compound candidates which met the requirements were shown as Table 4. cpd.8 and cpd.9 which were thought to activate LXR endogenously in liver and brain respectively[75] were also presented as reference ligands in Table 4. Compared with cpd.8, all screened compound candidates showed higher binding affinities of LXR β and most of them except cpd.4 attained lower binding affinities of LXR α , suggesting they are more likely to bind with LXR β . Moreover, values of HIA of most the candidates except cpd.2 were 1, indicating most candidates possessed potential to enter into internal environment and activate target protein. Among all candidates, cpd.5 was the most potential ligand for its performance in pharmacokinetics analysis and great gap in docking affinities with cpd.8. cpd.1 and cpd.4 were also considerable compounds for LXR β agonists. The former attained high value of Δ Score, which means that it highly selectively targets LXR β . The latter possessed high docking score of LXR β . Most of all, values of DILI of all screened compound candidates were 0, which means they all possessed essential properties of drugs targeting LXR β that is low toxicity. The discussion about cpd.9 will not proceed because it was a ligand found in brain.

20ns MD simulations for LXR-ligand were performed via Gromacs program in order to evaluate dynamic interactions between LXR β and ligand candidates. The calculated results of MD simulations were output in the form of MD trajectory. Then, assessment of backbone RMSD values for each MD trajectory was executed to provide a holistic sight to observe stability of LXR-ligand system. As illustrated in Figure 7, the backbone values of LXR α -ligand system were between 0.10 nm and 0.30 nm while that of LXR β -ligand system were from 0.075 nm to 0.275 nm. The reference ligand, cpd.8, rapidly reach platform at 2 ns in both LXR α -ligand system and LXR β -ligand system. cpd.1 and cpd.6 showed drastic fluctuation of around 0.20 nm and 0.225 nm in LXR α -ligand system and LXR α -cpd.7 system appeared stable and minimum equilibrium at 0.18nm. LXR β -cpd.6 system fluctuated steadily while LXR β -cpd.1 system showed fluctuation as drastic as LXR α -ligand system. LXR β -cpd.2 system was the most stable LXR β -ligand system, which fluctuated at 0.125 nm. Moreover, fluctuations of LXR α -cpd.2 system were on mean level among all LXR α -ligand systems. LXR β -cpd.4 was the most fluctuant LXR β -ligand system, which kept fluctuating during the 20 ns simulation experiment. However, LXR α -cpd.4 system showed stable equilibrium. Cpd.3 and cpd.5 presented increasingly large unstable fluctuation of RMSD values in LXR β -ligand system whereas the level of RMSD was moderately low in LXR α -ligand system. In order to screen ligands selectively targeting LXR β , compounds which fluctuated steadily in LXR β -ligand system but waved drastically in LXR α -ligand system were selected. It demonstrated that cpd.6 was the molecule that most fits the requirement, and cpd.1 and cpd.2 can also be candidates.

Calculation of MM/PBSA binding free energy

Because Vina score only compute the binding free energy of lowest-scoring conformation and exist empirical component, MM/PBSA binding energy calculation was introduced to the work in order to

assess binding affinity accurately between ligands and proteins[36,49]. The results of calculation of LXR α and LXR β were listed as Table 5 and Table 6, respectively. Here, ΔG_{bind} means comprehensive evaluation of binding free energy while ΔG_{vdw} , ΔG_{ele} , ΔG_{polar} and ΔG_{apolar} are key elements of binding free energy. The lower binding free energy suggests the binding mode between ligand and receptor is more stable. As shown in Table 5, cpd.2 and reference ligand cpd.8 presented similar level in ΔG_{bind} , which were $-45.84 \text{ kcal}\cdot\text{mol}^{-1}$ and $-45.46 \text{ kcal}\cdot\text{mol}^{-1}$, respectively. ΔG_{bind} of cpd.4 was $-40.67 \text{ kcal}\cdot\text{mol}^{-1}$, which means that interactions between cpd.4 and LXR α are the most unstable. cpd.7 was the most stable ligand that binds to LXR α . The rests were at the same and moderately stable level. As listed in Table 6, the reference compound, cpd.8, showed considerably high ΔG_{bind} of $-30.29 \text{ kcal}\cdot\text{mol}^{-1}$, which indicated that ΔG_{bind} was detrimental to binding to LXR β . ΔG_{bind} of cpd.6 was $-56.17 \text{ kcal}\cdot\text{mol}^{-1}$, which was the lowest value among all screened compound candidates, indicating cpd.6 possessed the highest binding affinity to LXR β . The binding affinity with LXR β of cpd.1 and cpd.7 were at similar and low level. ΔG_{bind} of cpd.4 and cpd.5 is the highest among all screened compound candidates. ΔG_{bind} of cpd.2 and cpd.3 were $-50.33 \text{ kcal}\cdot\text{mol}^{-1}$ and $-48.99 \text{ kcal}\cdot\text{mol}^{-1}$ respectively, which were at mean level of all candidates. In order to screen compounds selectively targeting LXR β , compounds which ΔG_{bind} of LXR β was higher than that of LXR α were eliminated. The result of the filter is that the cpd.1, cpd.2, cpd.4, cpd.6 are remained.

Table 5. Summary of binding free energy components for the LXR α complexes($\text{kcal}\cdot\text{mol}^{-1}$)

Molecules	ΔG_{vdw}	ΔG_{ele}	ΔG_{polar}	ΔG_{apolar}	ΔG_{bind}
cpd.1	-71.82 ± 3.32	-9.40 ± 1.50	37.32 ± 1.65	-6.35 ± 0.20	-50.26 ± 2.96
cpd.2	-71.25 ± 2.47	-10.60 ± 1.58	42.45 ± 2.41	-6.44 ± 0.19	-45.84 ± 2.73
cpd.3	-68.91 ± 2.30	-10.13 ± 1.83	35.01 ± 2.71	-6.86 ± 0.19	-50.89 ± 2.87
cpd.4	-61.92 ± 2.80	-0.58 ± 1.64	27.93 ± 1.64	-6.11 ± 0.19	-40.67 ± 3.64
cpd.5	-63.90 ± 2.70	-11.00 ± 2.15	31.24 ± 1.16	-6.10 ± 0.22	-49.75 ± 2.22
cpd.6	-71.78 ± 3.53	-6.81 ± 1.40	34.54 ± 2.93	-6.73 ± 0.19	-50.78 ± 3.32
cpd.7	-72.48 ± 3.23	-2.68 ± 1.18	30.11 ± 1.94	-6.69 ± 0.13	-51.73 ± 3.28
cpd.8	-62.86 ± 2.83	-4.98 ± 1.21	28.02 ± 1.96	-5.66 ± 0.14	-45.46 ± 2.35

Table 6. Summary of binding free energy components for the LXR β complexes($\text{kcal}\cdot\text{mol}^{-1}$)

