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REVIEW

Drug discovery targeting thyroid hormone receptor β (THRβ) for the treatment of liver diseases and other medical indications

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Abstract Thyroid hormone receptors (THRs), a crucial nuclear receptor protein family, primarily consist of two categories: α receptors and β receptors. Among them, THRβ is the primary subtype of thyroid hormone that confers benefits to the liver. In the last two decades, there have been efforts to develop THRβ agonists that selectively yield beneficial effects on the liver, such as lowering triglycerides and cholesterol, while reducing adverse effects on the heart, muscle, and bone. This paper systematically reviews strategies to enhance the safety of THRβ agonists for the treatment of MASH, with a focus on improving the selectivity of THRα and increasing the distribution of the drug in the liver. Additionally, we explore the potential application of this target in addressing other medical indications.

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1. Introduction

Thyroid hormones (THs) are amino acid derivatives secreted by the thyroid gland, comprising T3 (3,3′,5-triodo-L-thyronine) and T4 (3,3′,5,5′-tetraiodo-L-thyronine). Thyroid hormones promote metabolism and growth, enhance the excitability of the nervous system, accelerate heart rhythm, and increase heat production by binding to thyroid hormone receptors (THRs)[1−3]. THRs are members of a large family of nuclear hormone receptors in the body, sharing similarities with the retinoid acid receptors (RAR) and the retinoid X receptor (RXR), the vitamin D receptor (VDR), the peroxisome proliferators-activated receptor (PPAR), the constitutive androstane receptor (CAR), and some orphan receptors[4]. Regulated by the hypothalamic-pituitary-thyroid axis, the thyroid gland releases thyroid hormones T3 and T4, which enter peripheral tissues under the action of thyroid hormone transporters. In peripheral tissues, T4 is typically converted to more active T3 by the action of type I deiodinase (Dio1) and type II deiodinase (Dio2). Conversely, it is converted to the inactive rT3 by the action of type III deiodinase (Dio3). Thyroid hormone receptors share similar domains with typical nuclear receptors, comprising an amino-terminal domain, a DNA binding domain (DBD), a ligand binding domain (LBD), a hinge region connecting the DBD and LBD, and a carboxyl-terminal domain. Among these, the LBD is the key region where thyroid hormone receptor agonists bind to the thyroid hormone receptors. In the absence of activation, thyroid hormone receptors form heterodimers with the RXR and bind to the thyroid hormone response element (TRE) on DNA. These interactions recruit corepressors such as nuclear receptor corepressor 1 (N-CoR) and silencing mediator for retinoid and thyroid receptors (SMRT), along with their associated histone deacetylase (HDAC), to inhibit gene expression. When the thyroid gland releases thyroid hormones, these molecules bind to the LBD pocket of the thyroid hormone receptor, inducing a conformational change in the receptor. This change initiates the recruitment of coactivators such as steroid receptor coactivator-1 (SRC-1) and related histone acetyltransferase (HAT) to begin gene transcription, playing a crucial role in body growth, development, and metabolism.

For THR-mediated growth, this process is associated with the expression of insulin-like growth factor 1 (IGF-1) and the secretion of growth hormone (GH)[5,6]. Thyroid hormones activate IGF-1 gene expression through THR, promoting the growth of bones and muscles, and indirectly regulating body growth by influencing GH secretion. For THR-mediated development, thyroid hormones regulate the expression of myosin heavy chain (MYH) genes through THR, promoting muscle tissue development and differentiation. Additionally, by promoting the expression of RC3/neurogranin, they play a key role in brain development and neuron differentiation[7,8]. For THR-mediated metabolism, thyroid hormones regulate the expression of uncoupling protein 1 (UCP1), increasing thermogenesis and energy expenditure. They also influence glucose metabolism and insulin sensitivity by regulating the expression of glucose transporter type 4 (GLUT4)[9,10]. Excessive thyroid hormone may induce beneficial effects, including promoting growth and development, lowering plasma cholesterol levels, and improving mood. However, it can also result in adverse effects such as tachycardia, atrial arrhythmia, bone and muscle loss. In the human body, there are two subtypes of thyroid hormone receptors, including THRα and THRβ. Although there is clear evidence that thyroid hormones can promote oligodendrocyte lineage cells and myelin formation, it is unclear which THRs mediate these effects[11]. The most serious side effect of excessive endogenous thyroid hormone T3 on the human body is the induction of cardiac hypertrophy[12], which is mainly mediated by THRα[13,14]. In recent years, the beneficial effect of activation of THRβ in the liver on lipid metabolism has been widely revealed (Fig. 1).

THRα and THRβ share high homology with only a single amino acid difference in the LBD domain (Fig. 2A). Specifically, the 331 site of THRβ is L-asparagine, while the 277 site of THRα is serine[15]. There are two separate THR genes, α and β (NR1A1 and NR1A2). Each gene encodes two products generated from differential RNA splicing: THRα1, THRα2, THRβ1, and THRβ2, respectively[16]. THRα1 is a functional receptor that binds to thyroid hormones while THRα2 lacks the ability to bind to thyroid hormones but can antagonize their effects (Fig. 2B). Analysis of mRNA distribution in mice revealed differences in the distribution of THRα and THRβ. Specifically, *THRα1* mRNA is mainly expressed in the heart and brain, while *THRβ1* mRNA is higher in skeletal muscle, kidney, and liver than in other tissues. The expression of *THRβ2* mRNA in the brain, pituitary, retina and inner ear is higher than in other tissues[17].

THRβ agonists have demonstrated various beneficial effects on the liver, particularly for the treatment of metabolic dysfunction-associated steatotic liver disease (MASLD) and metabolic dysfunction-associated steatohepatitis (MASH). Furthermore, THRβ agonists have the potential to promote liver regeneration[18,19] and for the treatment of other indications, such as hepatocellular carcinoma, atherosclerosis, and central nervous system (CNS) diseases[20]. In this review, we systematically summarize strategies to enhance the safety of THRβ agonists by improving the selectivity of THRα and increasing the distribution of the drug in the liver. We also explore the potential applications of this target in various indications, aiming to provide a new research direction for the discovery of novel THRβ agonists.

2. THRβ activation in MASH treatment

Non-alcoholic fatty liver disease (NAFLD) is pathologically characterized by hepatocellular steatosis and ballooning, accompanied by lobular inflammation and pericellular fibrosis, encompassing non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH). NAFL is the hallmark of NAFLD, representing an early, reversible, non-progressive stage of the disease. NASH, in contrast, is the progressive stage of NAFLD, marked by chronic inflammation and liver fibrosis, which lead to irreversible liver damage and signify a critical point in the disease progression. Approximately 25% of NAFL patients may progress to NASH, and 5%−50% of NASH patients may advance to end-stage liver diseases such as cirrhosis, liver failure, and hepatocellular carcinoma. Moreover, NAFLD is closely associated with metabolic disorders such as insulin resistance, hypertension, dyslipidemia, and obesity. Consequently, at the 2023 European Association for the Study of the Liver (EASL) conference, several liver associations worldwide announced the adoption of MASLD (Metabolic dysfunction-associated steatotic liver disease) to replace the current term NAFLD. The new nomenclature emphasizes the close relationship between the disease and metabolic dysfunction. Correspondingly, MASH has been introduced as the replacement term for NASH. The key updates in MASLD include the following: the exclusion of the requirement to rule out concurrent liver diseases such as viral hepatitis and excessive alcohol