Molecules	ΔG_{vdw}	ΔG_{ele}	ΔG_{polar}	ΔG_{apolar}	ΔG_{bind}
cpd.1	-72.30 ± 3.02	-11.73 ± 2.03	38.88 ± 1.66	-6.62 ± 0.20	-51.77 ± 3.06
cpd.2	-72.07 ± 2.85	-17.66 ± 2.79	45.96 ± 3.78	-6.56 ± 0.18	-50.33 ± 3.81
cpd.3	-74.17 ± 2.73	-6.28 ± 1.14	38.15 ± 1.89	-6.69 ± 0.20	-48.99 ± 2.90
cpd.4	-67.67 ± 3.20	-5.52 ± 1.47	34.42 ± 1.98	-6.54 ± 0.22	-45.31 ± 2.32
cpd.5	-62.79 ± 3.25	-6.92 ± 1.49	31.25 ± 3.07	-6.00 ± 0.17	-44.46 ± 3.34
cpd.6	-72.95 ± 3.04	-8.05 ± 1.51	31.13 ± 1.71	-6.30 ± 0.24	-56.17 ± 2.81
cpd.7	-71.28 ± 2.72	-9.83 ± 2.02	36.65 ± 1.97	-6.64 ± 0.17	-51.10 ± 3.17
cpd.8	-58.01 ± 2.75	-9.63 ± 1.56	43.03 ± 2.53	-5.68 ± 0.14	-30.29 ± 2.85

Verifying of the LXR β selectivity of screened compounds

cpd.1, cpd.2, cpd.6 were selected as screened compounds for further analysis. cpd.4 was discarded for it fluctuated drastically in LXR β -cpd.4 system. The RMSF values of MD simulations, decomposition of binding free energy and binding model analysis were used to explore the binding mode of screened compounds and LXR β . Then, we applied the validation standard to verify whether screened molecules possessed selectivity.

RMSF analysis of the key amino acid residues

RMSF values for each MD trajectory were calculated in order to assess flexibility of proteins while higher values indicated better flexibility. Figure 8 are the results of RMSF values of LXR-ligand system. Low RMSF values mean that the system is stable, and therefore residues of this region may participate in the binding interactions of LXR-ligand system. It is clear that all compounds shared similar RMSF profiles in both LXR α -ligand system and LXR β -ligand system. Compared with LXR β -ligand system, LXR α -ligand system showed relatively higher RMSF values of average 0.1328 nm while that of LXR β -ligand system was at mean level of 0.1145 nm, which suggested screened compounds are more likely to bind to LXR β on the whole. In LXR β -ligand system, region Ser²⁵⁴-Leu²⁶⁴ was the most flexible, indicating the residues contribute less to binding of the system. RMSF values of region Ala³⁰⁶-Arg³¹⁹ and Gly³⁶⁹-Ile³⁷⁷ were lowest, suggesting the region are more responsible for binding of the system. Interestingly, the unique mutational residue Asn³⁷⁷ in LXR β was located on the region, which means that Asn³⁷⁷ contribute a lot to the binding of LXR β and it is a candidate screening target for LXR β . Region Ala³⁰⁶-Arg³¹⁹ was all situated on helix region except Ser³⁰⁷ and residues of protein-binding pocket Met³¹², Leu³¹³, Glu³¹⁵, Thr³¹⁶, Arg³¹⁹ also lay on the region. Met³¹² which has been proved to bind to LXR β such as T-0901317[52] is a part of validation standard for selective targeting LXR β binding residues. The RMSF values of all binding system on Met³¹² were at a low level, which indicates that binding between screened compounds and Met³¹² was stable. Arg³¹⁹ was a vital residue which participated in forming hydrogen bonds with ligands. Thr³¹⁶ is thought to be an active site of forming hydrogen bonds with LXR β [76]. cpd.1, cpd.2 and cpd.6 all possessed ability to bind with Thr³¹⁶ on LXR β in the form of hydrogen bonds.

Decomposition of MM/PBSA binding free energy

Binding free energy decomposition was applied to identify contributions of each residue in the LXR-ligand binding model. The contributions of each residue of LXR α and LXR β are depicted in Figure 9 and Figure 10, respectively. Low values of energy indicate an important role of residues in the LXR-ligand binding. As demonstrated in Figure 9, most residues showed values which were negligible to the system. Only LXR α -cpd.8 system had a moderately high positive value located on Arg³⁰⁵, which means that the residue contributes less to binding to LXR α . Phe²⁵⁷, Leu²⁶⁰, Ala²⁶¹, Met²⁹⁸, Leu³³¹, Trp⁴⁴³ (values of energy of selected residues are below -0.8 kcal·mol⁻¹) showed relatively larger contributions of binding to LXR α . As illustrated in Figure 10, most residues had little effect on LXR β -ligand binding. The reference, LXR β -cpd.8 system, showed two extremely high values of binding free energy of 8.21 kcal·mol⁻¹ and 3.71 kcal·mol⁻¹, which were located on Glu²⁸¹ and Glu³¹⁵, respectively. Energy of Arg³¹⁹ was high in LXR β -cpd.1 system

while the Phe²⁷¹, Phe³²⁹ and Trp⁴⁵⁷ showed the lowest values of binding free energy. Phe²⁷¹ and Phe³²⁹ have been proved an important contributor to hydrophobic interactions between LXR β and ligand, and Trp⁴⁵⁷ can form a close contact to His⁴³⁵ which is able to form polar interaction with ligand[52]. All of four residues are within validation standard and indicated cpd.1 possessed LXR β selectivity. Arg³¹⁹ is an inhibitive site which can form hydrogen bonds with ligands. Compared to the residues above, smaller change in ΔG_{MM} is direct cause of the higher energy value of Arg³¹⁹. ΔG_{bind} of Phe²⁷¹, Phe³²⁹ and Trp⁴⁵⁷ were lower than the rest residues because of lower ΔG_{MM} while ΔG_{polar} were even higher than the rest residues. ΔG_{MM} indicates potential energy in the vacuum which are composed of ΔG_{vdw} and ΔG_{ele} . It demonstrated that potential energy was a crucial factor that affects binding between cpd.1 and key residues in LXR β . Energy of Glu²⁸¹ and Phe³²⁹ were the maximum of 2.22 kcal·mol⁻¹ and minimum of -2.80 kcal·mol⁻¹ of LXR β -cpd.2 system, respectively. Glu²⁸¹, whose energy was highest in both LXR β -cpd.2 and LXR β -cpd.8 system, showed an extraordinary ΔG_{polar} of 2.48 kcal·mol⁻¹ and 8.80 kcal·mol⁻¹, respectively. Glu²⁸¹ was considered as a hydrophilic site which is able to bind to ligand in the form of hydrogen bonds[77]. Hydrogen bonds are usually considered as a set of dispersion force and intermolecular or interatomic electrostatic interaction[78], whose energy is represented by ΔG_{MM} . However, ΔG_{MM} of the two compounds were not low and therefore ΔG_{bind} were at a high level, suggesting the two compounds were less likely to form hydrogen bonds with LXR β at Glu²⁸¹. Nevertheless, the result was opposite for Phe³²⁹. Although ΔG_{polar} was at moderately high values at 2.17 kcal·mol⁻¹ in LXR β -cpd.2 system, ΔG_{MM} reached an extremely low value of -4.79 kcal·mol⁻¹, which counteracted the negative effect of ΔG_{polar} on binding. The ΔG_{apolar} of Phe³²⁹ in LXR β -cpd.2 system was also low. ΔG_{apolar} was calculated from solvent-accessible surface area that can be affected by hydrophobic effect. It indicates that cpd.2 possesses ability to participate in hydrophobic effect of LXR β . In LXR β -cpd.6 system, there was no high positive value. Phe²⁷¹, Leu²⁷⁴ and Trp⁴⁵⁷ provided key contributions of the lowest value of energy. Leu²⁷⁴ was considered as a hydrophobic site in the LXR β [59] and showed comparatively higher ΔG_{apolar} . The other hydrophobic residue, Phe²⁷¹, showed high values of ΔG_{polar} and low ΔG_{MM} . ΔG_{MM} value of Trp⁴⁵⁷ were at low level of -2.57 kcal·mol⁻¹, suggesting it has huge potential to form hydrogen bonds with cpd.6 while ΔG_{polar} and ΔG_{apolar} were at moderately low level, which means Trp⁴⁵⁷ would play an important role in LXR β -cpd.6 binding.

Binding model analysis of screened compounds

In order to investigate the differences of 2D and 3D LXR-ligands binding mode of screened compounds, Ligplot⁺[50] was introduced to identify key residues which interact with ligands in LXR β . Key residues of all screened compound candidates were predicted and they were shown as Supplementary Table 2. The results showed that most of them attained plentiful binding residues at helix3 and helix12, indicating they possessed high binding affinity with LXR β . Results of screened compounds and reference ligand was plotted in Figure 11. Circled residues were equivalent side chains, which were considered as key residues in binding free energy decomposition. The green line represented hydrogen bonds between ligands and

residues and residues who formed hydrogen bonds were highlighted particularly. As depicted in Figure 11, Phe²⁷¹, Thr³¹⁶, Ser²⁷⁸ and His⁴³⁵ formed hydrogen bonds with screened compounds. These residues were also considered very important for selective agonist binding with LXR β in our study. Interestingly, Thr³¹⁶, a residue found in both selective and nonselective agonists, presented in all screened compounds, indicating all the compounds may be potential agonists against LXR β . His⁴³⁵, which was proved an important residue that form hydrogen bonds to stabilize the protein binding pocket[52], could be found in all screened ligands. However, reference ligand can't bind with His⁴³⁵. Another residue, Trp⁴⁵⁷, which was located on helix 12 and could form contact with His⁴³⁵[52], had the same case. Arg³¹⁹ and Glu³¹⁵ were located in protein binding pocket and Arg³¹⁹ was an inhibitive site which can form hydrogen bonds. However, results of energy decomposition demonstrated that binding affinity between ligands and Arg³¹⁹ on LXR β was not high. And binding affinity between ligands and LXR β of Glu³¹⁵ was average.

The interactions between LXR β and ligands in 3D were illustrated as Figure 12 to show the hydrogen bonds intuitively. More hydrogen bonds and shorter length of hydrogen bonds indicate larger interaction intensity between ligands and receptor. It is clear that intensity of interaction between LXR β and screened compounds was higher than that of reference ligand, which was reflected on the number and length of hydrogen bonds between LXR β and ligands. All screened compounds could bind to Thr³¹⁶ and the distance between ligands and the residues was the nearest among all residues in Figure 12, indicating ligands possessed high binding affinity with Thr³¹⁶. Phe²⁷¹ was the residue which binds closest with cpd.1, suggesting the residue was important in LXR β -cpd.1 binding. Both His⁴³⁵ and Ser²⁷⁸ which were located on protein binding pocket had an average binding affinity, which means that all screened ligands could bind with the two residues. However, reference ligand, which was a kind of nonselective agonists in our body, didn't find the characters described above.

Discussion:

Previous studies showed that activation of LXR activity could reduce TC, which is responsible for hyperlipidemia[8, 1]. Many LXR β agonists have already been screened such as T0901317 and LXR623[62, 63]. However, the agonists are not selective, which can also activate LXR α . LXR α played an essential role in activating SREBP-1c protein, which was considered as a pivotal activator of lipogenic genes[9]. However, activation of LXR β had less impact on lipogenic synthesis pathway[14]. Therefore, screening a compound selectively targeting LXR β becomes urgent. *Alismatis Rhizoma*, a kind of TCM to treat dysuria, edema, obesity, diabetes, hyperlipidemia and hypertension was introduced to be the raw material of the study[18]. Terpenes from *Alismatis Rhizoma* were collected for they have been considered as active components to enhance transactivation for FXR which belongs to nuclear receptors superfamily along with LXR. Especially protostane-type triterpenes had been proved the major contributor to pharmacodynamic and bioactive compounds in *Alismatis Rhizoma*[21]. Then a series of virtual experiment was performed.

First of all, in order to understand the structural differences between LXR α and LXR β and specific residues of binding of selective agonists to LXR β , structural analysis of LXR α and LXR β was conducted and a validation standard was constructed. Pairwise sequence alignment of full length and LBD region was performed to identify regions of similarity of LXR α and LXR β . Results showed that LXR α and LXR β shared the same length of LBD region and the gaps of LBD were 0. The %similarity and %identity of LBD were higher than that of full length, suggesting LBD possessed a higher degree of homology. Alanine scanning was applied to evaluate stability effect of different residues in site-directed mutation. The Δ Stability of LXR α and LXR β was similar in general, with only some local differences. Residues whose Δ Stability below $-2 \text{ kcal}\cdot\text{mol}^{-1}$ were selected and LXR α was more stable than LXR β in mutations for Δ Stability of the residues of LXR α was slightly higher than that of LXR β in homologous residues. Moreover, the Lys²⁴⁰, Lys²⁴⁸, Gly²⁹⁶, Lys³³¹, Lys³³⁷, Asp³⁶⁶, Asn³⁷⁷, Pro³⁹⁸, Pro⁴¹² and Ser⁴⁵⁴ were unique mutational residues to LXR β . All of them was located on helix region except Lys²⁴⁸. Subsequently, 22 known selective and nonselective agonists were selected in order to analyze key residues that affected binding mode of LXR β and ligands. The Δ Score means that the docking score of agonists against LXR β minus that of LXR α and whether values below $-2 \text{ kcal}\cdot\text{mol}^{-1}$ was threshold that a compound possessed selectivity. Then, Ligplot⁺ was introduced to predict potential residues that interacted with ligands. Results showed that Phe²⁷¹, Ser²⁷⁸, Met³¹², His⁴³⁵ might be very important for selective binding of ligands due to their high engagement in selective agonists binding. Moreover, comparison between four representative selective agonists and four nonselective agonists was executed. Results showed that Trp⁴⁵⁷ was responsible for selective binding between ligands and LXR β . Finally, a standard we verify whether compounds possessed LXR β selectivity was set up, that is, ligands which bind with Phe²⁷¹, Ser²⁷⁸, Met³¹², His⁴³⁵, Trp⁴⁵⁷ in LXR β may be potential selective agonists targeting LXR β .