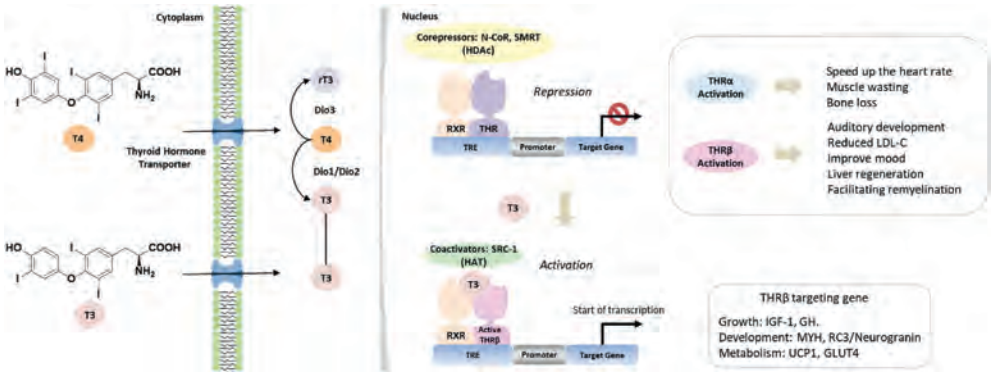


Figure 1 Mechanism of thyroid hormone receptor activation.

consumption; an emphasis on the significant roles of obesity, insulin resistance, dyslipidemia, type 2 diabetes, and systemic low-grade inflammation in the development and progression of fatty liver disease; a broader population coverage than NAFLD; the introduction of a clear definition of ethanol consumption levels; and the addition of the term MetALD (metabolic dysfunction-associated alcoholic liver disease) to describe MASLD patients with higher alcohol intake.

The challenge in MASH drug development primarily stems from the intricate pathogenic mechanism, involving multiple pathways, targets, and cell interactions. At present, the most convincing pathogenic mechanism has not been disclosed. In addition, the global drug regulatory authorities have strict standards for the clinical endpoints of MASH drug development. For instance, the US Food and Drug Administration (FDA) mandates that the alternative endpoint for phase III clinical trials of MASH drugs must involve the evaluation of liver biopsy results. It is required to observe (1) improvement of steatohepatitis and no deterioration of fibrosis, or (2) improvement of liver fibrosis and no deterioration of steatohepatitis, or (3) simultaneous improvement of steatohepatitis and liver fibrosis. Imaging evaluation and serological evaluation cannot be used as the main endpoints for the approval of MASH drugs, which further increases the difficulty of MASH drug development.

For over four decades, the landscape of MASH treatment has been characterized by a notable absence of effective medications, leaving the MASH patient population largely without recourse. However, in March of this year, a significant breakthrough emerged with the FDA's approval of Resmetirom, a THRβ agonist, marking the debut of a novel drug for MASH treatment. This milestone underscores the immense promise held by THRβ in addressing MASH. Table 1 lists the overview of THRβ agonists identified and investigated in recent years.

The mechanism of THRβ agonists for the treatment of MASH is not entirely clear, but there may be the following signaling pathways involved (Fig. 3)[21,22]. In the high-density lipoprotein (HDL) pathway, THRβ agonists stimulate the secretion of HDL precursor particles containing apolipoprotein A1 (APOA1) into the circulation. Cholesterol is then absorbed from atherosclerotic plaques and other sources, leading to the formation of mature HDL particles. Additionally, THRβ agonists facilitate the absorption of high-density lipoprotein particles by the liver through interaction with liver high-density lipoprotein receptors (HDLR), including scavenger receptor B1 (SRB1). In the low-density lipoprotein (LDL) pathway, THRβ agonists enhance the entry of low-density lipoprotein into the liver through interaction with low-density lipoprotein receptors (LDLR) and increase the conversion of cholesterol to bile acids by up-regulating cytochrome P450 enzyme 7A1 (CYP7A1). THRβ agonists inhibit triglyceride synthesis by down-regulating the transcription factor sterol response element-binding protein 1 (SREBP1). The delivery of triglycerides to lysosomes in the liver is mediated by an autophagy

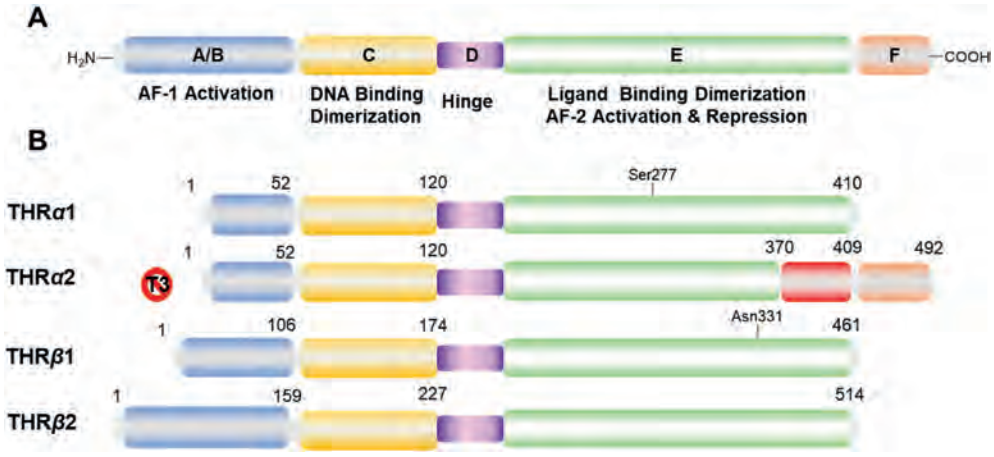
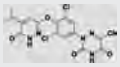
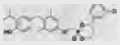
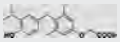
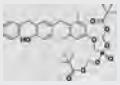
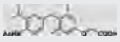
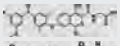
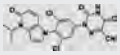


Figure 2 (A) Domain organization of thyroid hormone receptors (THRs) and functional regions. (B) Schematic representation of the different isoforms of thyroid hormone receptors.

Table 1 Discovery of THRβ agonists in recent years.