Then, a series means of computer aided drug design were executed to evaluate agonists against LXR β from 112 terpenes of *Alismatis Rhizoma*. Suitable ADMET properties was the first hurdle for most ligand candidate[29] and unfavorable ADMET properties would lead that drugs possesses low bioavailability. RO5, DILI and HIA were used to assess whether properties of ADMET are favorable. Results showed that most of compounds possessed favorable ADMET properties. The ADMET properties of screened compound candidates in the study were all favorable except the absorption of cpd.2 was not high. Subsequently, molecular docking was employed to screen ligand whose binding affinity of LXR β was far higher than that of LXR α . High values of AUC of ROC curves of docking functions and high RMSD value between redock structure and its initial structure of LXR β suggested that the virtual screening model was reliable for screening active ligands effectively from compounds database. Results of molecular docking showed that most terpenes have favorable binding affinity with LXR β . The Δ Score value of $-2 \text{ kcal}\cdot\text{mol}^{-1}$ which was considered as the threshold of selective agonists and nonselective agonists is used to screen compounds targeting LXR β and seven compound candidates were labeled as cpd.1 to 7. In order to further explore and verify the result of molecular docking, RMSD values analysis of MD simulations and calculation of MM/PBSA binding energy were followed. Results of MD simulation were used to evaluate dynamic interactions between screened compound candidates and LXR. Compounds whose RMSD curve

of MD trajectory was stable were considered to possess high binding affinity with LXR. In this study, molecules with a stable trajectory of LXR β and fluctuating trajectory of LXR α were thought to possess high dynamics binding affinities to LXR β . It demonstrated that cpd.6 was the candidate that most fits the requirement and dynamic binding affinity of cpd.1 and cpd.2 was also favorable. Cpd.4 showed dramatically fluctuant during the specified simulative time of LXR β -ligand system, which means that the molecule is averse to binding to LXR β . 2 ns MD trajectory was extracted to calculate the MM/PBSA binding free energy. Compared to result of docking, result of binding free energy possesses distinct distribution patterns. Some compounds of high Δ Score values possessed binding free energy of LXR α higher than that of LXR β . Because Vina score only compute the binding free energy of lowest-scoring conformation and exist empirical component, we adopted computation of g_mmpbsa which combines MM energy of the gas phase and solvation free energy as the results of binding free energy to simulate ΔG_{bind} in vivo[36, 49]. Screened compound candidates whose ΔG_{bind} of LXR β were higher than that of LXR α were eliminated and cpd.1, cpd.2, cpd.4 and cpd.6 were remained. Taken together, we screened three compounds through a series of experiments, namely cpd.1, cpd.2 and cpd.6. The HIA properties of cpd.2 was unfavorable, and cpd.4 didn't meet the standard for it didn't pass the screening of MD simulations.

Later on, RMSF analysis of MD simulations, binding free energy decomposition and binding model analysis were carried out to find the differences underlying the binding mode of agonists between LXR α and LXR β . The standard we proposed earlier was used to verify whether screened compounds possessed LXR β selectivity. The RMSF values of molecular dynamics simulations provided flexibility of proteins. RMSF values of region Ala³⁰⁶-Arg³¹⁹ and Gly³⁶⁹-Ile³⁷⁷ were lowest, suggesting the regions are more responsible for binding between LXR β and the screened compounds. It is worth noting that Met³¹², Thr³¹⁶ and Asn³⁷⁷ were on the region. Thr³¹⁶ is an active site of forming hydrogen bonds with LXR β [76] and it can be found in both selective and nonselective agonists. Asn³⁷⁷ is specific residues of LXR β in structural analysis of alanine mutations. It demonstrated that screened compounds might possess the ability of agonists and bind active residues on LXR β . Met³¹² was a hydrophobic site which interact with ligand by hydrogen bonds[52] and it was one of residues of verification standard. RMSF values of Met³¹² were one of the lowest on the entire residues, suggesting the three compounds might possess LXR β selectivity. Decomposition of MM/PBSA binding-energy was also executed to calculate contributions of each residue in the LXR-ligand binding model. Results showed that the residues of lowest binding free energy of LXR β -cpd.1 system were Phe²⁷¹, Phe³²⁹ and Trp⁴⁵⁷. The residues of lowest binding free energy of LXR β -cpd.2 system was Phe³²⁹, and that of LXR β -cpd.6 system was Phe²⁷¹, Leu²⁷⁴ and Trp⁴⁵⁷. Leu²⁷⁴ and Phe³²⁹ participated in LXR β binding for all selective and nonselective agonists, indicating all of screened compounds possessed potential to be agonists. Phe²⁷¹ has been proved an important contributor to hydrophobic interactions between LXR β and ligand, and Trp⁴⁵⁷ can form a close contact to His⁴³⁵ which is able to form polar interaction with ligand[52]. They are also the members of verification standard. As mentioned earlier, cpd.1 and cpd.6 could bind to all of them, indicating the two compounds might possess LXR β selectivity. The binding mode between LXR β and screened compounds were calculated by

Ligplot⁺ and PyMOL. Results showed that cpd.1 could form hydrogen bonds to Phe²⁷¹, Ser²⁷⁸, Phe³²⁹ and His⁴³⁵. Cpd.2 could form hydrogen bonds to Thr³¹⁶ and His⁴³⁵. Cpd.8 could form hydrogen bonds to Phe²⁷¹, Ser²⁷⁸ and Thr³¹⁶. Ser²⁷⁸ was one of residues that we thought contribute more to selective agonist binding with LXR β . His⁴³⁵ which was the center of a protein binding pocket and could form hydrogen bonds to agonists[52], was one of residues of verification standard. It demonstrated that all of the three compounds might possess LXR β selectivity for they can form hydrogen bonds with Ser²⁷⁸ or His⁴³⁵. Ligand binding to Phe²⁷¹, Ser²⁷⁸, Met³¹², His⁴³⁵ and Trp⁴⁵⁷ was considered as verification standard whether the ligand possesses LXR β selectivity in the study. Results of verifying of the LXR β specificity showed cpd.1 could interact with Phe²⁷¹, Ser²⁷⁸, Met³¹², His⁴³⁵ and Trp⁴⁵⁷. Cpd.2 could interact with Met³¹² and His⁴³⁵. Cpd.6 could interact with Phe²⁷¹, Ser²⁷⁸, Met³¹², and Trp⁴⁵⁷. It indicated that cpd.1 and cpd.6 possessed LXR β selectivity. Cpd.2 is not considered to have LXR β selectivity for it didn't meet the standard of screening and unfavorable pharmacokinetics properties.