Drug	Structure	Condition	R & D status	Organization
Resmetirom (MGL-3196)[54]		MASLD; MASH; Cirrhosis	Approval	Madrigal Pharmaceuticals
MB07811 (VK2809)[63]		MASLD; MASH; dyslipidemia	Phase II	Viking Therapeutics
ASC-41	Not disclosed	MASLD; MASH; hyperlipidemia; Obesity; overweight	Phase II	Gannex Pharma; Ascletis Pharmaceutical
HSK-31679	Not disclosed	MASLD; MASH; primary hypercholesterolemia	Phase II	Haisco Pharmaceuticals
TERN-501	Not disclosed	MASLD; MASH	Phase II	Terns Pharmaceuticals
Sobetirome (GC-1)[29]		X-linked adrenoleukodystrophy; 2019-novel coronavirus infection; Idiopathic pulmonary fibrosis	Phase I	NeuroVia Inc.
VK0214		X-linked adrenoleukodystrophy	Phase I	Viking Therapeutics
ALG-055009	Not disclosed	MASLD; MASH	Phase I	Aligos Therapeutics
ECC-4703	Not disclosed	MASLD; MASH; dyslipidemia	Phase I	Shanghai Chengyi Biological Technology
Kylo-0603	Not disclosed	MASLD; MASH	Phase I	Kylonova (Xiamen) Biopharma
ABX-002	Not disclosed	Severe depression; X-linked adrenoleukodystrophy; multiple sclerosis	Phase I	Autobahn Therapeutics
Eprotirome (KB2115)[44]		Hyperlipidemia; liver regeneration	Pre-clinical	Karo Bio Pharmaceuticals
IS25[47]		Liver regeneration; MASLD; MASH; HCC; MS	Pre-clinical	University of Pisa
TG68[47]		Liver regeneration; MASLD; MASH; HCC; MS	Pre-clinical	University of Pisa
16g[53]		MASLD; MASH	Pre-clinical	Chinese Academy of Sciences
Compound **15**[61]		MASLD; MASH; hyperlipidemia	Pre-clinical	Chinese Academy of Sciences

process called lipophagy. Lipophagy involves the autophagic engulfment of triacylglycerol stored in lipid droplets, followed by autophagosome-lysosome fusion, and the transport of triacylglycerol to lysosomes for degradation and hydrolysis into free fatty acids (FFAs). Studies have shown that thyroid hormones enhance the process of lipophagy in a THR-dependent manner, increasing the number of lipid autophagosomes and lysosomes in human hepatocytes and mouse livers[23]. Mitochondria are the main site of fatty acid metabolism and a classic target of liver thyroid hormones. Thyroid hormones can mediate mitochondrial biological function and fatty acid β-oxidation to achieve a lipid-lowering effect. The nuclear regulation of mitochondrial content by thyroid hormones is primarily due to the regulation of the PPARγ coactivator 1α (PGC1α)—nuclear respiratory factor 1 (NRF1)—transcription factor A, mitochondrial (mtTFA) axis[24]. Thyroid hormones are known to increase protein levels of PGC1α, which acts as a co-transcriptional regulatory factor that induces mitochondrial biogenesis by activating NRF1 to promote the expression of mtTFA. Thyroid hormones have also been reported to localize within mitochondria and to regulate transcription from the mitochondrial genome[25]. Additionally, thyroid hormones increase the expression of mitochondrial enzymes needed for fatty acid β-oxidation, including medium-chain acyl-CoA dehydrogenase (MCAD), pyruvate dehydrogenase kinase isoform 4 (PDK4), and mitochondrial uncoupling protein 2 (UCP2)[26–28].

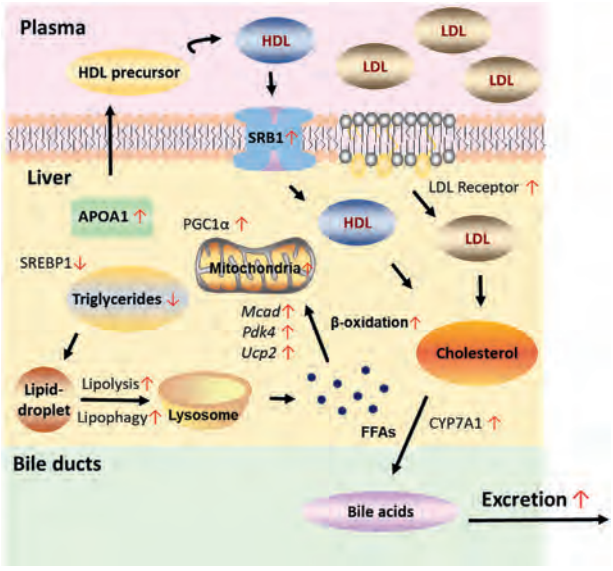


Figure 3 The mechanism of THRβ agonists in the treatment of MASH.

3. Strategy for the discovery of THRβ agonists

The early modification of the regulatory structure–activity relationship (SAR) began with endogenous hormone T3. T3 can activate both THRα and THRβ with an EC_{50} of 0.06 nmol/L. The non-selectivity of T3 to THRβ may be the cause of its

cardiotoxicity. From a structural standpoint, T3 occupies the pocket of both THRα and THRβ ligand binding domains, rendering it nonselective. The head of the THR pocket is primarily occupied by hydrophobic amino acid residues and contains a small amount of polar amino acid residues, such as His381/435 (THRα/THRβ), which interact with the hydroxyl group of T3 to form hydrogen bonds. This amino acid residue is crucial for the efficacy of most thyroid hormone-like drugs. The iodine atoms of T3 form halogen bonds with Phe218/272 (THRα/THRβ). Many hydrophilic polar amino acid residues are present in the tail of the THR pocket. The carboxyl group of the T3 tail forms hydrogen bond interactions with Arg228/282 (THRα/THRβ), and Asn331 (THRβ) (Fig. 4). Therefore, how to modify the structure of T3 to bring about subtype selectivity has become the direction of drug development.

The general scaffold of the thyroid hormone analogs is shown in Fig. 5. The rigid ring containing R_1 and R_2 is called the 'hydrophobic head', another rigid ring containing R_3 and R_4 side chain is called 'hydrophilic tail', and Y connecting two rigid fragments is called 'Linker'. For T3, R_1 in 3′ position of the hydrophobic head is the iodine atom, and R_2 in 4′ position is OH, while the Linker is an oxygen atom, the hydrophilic tail R_3 is substituted by an iodine atom, and the R_4 position is α-alanine.

The hydrogen bond interaction between phenolic group (R_2 = 4′-OH) and His435 in the receptor is important for binding and functional activity. The substituents at the R_1 positions interact with the hydrophobic region of the receptor, and the lack of hydrophobic groups at these positions will lead to a loss of affinity. The vertical orthogonal relationship between the two

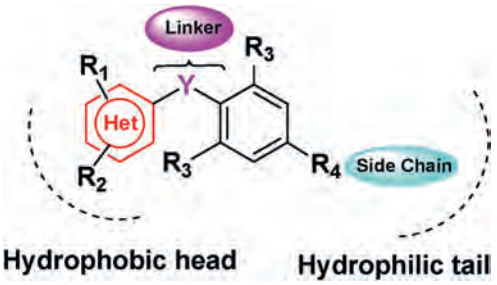


Figure 5 General scaffold of the thyroid hormone analogs.

aromatic rings of the ligand's biphenyl ether scaffold is important for the active conformation, which is enhanced by substitution on R_3, usually halogen substitution or methyl substitution. The R_4 position points to the receptor polar pocket containing several arginine residues. The requirement for structural substitution at the R_4 position is low because the arginine residue is highly flexible. Therefore, the changes in the polar side chains of T3 analogs can be very diverse, without significant loss of affinity for THR, but it is better to be replaced by acidic functions, such as the α-amino acid group of T3. Since the R_4 position structure can be replaced by various acidic functional groups, we divided thyroid hormone analogs into carboxylic acid THRβ agonists and cyano-substituted nitrogen uracil THRβ agonists according to the R_4 fragments. To improve liver targeting, researchers have also pioneered a new class of THRβ agonists using prodrug strategies, which will be discussed in detail next.

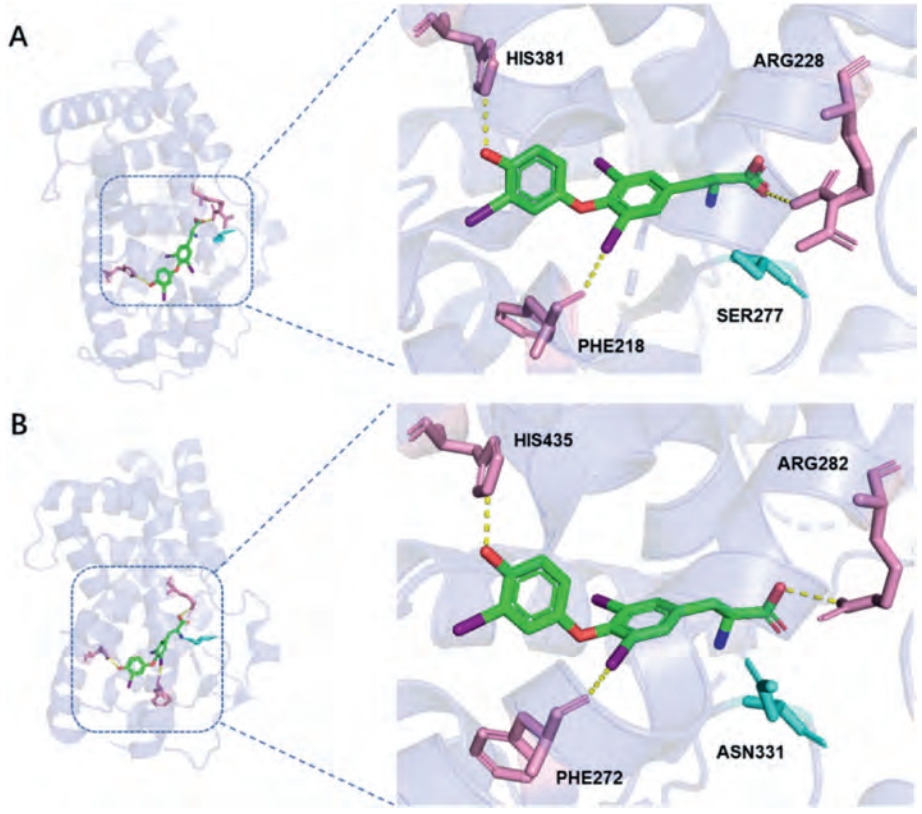


Figure 4 (A) T3 crystal structure in complex with the THRα pocket (PDB:4LNW). (B) T3 crystal structure in complex with the THRβ pocket (PDB:3GWS). Hydrogen bonds and halogen bonds are illustrated in yellow.

3.1. Carboxylic acid THRβ agonists

3.1.1. Sobertirome (GC-1)

Due to deiodination by deiodinase, modifications were made at the hydrophobic groups in both R_1 and R_3 positions. Specifically, the iodine in the R_1 position was substituted with methylenepyrazinone group, and the iodine in the R_3 position was replaced with the bromine group, resulting in the synthesis of compound **1**. In comparison to T3, compound **1** exhibits reduced binding in the heart and increased binding in the liver. Specifically, this compound exhibits 18% T3 activity in the liver, whereas it shows only 0.1% T3 activity in the heart. To enhance THRβ selectivity, substituents at the R_1 position were replaced with isopropyl group, the R_3 position with methyl group, and the R_4 position side chain with oxyacetic acid (Fig. 5). This synthesis resulted in compound **2**, exhibiting 50-fold selectivity for THRβ over THRα[29−34]. Then, GC-1, also known as Sobetirome (3,5-dimethyl-4-(4′-hydroxy-3′-isopropyl-benzyl) phenoxy acetic acid), was synthesized by the University of California in 1998. Its binding affinity for THRβ is comparable to that of T3, the K_d of T3 binding to THRβ is 81 pmol/L, while the K_d of GC-1 binding to THRβ is 67 pmol/L. The binding affinity of GC-1 for THRα is approximately 1/7 of its affinity for THRβ, the K_d of GC-1 binding to THRα is 440 pmol/L, while the K_d of T3 binding to THRα is 58 pmol/L. Thus, GC-1 demonstrates a 10-fold selectivity compared to T3.

To elucidate the selectivity of GC-1, we analyzed its crystal complex structures with both THRα and THRβ proteins. In the THRβ pocket, the side chain at the R_4 position of GC-1 occupies the hydrophilic pocket, forming two hydrogen bond interactions with Arg282 and Arg320. Additionally, the hydroxyl group at the R_2 position establishes a hydrogen bond interaction with His435 (Fig. 6B). Within the THRα pocket, the side chain at the R_4 position of GC-1 lacks hydrogen bond interactions with arginine residues but does form a hydrogen bond interaction solely with Ser277 (Fig. 6C)[35]. Additionally, GC-1 exhibited a 16-fold increase in the ratio of liver to heart. After intravenous injection of 10 μmol/kg T3 and GC-1, the distribution of T3 in plasma, liver and heart was 3188 ± 445; 18,690 ± 6148 and 11,511 ± 1100 ng/mL, respectively; the distribution of GC-1 in plasma, liver and heart was 42.5 ± 8.2; 90.4 ± 23.2 and 5.6 ± 1.2 ng/mL, respectively[36]. In comparison to T3, GC-1 demonstrated a higher liver-to-heart ratio. Its preferential accumulation in the liver might be attributed to the high first-pass uptake rate of the liver and variations in cellular uptake and retention mechanisms.

Thyroid hormones play a pivotal role in regulating processes, such as lipogenesis, fatty acid β-oxidation, cholesterol synthesis, and the reverse cholesterol transport pathway through diverse mechanisms[37]. Individuals diagnosed with hyperthyroidism typically present with decreased serum levels of low-density lipoprotein cholesterol (LDL-C) and an accelerated heart rate. In contrast, those with hypothyroidism often display elevated serum LDL-C levels coupled with bradycardia. These results indicate that thyroid hormone analogs, such as GC-1, have potential lipid-lowering effects. GC-1 demonstrated a dose-dependent reduction in cholesterol in the cholesterol-fed rats, with an effective dose of 1 μg/kg·day. Notably, while the administration of T3 led to a dose-dependent increase in heart rate corresponding to the cholesterol reduction dose range, GC-1 did not exert any influence on heart rate, indicating a more favorable safety profile. Consistent findings emerged from studies involving hypothyroid mice, high-fat choline methionine deficiency (CMD) diet rats, and cynomolgus monkeys[38−40]. Phase I clinical trials revealed that oral administration of GC-1 at a dosage of 100 μg/day led to a significant 41% reduction in serum LDL-C levels in healthy participants during multi-dose administration studies. Unfortunately, GC-1 was discontinued following the completion of Phase I clinical trials in 2008. Nonetheless, owing to its selectivity and potent lipid-lowering efficacy, researchers continued their exploration of the lipid-lowering mechanisms of GC-1 and its potential therapeutic applications.