Conclusion:

In summary, the present study provides an alternative strategy for screening selective agonists targeting LXR α and LXR β from terpenes. First of all, a standard we verify agonists whether possess LXR β selectivity was constructed by analyzing differences of residues between developed selective and nonselective agonists. Then, a series of computational tools such as ADMET analysis, molecular docking, calculation of binding free energy and MD simulations were applied to evaluate physicochemical and biological properties of 112 compounds. Moreover, key residues and the binding mode of screened compounds were analyzed to confirm whether they possessed selectivity. Our result indicated that 16-hydroxy-alisol B 23-acetate and Alisol M 23-acetate were identified as the most potential agonists. Phe²⁷¹, Ser²⁷⁸, Met³¹², His⁴³⁵, Trp⁴⁵⁷ were considered as the major contributor to binding between LXR β and screened compounds. Finally, favorable physicochemical properties of screened compounds and remarkable binding affinity to residues which are favor of binding in previous study indicates that the result of the study is reliable. Thus, the screening strategies used in the study may guide the development of LXR β -agonists from TCM.

Declarations

Funding:

This work was supported in part by National Natural Science Foundation of China (Nos 81373311, 31300674, 81173093, 30970643 and J1103518), the Special Program for Youth Science and the Technology Innovative Research Group of Sichuan Province, China (No. 2011JTD0026), and Graduate Student's Research and Innovation Found of Sichuan University (No. 2018YJSY055).

Availability of data and material:

All data and material are included in this article.

Code availability:

All code used in the study are mentioned in this article or can obtain from official website of the software.

Compliance with ethical standards:

Conflicts of interest/Competing interests:

The authors declare that they have no conflict of interest.

Ethics approval:

N/A

Consent to participate:

N/A

Consent for publication:

N/A

Authors' contributions:

Chuanfang Wu, Jinku Bao conceived and designed the experiments; Chuanjiong Lin performed the experiment and Jianzong Li analyzed the data; and Chuanjiong Lin wrote this manuscript; All authors read and approved the final manuscript.

References

1. Lan T, Li Q, Chang M, Yin C, Zhu D, Wu Z, Li X, Zhang W, Yue B, Shi J, Yuan H, Su Z, Guo H (2020) Lei-gong-gen formula granule attenuates hyperlipidemia in rats via cGMP-PKG signaling pathway. *J Ethnopharmacol* 260:112989. doi:10.1016/j.jep.2020.112989
2. Jeun J, Kim S, Cho SY, Jun HJ, Park HJ, Seo JG, Chung MJ, Lee SJ (2010) Hypocholesterolemic effects of *Lactobacillus plantarum* KCTC3928 by increased bile acid excretion in C57BL/6 mice. *Nutrition* 26(3):321–330. doi:10.1016/j.nut.2009.04.011
3. Wang K, Liao M, Zhou N, Bao L, Ma K, Zheng Z, Wang Y, Liu C, Wang W, Wang J, Liu SJ, Liu H (2019) *Parabacteroides distasonis* Alleviates Obesity and Metabolic Dysfunctions via Production of Succinate and Secondary Bile Acids. *Cell Rep* 26(1):222–235 e225. doi:10.1016/j.celrep.2018.12.028
4. Kim SJ, Park SH, Sin HS, Jang SH, Lee SW, Kim SY, Kwon B, Yu KY, Kim SY, Yang DK (2017) Hypocholesterolemic Effects of Probiotic Mixture on Diet-Induced Hypercholesterolemic Rats. *Nutrients* 9 (3). doi:10.3390/nu9030293

5. Trinick TR, Duly EB (2013) Hyperlipidemia: Overview. In: Encyclopedia of Human Nutrition. pp 442–452. doi:10.1016/b978-0-12-375083-9.00148-3
6. Millán Núñez-Cortes JE, Millán Pérez JJ (2019) Classification of Hyperlipidemias and Dyslipidemias. In: Encyclopedia of Endocrine Diseases. pp 275–281. doi:10.1016/b978-0-12-801238-3.65816-6
7. Gao S, Hu G, Li D, Sun M, Mou D (2020) Anti-hyperlipidemia effect of sea buckthorn fruit oil extract through the AMPK and Akt signaling pathway in hamsters. *Journal of Functional Foods* 66. doi:10.1016/j.jff.2020.103837
8. Chen K, Ma Z, Yan X, Liu J, Xu W, Li Y, Dai Y, Zhang Y, Xiao H (2020) Investigation of the Lipid-Lowering Mechanisms and Active Ingredients of Danhe Granule on Hyperlipidemia Based on Systems Pharmacology. *Front Pharmacol* 11:528. doi:10.3389/fphar.2020.00528
9. Wrobel JSR, Bowen SM et al (2008) Indazole-based liver X receptor (LXR) modulators with maintained atherosclerotic lesion reduction activity but diminished stimulation of hepatic triglyceride synthesis [J]. *J Med Chem* 51:7161–7168. doi:10.1021/jm800799q
10. Ullrich JW, Morris R, Bernotas RC, Travins JM, Jetter J, Unwalla R, Quinet E, Nambi P, Feingold I, Huselton C, Enroth C, Wilhelmsson A, Goos-Nilsson A, Wrobel J (2010) Synthesis of 4-(3-biaryl)quinoline sulfones as potent liver X receptor agonists. *Bioorg Med Chem Lett* 20(9):2903–2907. doi:10.1016/j.bmcl.2010.03.031
11. Swahn BM, Macsari I, Viklund J, Ohberg L, Sjodin J, Neelissen J, Lindquist J (2009) Liver X receptor agonists with selectivity for LXRbeta; N-aryl-3,3,3-trifluoro-2-hydroxy-2-methylpropionamides. *Bioorg Med Chem Lett* 19(7):2009–2012. doi:10.1016/j.bmcl.2009.02.039
12. Grefhorst A, Elzinga BM, Voshol PJ, Plosch T, Kok T, Bloks VW, van der Sluijs FH, Havekes LM, Romijn JA, Verkade HJ, Kuipers F (2002) Stimulation of lipogenesis by pharmacological activation of the liver X receptor leads to production of large, triglyceride-rich very low density lipoprotein particles. *J Biol Chem* 277(37):34182–34190. doi:10.1074/jbc.M204887200
13. Repa JJ, Liang G, Ou J, Bashmakov Y, Lobaccaro JM, Shimomura I, Shan B, Brown MS, Goldstein JL, Mangelsdorf DJ (2000) Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRalpha and LXRbeta. *Genes Dev* 14(22):2819–2830. doi:10.1101/gad.844900
14. Larkin JC, Sears SB, Sadovsky Y (2014) The influence of ligand-activated LXR on primary human trophoblasts. *Placenta* 35(11):919–924. doi:10.1016/j.placenta.2014.09.002
15. Kazeminasab F, Marandi SM, Ghaedi K, Esfarjani F, Moshtaghian J (2018) Endurance training and LXR activation increased LXRα and ABCA1 transcripts in Wistar male rats. *Sci Sports* 33(3):152–157. doi:10.1016/j.scispo.2018.02.005
16. Shen Q, Bai Y, Chang KC, Wang Y, Burris TP, Freedman LP, Thompson CC, Nagpal S (2011) Liver X receptor-retinoid X receptor (LXR-RXR) heterodimer cistrome reveals coordination of LXR and AP1 signaling in keratinocytes. *J Biol Chem* 286(16):14554–14563. doi:10.1074/jbc.M110.165704
17. Tian T, Chen H, Zhao YY (2014) Traditional uses, phytochemistry, pharmacology, toxicology and quality control of *Alisma orientale* (Sam.) Juzep: a review. *J Ethnopharmacol* 158 Pt A:373–387.