3.1.2. Eprotirome (KB2115)

Karo Bio has also developed a series of THRβ selective agonists. In the initial stages, the company conducted SAR studies and observed that by replacing the R_4 side chain α-alanine of T3 with acetic acid, the selectivity of compound **3** increased from 0.92 to 2.9 times (Fig. 7A). Substituting the R_1 position of compound **4**

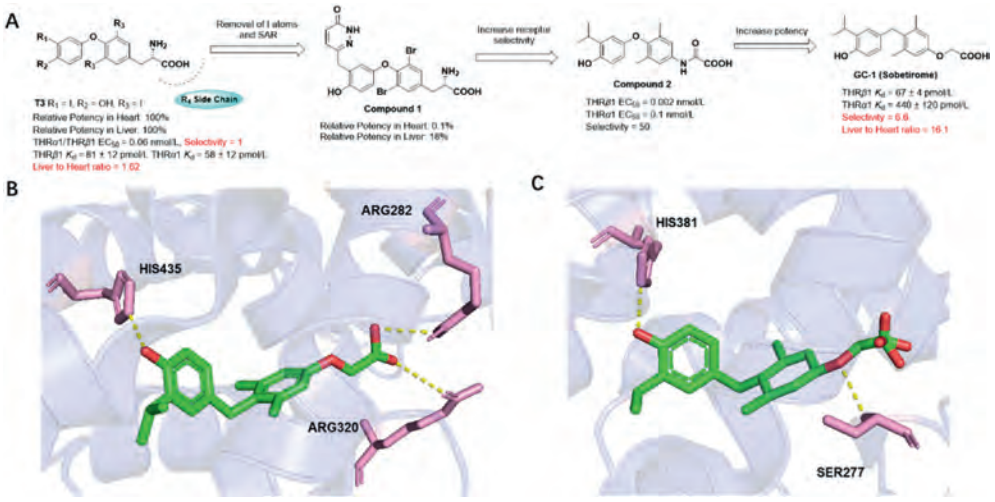


Figure 6 (A) Discovery of GC-1 based on T3. (B) Crystal structure of GC-1 in complex with the THRβ pocket (PDB: 3IMY). (C) Crystal structure of GC-1 in complex with the THRα pocket (PDB: 3ILZ). Hydrogen bonds are illustrated in yellow.

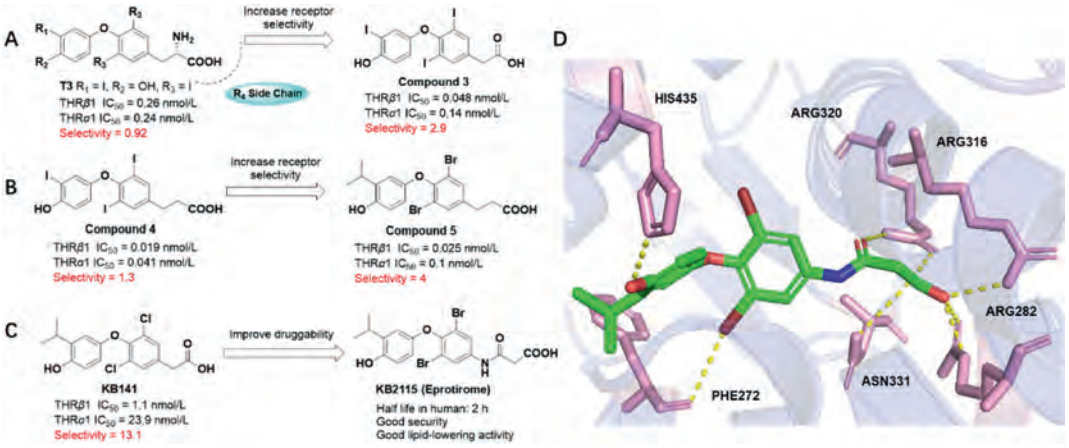


Figure 7 (A) Discovery of compound **3** based on T3. (B) The structure and activity data of compound **4** and compound **5**. (C) Discovery of KB141 and KB2115 based on T3. (D) Binding mode of KB2115 in complex with THRβ pocket. The results were obtained by docking the ligand with the protein crystal complex (PDB: 1N46) using Maestro Schrödinger software. Hydrogen bonds and halogen bonds are illustrated in yellow.

with an isopropyl group to obtain compound **5** resulted in an additional improvement in selectivity while maintaining activity (Fig. 7B). Compound **4** exhibits an IC_{50} of 0.019 nmol/L for THRβ1, while compound **5** shows an IC_{50} of 0.025 nmol/L for THRβ1. Compound **4** demonstrates a selectivity of 1.3 times, whereas compound **5** exhibits a selectivity of 4 times. By combining the advantages of the two types of substitutions, KB141 was synthesized and exhibits a remarkable 13-fold higher affinity for THRβ compared to THRα (Fig. 7C)[41]. KB141 significantly reduced cholesterol levels in various models, including THR$\alpha^{-/-}$ mice, cholesterol-fed rats, and cynomolgus monkeys. Impressively, its therapeutic efficacy remains devoid of tachycardia induction[42]. KB141 reduced plasma cholesterol by approximately 35% at the highest dose (924 nmol/kg·day) in cynomolgus monkeys. After 21 days of administrating KB141, the highest dose group (0.547 mg/kg) demonstrated a 14.8% reduction in body weight and cholesterol levels without inducing tachycardia in obese Zucker *fa/fa* rats. In a 7-day study, KB141 administration at the dosage of 0.547 mg/kg resulted in a 36% decrease in serum total cholesterol, a 28% decrease in triglycerides, an 18% decrease in serum non-esterified fatty acids (NEFA), and a 30% reduction in liver TC levels in *ob/ob* mice[43]. In general, the lipid-lowering effect of KB141 in animals is not as good as GC-1, which may be related to the difference in the distribution of the drug in the liver. Therefore, Karo Bio continued to discover Eprotirome, also known as 3-[3,5-dibromo-4-(4-hydroxy-3-isopropyl-phenoxy) anilino]-3-oxo-propanoic acid and KB2115.

KB2115 demonstrates favorable pharmacokinetic properties. The drug was rapidly absorbed with a half-life approximating 2 h in human subjects with moderately elevated total plasma cholesterol levels (mean total cholesterol 6.6 mmol/L or 255 mg/dL) and body weights (mean body mass index of 31 kg/m^2), and no accumulation was observed. Single oral doses of KB2115 were administered at 50, 200, 500, and 2000 μg/kg. No cardiac side effects were observed at the highest doses, indicating a favorable safety profile. In a two-week multi-dose trial, the highest dose group (200 μg/kg) of KB2115 demonstrated a 40% reduction in serum total cholesterol, apolipoprotein B (ApoB) levels, and LDL cholesterol. Additionally, KB2115 has demonstrated the ability to promote bile acid synthesis at high doses without impacting cholesterol elevation, showcasing a mechanism of action distinct from statins[44]. To investigate the potential of reducing the risk of atherosclerotic cardiovascular disease caused by lipid abnormalities, researchers combined KB2115 with statins. KB2115 was added to patients who had been receiving simvastatin ($\leq$40 mg/day) or atorvastatin ($\leq$20 mg/day) for at least 3 months at doses of 25, 50, or 100 μg/day for 12 weeks. The patients exhibited further reductions in serum LDL-C and lipoprotein(a) [Lp(a)], associated with atherosclerosis progression. These findings suggest that the combination of KB2115 with statins may be effective[45].

To elucidate the good selectivity of KB2115, a molecular docking analysis of KB2115 with THRβ binding pocket has been performed. The results showed that the hydrophobic region of THRβ was occupied by the hydrophobic head of KB2115 with R_1 substituted by isopropyl and R_2 substituted by hydroxyl, and His435 formed hydrogen bond interaction with hydroxyl. Arg282, Arg316, Arg320 and Asn331 in the hydrophilic region of THRβ form multiple hydrogen bond interactions with the R_4 side chain of KB2115. One of the bromine substituents at the R_3 position of KB2115 forms a halogen bond with Phe272. These intermolecular interactions elucidate the selectivity of KB2115 to THRβ (Fig. 7D). Unfortunately, although KB2115 reduced LDL-C levels in patients with familial hypercholesterolemia during Phase III clinical trials (NCT01410383), concerns emerged regarding its potential hepatotoxicity[46]. Additionally, preclinical investigations suggested that KB2115 might lead to cartilage damage in dogs. As a result, the clinical study of KB2115 was discontinued.

3.1.3. IS25 and TG68

Recently, a new halogen-free THRβ agonist, 2-(4-(4-amino-3-isopropylbenzyl)-3,5-dimethylphenoxy)acetic acid (IS25), and its prodrug, 2-(4-(4-acetamido-3-isopropylbenzyl)-3,5-dimethylphenoxy)acetic acid (TG68), were discovered (Fig. 8A)[47]. The R_1 position of the hydrophobic head of the IS25 is replaced by isopropyl, the R_2 position is replaced by amino, the Y position of the linker is replaced by methylene, the R_3 position is replaced by methyl, and the R_4 side chain is replaced by oxyacetic acid. TG68 is an acetamide prodrug at its R_2 position. In *in vitro* off-target and ADME-Tox profiling[48], both compounds IS25 and TG68 exhibited almost non-toxicity. IS25 exhibits a high selectivity for THRβ, with an EC_{50} value of 458 nmol/L, and THRα activation is limited to 28% at a concentration of 1 μmol/L.

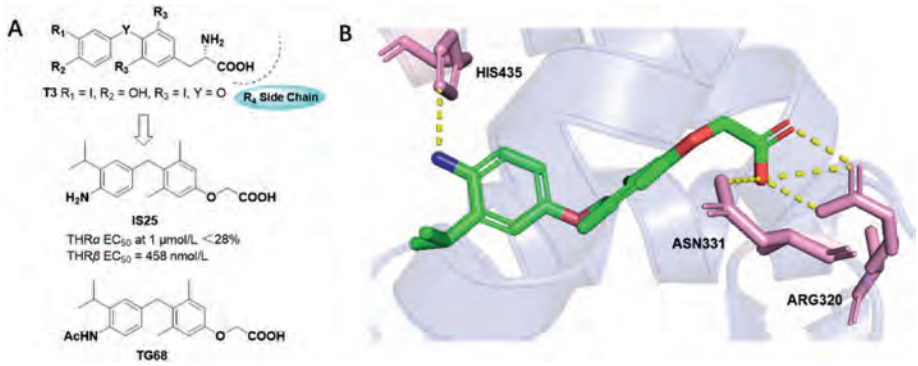


Figure 8 (A) Chemical structures of IS25 and TG68. (B) Binding mode of IS25 in complex with THRβ pocket. The results were obtained by docking the ligand with the protein crystal complex (PDB: 1N46) using Maestro Schrödinger software.

Molecular docking studies elucidate that the carboxylic acid group of IS25 forms hydrogen bonds with Arg320 and Asn331. And, the amino group of IS25 forms a hydrogen bond with His435 (Fig. 8B). Further investigations revealed that IS25 effectively reduced lipid accumulation in HepG2 cells. This mechanism is intricately linked to the stimulation of AMPK phosphorylation, subsequently culminating in the phosphorylation and deactivation of acetyl coenzyme A carboxylase (ACC), a primary regulator of fatty acids synthesis. No hepatic toxicity or cardiac hypertrophy was observed in F344 male rats at the highest dosage (1 mg/kg) after daily intragastric administration of IS25, and there were no changes in serum levels of markers indicative of liver injury, such as transaminases or bilirubin. In high-fat diet (HFD) mice, TG68 oral administration for 2 or 3 weeks (9.35 mg/kg in drinking water) significantly reduced liver steatosis and serum transaminases, but had no significant adverse effects on extrahepatic tissues such as kidneys or hearts. Intriguingly, although most of the effects of TG68 were almost the same as those of MGL-3196, only TG68 significantly reduced circulating TG levels[49].

*3.1.4. **16g***

All known thyroid hormone analogs formed hydrogen bonds with His435 in the THRβ pocket, and His435 is a crucial amino acid residue for maintaining their THRβ agonistic activity. Mutations in His435 are the primary cause of thyroid hormone resistance syndrome (SRTH), also known as thyroid hormone insensitivity syndrome (THIS), which is a rare disease. SRTH has a low prevalence, estimated between 0.001% and 0.002%[50,51]. Among the reported cases, familial instances account for approximately two-thirds, with sporadic cases constituting the remainder. Primarily afflicting children and adolescents, SRTH can even manifest in newborns and affects both genders. Clinically, patients typically present elevated serum-free T4 (FT4) and serum-free T3 (FT3) levels, while thyroid-stimulating hormone (TSH) levels remain within the normal range. Currently, there are no investigational drugs for the treatment of SRTH.

In recent years, Xiamen University has discovered that the hypoxia-inducible factor alanine hydroxylase (HIF-PHD) inhibitor compound **6** (Fig. 9A) can serve as a potent agonist for THRα and THRβ, with EC_{50} values of 32.7 and 12.9 nmol/L, respectively. Utilizing a structure-guided design approach, the authors optimized the structure of compound **6** to generate compound **7**, eliminating PHD2 inhibition. During this optimization, it was observed that the isoquinoline skeleton and glycine side chain of the compound are crucial for maintaining THRβ agonistic activity. Previous exploration revealed that the hydrophobic pocket of THRβ is more flexible than that of THRα, offering an opportunity for developing selective THRβ agonists[52]. Building upon compound **7**, the authors introduced a large sterically hindered hydrophobic group on the left side of the isoquinoline ring, leading to compound **8**, which demonstrated selectivity for THRβ (EC_{50} of 19.8 nmol/L, inactive for THRα). Besides EC_{50} values, the researchers also evaluated the maximum activation ability of the compounds in comparison to T3. **16g**, achieved through additional structural optimization, retained potent THRβ activity (EC_{50} of 21 nmol/L) and exhibited the highest maximum activation ability (85%, compared to 44.5% for compound **8**) in this series. Notably, **16g** maintained selectivity for THRβ and demonstrated a moderate agonist effect against the THRβ H435R mutant, with an EC_{50} value of 1344.0 nmol/L[53].To gain a comprehensive understanding of the binding mode of **16g** with THRβ, docking studies were conducted. Docking analysis revealed that the carboxyl group of the **16g** establishes hydrogen bonds with Arg282 and Arg316. The nitrogen atom and oxygen atom of the amide fragment form hydrogen bond interactions in Met313 and Asn331, respectively. Additionally, the conserved hydroxyl group establishes hydrogen bonds with the side chain of Asn331 (Fig. 9B). These interactions stabilize the **16g** conformation, ensuring good selectivity and activity. The docking results of **16g** also support the previous view that the hydrophobic subpocket of THRβ is more flexible compared to that of THRα. In the pharmacodynamic study, **16g** significantly reduced the levels of TG and total cholesterol (TC) in HepG2 cells. Consistent with these results of the HepG2, **16g** significantly decreased TG and TC levels in primary mouse hepatocytes and reduced lipid accumulation. This observed effect can be attributed to the stimulation of AMPK phosphorylation, resulting in the phosphorylation-induced inactivation of ACC and an increase in carnitine palmitoyltransferase (CPT1).