doi:10.1016/j.jep.2014.10.061

18. Huang MQ, Xu W, Wu SS, Lu JJ, Chen XP (2013) A 90-day subchronic oral toxicity study of triterpene-enriched extract from *Alismatis Rhizoma* in rats. *Food Chem Toxicol* 58:318–323. doi:10.1016/j.fct.2013.05.009
19. Hur JM, Park JWC, JC (2007) Effects of methanol extract of *Alisma orientale* rhizome and its major component, alisol B 23-acetate, on hepatic drug metabolizing enzymes in rats treated with bromobenzene. *Arch Pharm Res* 30:1543–1549. doi:10.1007/BF02977323
20. Hiebl V, Ladurner A, Latkolik S, Dirsch VM (2018) Natural products as modulators of the nuclear receptors and metabolic sensors LXR, FXR and RXR. *Biotechnol Adv* 36(6):1657–1698. doi:10.1016/j.biotechadv.2018.03.003
21. Wang L, Li S, Li J, Cheng Z, Feng Y, Ouyang H, Du Z, Jiang H (2020) Comprehensive metabolic profiling of *Alismatis Rhizoma* triterpenes in rats based on characteristic ions and a triterpene database. *Journal of Pharmaceutical Analysis*. doi:10.1016/j.jpha.2020.03.010
22. Li S, Jin S, Song C, Jia S, Zhang Y, Feng Y, Du Z, Jiang H (2017) The strategy for establishment of the multiple reaction monitoring based characteristic chemical profile of triterpenes in *Alismatis rhizoma* using two combined tandem mass spectrometers. *J Chromatogr A* 1524:121–134. doi:10.1016/j.chroma.2017.09.057
23. Li Q, Qu H (2012) Study on the hypoglycemic activities and metabolism of alcohol extract of *Alismatis Rhizoma*. *Fitoterapia* 83(6):1046–1053. doi:10.1016/j.fitote.2012.05.009
24. Zhang LL, Xu W, Xu YL, Chen X, Huang M, Lu JJ (2017) Therapeutic potential of *Rhizoma Alismatis*: a review on ethnomedicinal application, phytochemistry, pharmacology, and toxicology. *Ann N Y Acad Sci* 1401(1):90–101. doi:10.1111/nyas.13381
25. Sussman JL, Lin DW, Jiang JS, Manning NO, Prilusky J, Ritter O, Abola EE (1998) Protein Data Bank (PDB): Database of three-dimensional structural information of biological macromolecules. *Acta Crystallogr Sect D-Biol Crystallogr* 54:1078–1084. doi:10.1107/s09074444998009378
26. Madeira F, Park YM, Lee J, Buso N, Gur T, Madhusoodanan N, Basutkar P, Tivey ARN, Potter SC, Finn RD, Lopez R (2019) The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic Acids Res* 47(W1):W636–W641. doi:10.1093/nar/gkz268
27. UniProt C (2015) UniProt: a hub for protein information. *Nucleic Acids Res* 43(Database issue):D204–D212. doi:10.1093/nar/gku989
28. Schymkowitz J, Borg J, Stricher F, Nys R, Rousseau F, Serrano L (2005) The FoldX web server: an online force field. *Nucleic Acids Res* 33 (Web Server issue):W382–W388. doi:10.1093/nar/gki387
29. Balani SK, Miwa GT, Gan LS, Wu JT, Lee FW (2005) Strategy of utilizing in vitro and in vivo ADME tools for lead optimization and drug candidate selection. *Curr Top Med Chem* 5(11):1033–1038. doi:10.2174/156802605774297038
30. Daina A, Michielin O, Zoete V (2017) SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep* 7:42717. doi:10.1038/srep42717

31. Dong J, Wang NN, Yao ZJ, Zhang L, Cheng Y, Ouyang D, Lu AP, Cao DS (2018) ADMETlab: a platform for systematic ADMET evaluation based on a comprehensively collected ADMET database. *J Cheminform* 10(1):29. doi:10.1186/s13321-018-0283-x
32. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* 23(1–3):3–25. doi:10.1016/s0169-409x(96)00423-1
33. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings (Reprinted from *Advanced Drug Delivery Reviews*, vol 23, pg 3–25, 1997). *Adv Drug Deliv Rev* 46 (1–3):3–26. doi:10.1016/s0169-409x(00)00129-0
34. Cereto-Massague A, Guasch L, Valls C, Mulero M, Pujadas G, Garcia-Vallve S (2012) DecoyFinder: an easy-to-use python GUI application for building target-specific decoy sets. *Bioinformatics* 28(12):1661–1662. doi:10.1093/bioinformatics/bts249
35. Li J, Wang H, Li J, Bao J, Wu C (2016) Discovery of a Potential HER2 Inhibitor from Natural Products for the Treatment of HER2-Positive Breast Cancer. *Int J Mol Sci* 17 (7). doi:10.3390/ijms17071055
36. Trott O, Olson AJ (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem* 31(2):455–461. doi:10.1002/jcc.21334
37. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE (2004) UCSF Chimera—a visualization system for exploratory research and analysis. *J Comput Chem* 25(13):1605–1612. doi:10.1002/jcc.20084
38. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ (2009) AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J Comput Chem* 30(16):2785–2791. doi:10.1002/jcc.21256
39. Wang Z, Sun H, Yao X, Li D, Xu L, Li Y, Tian S, Hou T (2016) Comprehensive evaluation of ten docking programs on a diverse set of protein-ligand complexes: the prediction accuracy of sampling power and scoring power. *Phys Chem Chem Phys* 18(18):12964–12975. doi:10.1039/c6cp01555g
40. Lang PT, Brozell SR, Mukherjee S, Pettersen EF, Meng EC, Thomas V, Rizzo RC, Case DA, James TL, Kuntz ID (2009) DOCK 6: combining techniques to model RNA-small molecule complexes. *RNA* 15(6):1219–1230. doi:10.1261/rna.1563609
41. Pronk S, Pall S, Schulz R, Larsson P, Bjelkmar P, Apostolov R, Shirts MR, Smith JC, Kasson PM, van der Spoel D, Hess B, Lindahl E (2013) GROMACS 4.5: a high-throughput and highly parallel open source molecular simulation toolkit. *Bioinformatics* 29(7):845–854. doi:10.1093/bioinformatics/btt055
42. Wang JWR, Caldwell JW et al (2004) Development and testing of a general Amber force field. *J Comput Chem* 25:1157–1174. doi:10.1002/jcc.20035
43. Wang J, Wang W, Kollman PA, Case DA (2006) Automatic atom type and bond type perception in molecular mechanical calculations. *J Mol Graph Model* 25(2):247–260.