3.2. Cyano-substituted aziridine THRβ agonists

3.2.1. Resmetirom (MGL-3196)

2-[3,5-Dichloro-4-(5-isopropyl-6-oxo-1,6-dihydropyridazin-3-yloxy) phenyl]-3,5-dioxo-2,3,4,5-tetrahydro[1,2,4]triazine-6-carbonitrile, known as Resmetirom or MGL-3196, is a potent oral selective THRβ agonist. Recognized by the FDA as a breakthrough therapy, MGL-3196 is now used for the treatment of MASH patients with liver fibrosis. The hydrophobic head of many T3 analogs containing R_1 and R_2 substituents contains a phenol

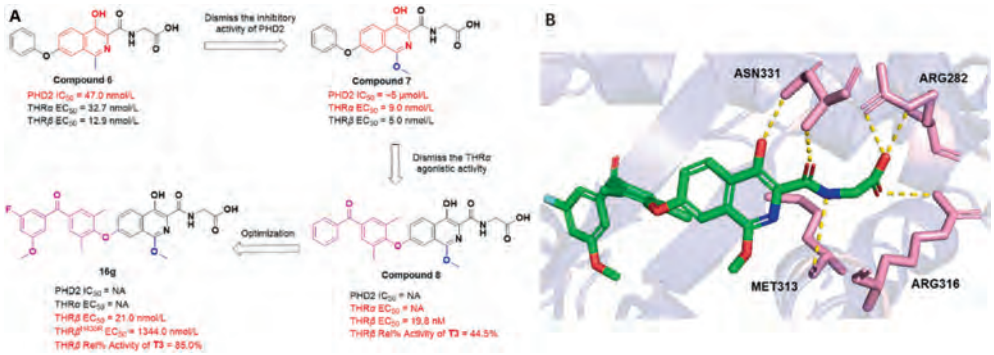


Figure 9 (A) Discovery of **16g** based on compound **6**. (B) Binding mode of **16g** in complex with THRβ pocket. The results were obtained by docking the ligand with the protein crystal complex (PDB: 7WML) using Maestro Schrödinger software.

structure. This structure and nitrite can undergo free radical nitration at gastric pH to form toxic nitration derivatives, which has potential metabolic and safety risks. Initially, the phenol structure was replaced by a heterocyclic ring, and it was found that the selectivity of the electron-deficient pyridazine analog compound **9** reached 10.23-fold (Fig. 10A). Subsequent optimization of the R_4-side chain revealed that the azauracil substituted with a cyano group exhibited the better selectivity (28.29-fold) and EC_{50} (EC_{50} of THRβ is 0.21 μmol/L), leading to its identification as MGL-3196[54]. The binding mode of MGL-3196 and other thyroid hormone analogs with THRβ are remarkably similar. The most notable difference lies in their interactions with Arg320. When the side chain at the R_4 position is a linear acid substitution, its hydrogen bond interaction with Arg320 is often mediated by a water bridge, while the cyclic acid group substitution of MGL-3196 (negatively charged) can directly interact with Arg320. Upon substituting the R_4-position of azauracil with a cyano group, the larger and more diffuse negatively charged heterocycle may provide specific benefits over traditional carboxylic acids, since it displaces several waters and would be expected to suffer a smaller binding desolvation penalty relative to a carboxylic acid (Fig. 10B). Further investigations have shown that MGL-3196 has certain liver tissue targeting (liver to serum ratio = 6:1) and its favorable safety profile. This may be because the action of MGL-3196 requires the expression of OAT1B1, a hepatocyte-specific organic anion transporting polypeptide family (OATP)[55]. C57BL/6J diet-induced obesity (DIO) mice treated with MGL-3196 at a dosage of 3 mg/kg for 8 weeks. This led to significant reductions in liver weight, hepatic steatosis, plasma alanine aminotransferase levels, as well as liver and plasma cholesterol. Moreover, blood glucose levels were notably decreased. There was a significant improvement in the NAFLD activity score (NAS), and a reduction in the degree of liver fibrosis was observed[56].

The Phase I clinical trial included a single ascending dose study (NCT01367873), and a two-week multiple-dose investigation (5, 20, 50, 80, 100, and 200 mg/day) in healthy individuals with slightly elevated LDL-C (>110 mg/dL) (NCT01519531). MGL-3196 demonstrated excellent tolerability across all doses, with no dose-dependent adverse events observed. The optimal lipid-lowering effects were observed at a dosage of 80 mg daily, demonstrating highly statistically significant reductions when compared to the placebo. These reductions encompassed a 30% decline in LDL-C, a 28% reduction in non-high density lipoprotein cholesterol (non-HDL-C), a 24% reduction in ApoB, and a 60% reduction in TG[57]. One of the Phase II clinical trials of MGL-3196 focused on MASH patients exhibiting stage 1−3 fibrosis. Evaluations at weeks 12 and 36 using MRI proton density fat fraction (MRI-PDFF) (NCT02912260), highlighted that administration of 80 mg/kg MGL-3196 resulted in a significant reduction in various atherosclerotic markers, including LDL-C, ApoB, TG, Lp(a), and apolipoprotein CIII (ApoCIII). Additionally, it exhibited improvements in liver injury and fibrosis, reduced NAS scores, and demonstrated good tolerance and safety[58]. In another 12-week phase II clinical study for patients with heterozygous familial hypercholesterolemia (HeFH), the oral administration of MGL3196 (at dosages of 100 and 60 mg/kg in a crossover design) significantly reduced LDL-C and other atherosclerotic lipids or lipoprotein levels in HeFH patients, demonstrating good safety (NCT03038022)[59].

Phase III clinical trials, including MAESTRO-MASLD (NCT04197479), MAESTRO-MASH (NCT03900429), MAESTRO-MASLD-OLE (NCT04951219), and MAESTRO-MASH-OUTCOMES (NCT05500222), have been pivotal in evaluating the efficacy and safety of MGL-3196 for the treatment of MASLD and MASH. The MAESTRO-MASLD trail is a 52-week study involving more than 950 patients who underwent a series of liver biopsies designed to evaluate the safety and efficacy of MGL-3196 at doses of 80 and 100 mg/kg in adults, MGL-3196 demonstrated promising outcomes. Notably, the study revealed no significant difference in treatment-emergent adverse events (TEAEs) between MGL-3196 and the placebo after 52 weeks treatment period. Moreover, dosage evaluations yielded significant reductions in key parameters. Specifically, MGL-3196 at 80 mg/kg and 100 mg/kg exhibited notable decreases in LDL-C (−11.1%, −12.6%), ApoB (−15.6%, −18.0%), TG (−15.4%, −20.4%), liver fat (−34.9%, −38.6%) at 16 weeks, liver stiffness (−1.02, −1.70), and liver fat (−28.8%, −33.9%) at 52 weeks, respectively[60]. On March 5th of this year, the European Medicines Agency (EMA) approved MGL-3196 for the treatment of MASH and liver cirrhosis. Similarly, on the 15th of the same month, the FDA approved this drug for the treatment of MASH, marketed by Madrigal Pharmaceuticals Inc. under the name Rezdiffra® (NDA217785).