doi:10.1016/j.jmngm.2005.12.005

44. Da Silva AWSVW (2012) ACPYPE—Antechamber Python parser interface. BMC Res Notes 5:367. doi:10.1186/1756-0500-5-367
45. Van Der Spoel D, Lindahl E, Hess B, Groenhof G, Mark AE, Berendsen HJ (2005) GROMACS: fast, flexible, and free. J Comput Chem 26(16):1701–1718. doi:10.1002/jcc.20291
46. Hess B BH, Berendsen HJ et al (1997) LINCS: a linear constraint solver for molecular simulations. J Comput Chem 18:1463–1472. doi:10.1002/(SICI)1096-987X(199709)18:12<1463::AID-JCC4>3.0.CO;2-H
47. Essmann U, Perera L, Berkowitz ML, Darden T, Lee H, Pedersen LG (1995) A smooth particle mesh Ewald method. J Chem Phys 103(19):8577–8593. doi:10.1063/1.470117
48. Miyamoto SK, P.A (1992) Settle: An analytical version of the shake and rattle algorithm for rigid water models. J Comput Chem 13:952–962. doi:10.1002/jcc.540130805
49. Massova IKP (2000) Combined molecular mechanical and continuum solvent approach (MM-PBSA/GBSA) to predict ligand binding. Perspect Drug Discov Des 18:113–135. doi:10.1023/A:1008763014207
50. Laskowski RA, Swindells MB (2011) LigPlot+: Multiple Ligand-Protein Interaction Diagrams for Drug Discovery. J Chem Inf Model 51(10):2778–2786. doi:10.1021/ci200227u
51. Farnegardh M, Bonn T, Sun S, Ljunggren J, Ahola H, Wilhelmsson A, Gustafsson JA, Carlquist M (2003) The three-dimensional structure of the liver X receptor beta reveals a flexible ligand-binding pocket that can accommodate fundamentally different ligands. J Biol Chem 278(40):38821–38828. doi:10.1074/jbc.M304842200
52. Hoerer S, Schmid A, Heckel A, Budzinski RM, Nar H (2003) Crystal structure of the human liver X receptor beta ligand-binding domain in complex with a synthetic agonist. J Mol Biol 334(5):853–861. doi:10.1016/j.jmb.2003.10.033
53. Belorusova AY, Evertsson E, Hovdal D, Sandmark J, Bratt E, Maxvall I, Schulman IG, Akerblad P, Lindstedt EL (2019) Structural analysis identifies an escape route from the adverse lipogenic effects of liver X receptor ligands. Commun Biol 2:431. doi:10.1038/s42003-019-0675-0
54. Marino JP Jr, Kallander LS, Ma C, Oh HJ, Lee D, Gaitanopoulos DE, Krawiec JA, Parks DJ, Webb CL, Ziegler K, Jaye M, Thompson SK (2009) The discovery of tertiary-amine LXR agonists with potent cholesterol efflux activity in macrophages. Bioorg Med Chem Lett 19(19):5617–5621. doi:10.1016/j.bmcl.2009.08.036
55. Collins JL, Fivush AM, Watson MA, Galardi CM, Lewis MC, Moore LB, Parks DJ, Wilson JG, Tippin TK, Binz JG, Plunket KD, Morgan DG, Beaudet EJ, Whitney KD, Kliewer SA, Willson TM (2002) Identification of a nonsteroidal liver X receptor agonist through parallel array synthesis of tertiary amines. J Med Chem 45(10):1963–1966. doi:10.1021/jm0255116
56. Toporova L, Grimaldi M, Boulahtouf A, Balaguer P (2020) Assessing the Selectivity of FXR, LXRs, CAR, and RORgamma Pharmaceutical Ligands With Reporter Cell Lines. Front Pharmacol 11:1122. doi:10.3389/fphar.2020.01122

57. Mita MM, Mita AC, Chmielowski B, Hamilton EP, Pant S, Waltzman RJ, Rowinsky EK, Szarek M, Gonsalves F, Kurth I, Andreu C, Busby R, Darst DM, Tavazoie SS, Tavazoie M, Postow MA (2018) Pharmacodynamic and clinical activity of RGX-104, a first-in-class immunotherapy targeting the liver-X nuclear hormone receptor (LXR), in patients with refractory malignancies. *J Clin Oncol* 36(15_suppl):3095–3095. doi:10.1200/JCO.2018.36.15_suppl.3095
58. Hu B, Unwalla RJ, Goljer I, Jetter JW, Quinet EM, Berrodin TJ, Basso MD, Feingold IB, Nilsson AG, Wilhelmsson A (2010) Identification of phenylsulfone-substituted quinoxaline (WYE-672) as a tissue selective liver X-receptor (LXR) agonist. *Journal of medicinal chemistry* 53(8):3296–3304. doi:10.1021/jm100034x
59. Nomura S, Endo-Umeda K, Fujii S, Makishima M, Hashimoto Y, Ishikawa M (2018) Structural development of tetrachlorophthalimides as liver X receptor beta (LXRbeta)-selective agonists with improved aqueous solubility. *Bioorg Med Chem Lett* 28(4):796–801. doi:10.1016/j.bmcl.2017.12.024
60. Molteni V, Li X, Nabakka J, Liang F, Wityak J, Koder A, Vargas L, Romeo R, Mitro N, Mak PA (2007) N-Acylthiadiazolines, a new class of liver X receptor agonists with selectivity for LXR β . *Journal of medicinal chemistry* 50(17):4255–4259. doi:10.1021/jm070453f
61. Van Der Hoorn JW, Lindén D, Lindahl U, Bekkers M, Voskuilen M, Nilsson R, Oscarsson J, Lindstedt E, Princen HM (2011) Low dose of the liver X receptor agonist, AZ876, reduces atherosclerosis in APOE*3Leiden mice without affecting liver or plasma triglyceride levels. *Br J Pharmacol* 162(7):1553–1563. doi:10.1111/j.1476-5381.2010.01168.x
62. Nambi P, Basso MD, Chen L, Liu Q-Y, Halpern A, Clerin V, Resmini C, Keith JC Jr, Feingold I, Steffan RJ (2007) LXR-623, a novel liver X receptor modulator, displays neutral lipid effects in cholesteryl ester transfer protein-expressing species and inhibits atherosclerotic lesion progression in low density lipoprotein receptor knockout mice. *Am Heart Assoc.* doi:10.1161/circ.116.suppl_16.II_299-a
63. Houck KA, Borchert KM, Hepler CD, Thomas JS, Bramlett KS, Michael LF, Burris TP (2004) T0901317 is a dual LXR/FXR agonist. *Mol Genet Metab* 83(1–2):184–187. doi:10.1016/j.ymgme.2004.07.007
64. Hu B, Collini M, Unwalla R, Miller C, Singhaus R, Quinet E, Savio D, Halpern A, Basso M, Keith J (2006) Discovery of phenyl acetic acid substituted quinolines as novel liver X receptor agonists for the treatment of atherosclerosis. *Journal of medicinal chemistry* 49(21):6151–6154. doi:doi.org/10.1002/jcc.20035
65. Ntie-Kang F (2013) An in silico evaluation of the ADMET profile of the StreptomeDB database. *SpringerPlus* 2:11. doi:10.1186/2193-1801-2-353
66. 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. *J Chem Inf Model* 54(12):3284–3301. doi:10.1021/ci500467k
67. Cheng TJ, Zhao Y, Li X, Lin F, Xu Y, Zhang XL, Li Y, Wang RX, Lai LH (2007) Computation of octanol-water partition coefficients by guiding an additive model with knowledge. *J Chem Inf Model* 47(6):2140–2148. doi:10.1021/ci700257y

68. Wildman SA, Crippen GM (1999) Prediction of physicochemical parameters by atomic contributions. *J Chem Inf Comput Sci* 39(5):868–873. doi:10.1021/ci990307l
69. Delaney JS (2004) ESOL: Estimating aqueous solubility directly from molecular structure. *J Chem Inf Comput Sci* 44(3):1000–1005. doi:10.1021/ci034243x
70. Fan J, Upadhye S, Worster A (2006) Understanding receiver operating characteristic (ROC) curves. *Cjem* 8(1):19–20. doi:10.1017/S1481803500013336
71. Bradley AP (1997) The use of the area under the roc curve in the evaluation of machine learning algorithms. *Pattern Recognit* 30(7):1145–1159. doi:10.1016/s0031-3203(96)00142-2
72. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, Olson AJ (1998) Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *Journal of Computational Chemistry* 19 (14):1639–1662. doi:10.1002/(sici)1096-987x(19981115)19:14<1639::Aid-jcc10>3.0.Co;2-b
73. Agrawal MR, Ozarkar AD, Gupta S, Deobagkar DN, Deobagkar DD (2014) Comparative study of Plasmodium falciparum erythrocyte membrane protein 1-DBLalpha domain variants with respect to antigenic variations and docking interaction analysis with glycosaminoglycans. *Mol Biosyst* 10(9):2466–2479. doi:10.1039/c4mb00274a
74. Brozell SR, Mukherjee S, Balius TE, Roe DR, Case DA, Rizzo RC (2012) Evaluation of DOCK 6 as a pose generation and database enrichment tool. *J Comput Aided Mol Des* 26(6):749–773. doi:10.1007/s10822-012-9565-y
75. Lehmann JM, Kliewer SA, Moore LB, SmithOliver TA, Oliver BB, Su JL, Sundseth SS, Winegar DA, Blanchard DE, Spencer TA, Willson TM (1997) Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway. *J Biol Chem* 272(6):3137–3140. doi:10.1074/jbc.272.6.3137
76. Haque MW, Bose P, Siddique MUM, Sunita P, Lapenna A, Pattanayak SP (2018) Taxifolin binds with LXR (alpha & beta) to attenuate DMBA-induced mammary carcinogenesis through mTOR/Maf-1/PTEN pathway. *Biomed Pharmacother* 105:27–36. doi:10.1016/j.biopha.2018.05.114
77. Chen H, Chen Z, Zhang Z, Li Y, Zhang S, Jiang F, Wei J, Ding P, Zhou H, Gu Q, Xu J (2020) Discovery of new LXRBeta agonists as glioblastoma inhibitors. *Eur J Med Chem* 194:112240. doi:10.1016/j.ejmech.2020.112240
78. Ahmed I, Jhung SH (2017) Applications of metal-organic frameworks in adsorption/separation processes via hydrogen bonding interactions. *Chem Eng J* 310:197–215. doi:10.1016/j.cej.2016.10.115

Figures

Figure 1. Plot of pairwise sequence alignment of LXR α and LXR β . (A) Statistic data of alignment results. (B) Details of LBD sequence alignment.

Figure 1

Caption found in figure.

Figure 2. Distribution of Δ Stability against Residue Number. (A) Δ Stability against Residue Number in LXR α (B) Δ Stability against Residue Number in LXR β .

Figure 2

Caption found in figure.

Figure 3. Plots of LXR α and LXR β for the Δ Stability and residue number. (A) LXR α and residues whose Δ Stability is less than $-2 \text{ kcal}\cdot\text{mol}^{-1}$. (B) LXR β and residues whose Δ Stability is below $-2 \text{ kcal}\cdot\text{mol}^{-1}$.

Figure 3

Caption found in figure.

Figure 4. Plot of binding model between selective agonists and LXR β . (A) BDBM27173. (B) BDBM27174. (C) BDBM50300572. (D) RGX-104.

Figure 4

Caption found in figure.

Figure 5. Plot of binding model between nonselective agonists and LXR β . (A) LXR623. (B) WAY-254011. (C) Quinoline 16. (D) AZ876.

Figure 5

Caption found in figure.

Figure 6. Plots of results of molecular docking model validation (A) ROC curves of docking model. (B) the results of redock, carbon skeleton shown as white is the original structure while carbon skeleton shown as green is the redocking structure. (C) the binding pocket of LXR β .

Figure 6

Caption found in figure.

Figure 7. Distribution of RMSD values of LXR-ligand system. A and B represent RMSD values of LXR α -ligand system while C and D mean that of LXR β -ligand system.

Figure 7

Caption found in figure.

Figure 8. Plots of RMSF values of LXR-ligand system. (A) RMSF of LXR α -ligand system. (B) RMSF of LXR β -ligand system. (C) RMSF of LXR β -ligand system in region Ala³⁰⁶-Arg³¹⁹. (D) RMSF of LXR β -ligand system in region Gly³⁶⁹-Ile³⁷⁷.

Figure 8

Caption found in figure.

Figure 9. Binding free energy per residue for LXR α -ligand system (Error bar of residues with an absolute value of energy less than 0.1 kcal·mol⁻¹ was not shown). (A) LXR α -cpd.8 system. (B) LXR α -cpd.1 system. (C) LXR α -cpd.2 system. (D) LXR α -cpd.6 system.

Figure 9

Caption found in figure.

Figure 10. Binding free energy decomposition of LXR β -ligand model (Error bar of residues with an absolute value of energy less than 0.1 kcal·mol⁻¹ was not shown). (A) LXR β -cpd.8 system. (B) LXR β -cpd.1 system. (C) LXR β -cpd.2 system. (D) LXR β -cpd.6 system.

Figure 10

Caption found in figure.

Figure 11. Diagrams of interactions between LXR β and ligands. (A) cpd.8 (B) cpd.1 (C) cpd.2 (D) cpd.6

Figure 11

Caption found in figure.

Figure 12. Plots of interaction between LXR β and ligands in 3D. (A) cpd.8 (B) cpd.1 (C) cpd.2 (D) cpd.6

Figure 12

Caption found in figure.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementaryinformation.docx](#)