3.2.2. *Compound **15***

Based on the docking diagram of MGL-3196 and THRβ pocket, the researchers of Shanghai Institute of Medicine, Chinese Academy of Sciences speculated that there is a space between the two phenyl groups of MGL-3196 that can accommodate a moderately sized ring. Therefore, on this basis, MGL-3196 was

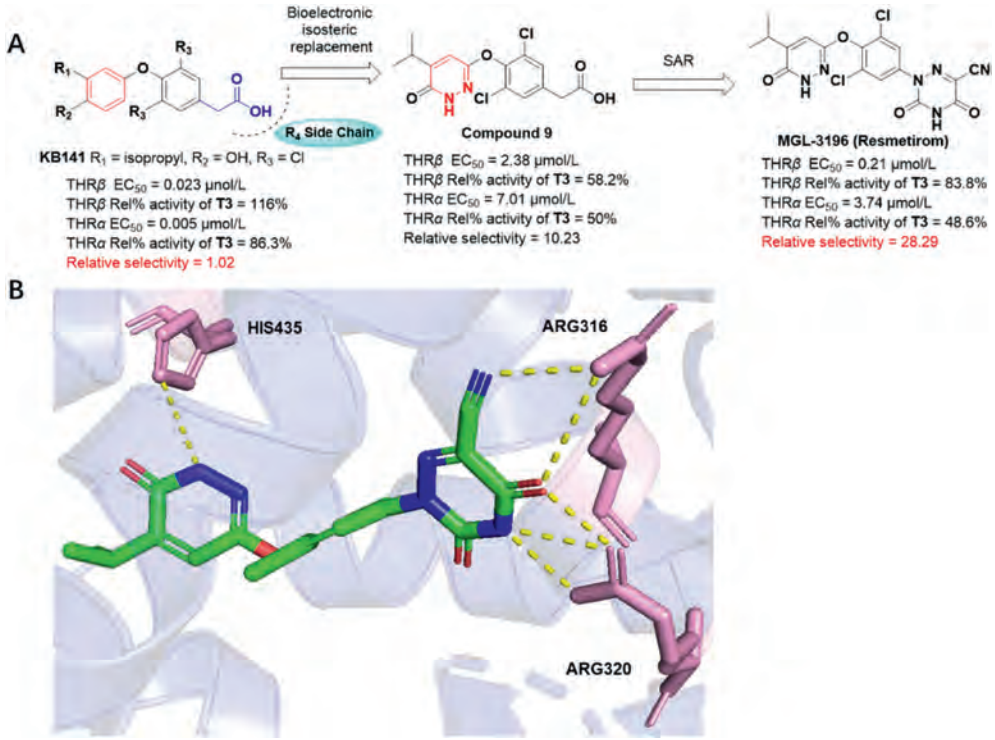


Figure 10 (A) Discovery of MGL-3196 based on KB141. (B) Binding mode of MGL-3196 in complex with THRβ pocket. The results were obtained by docking the ligand with the protein crystal complex (PDB: 1N46) using Maestro Schrödinger software.

cyclized to construct a rigid ring skeleton to obtain compound **10**. Compound **10** showed high THRβ activity (EC_{50} of THRβ is 0.03 μmol/L) and moderate selectivity (2.9-fold). Considering that the hydroxyl phenyl group is the site that forms a hydrogen bond interaction with His435, it is replaced with the pyridone structure, and the hydrophobic group is further introduced for structural optimization to obtain the optimal compound **15**[61] (Fig. 11A). Compound **15**, featuring a pyrrolo[3,2-*b*] pyridine-5-one skeleton, stands out as a novel THRβ agonist with commendable efficacy, selectivity, and liver targeting capabilities. The EC_{50} values for THRα and THRβ are 0.22 ± 0.04 and 0.03 ± 0.01 μmol/L, respectively, resulting in a relative selectivity of 11.2 ± 4.2 fold. Pharmacokinetic evaluation of compound **15** reveals excellent oral exposure in the liver following a 10 mg/kg oral administration, with an AUC value of 1,124,265 ng h/mL. Importantly, the serum exposure of compound **15** is significantly lower than in the liver, boasting a liver-to-serum ratio of 93:1. This notable liver accumulation suggests a potential mitigation of the multiple adverse effects associated with THR activation. Docking results highlight key interactions, with the carbonyl group on the pyridine ring forming a hydrogen bond with His435, and the acidic group forming a hydrogen bond interaction with Arg320 and Arg316, ensuring the adhesion of the molecule in the THRβ pocket (Fig. 11B).

Compound **15** demonstrates remarkable efficacy in reducing TC and serum LDL-C levels in ICR mice, with a notable TC reduction exceeding 30% at a 30 mg/kg dose. Its effectiveness in lowering serum LDL-C levels at 3 and 10 mg/kg is better than that of MGL-3196. Compound **15** also up-regulates the expression of THR target genes *Dio1*, *Thrsp*, and *Me1* in the liver, with no significant impact on heart-related genes observed. *In vivo* effects of compound **15** were assessed in a high-fat diet combined with a CCl_4-induced MASH model. Results revealed a significant, dose-dependent reduction in serum total cholesterol and LDL-C levels. Additionally, there is a significant decrease in hepatic steatosis, inflammation score, and NAS value. The compound also hinders the progression of fibrosis, suggesting that compound **15** effectively ameliorates symptoms in MASH-afflicted animals and holds promise as a potential drug candidate for the treatment of MASH.

3.3. Using hep-direct prodrug strategy THRβ agonists

T3 analogs, including GC-1 and KB2115, exhibit tissue selectivity, with a preference for distribution in the liver. THRβ is primarily distributed in the liver. Activation of THRβ in the liver offers beneficial outcomes on plasma cholesterol and lipoprotein levels, achieved through various mechanisms. However, activation of THRs in other tissues might lead to cardiovascular alterations, disrupt the thyroid axis, induce muscle atrophy, and contribute to bone demineralization. Theoretically, the potential adverse effects could be ameliorated by reducing the circulating levels of THR agonists and/or restricting their absorption by non-hepatic tissues.

Generally, the pK_a values of drugs containing phosphate groups are mostly between 1 and 2. At the physiological pH range of 7.0−7.4, these molecules readily undergo deprotonation, assuming a high negative charge. Medicinal chemists have extensively explored phosphate drugs, discovering that the introduction of such groups enhances drug transportation while alleviating challenges associated with poor membrane permeability. Furthermore, the introduction of phosphate groups into drugs is characterized by limited aqueous solubility. Erion and his co-workers[62] discovered a novel phosphate prodrug, the hep-direct prodrug, which undergoes specific metabolism by hepatocyte CYP450 enzymes, liberating active compounds tailored

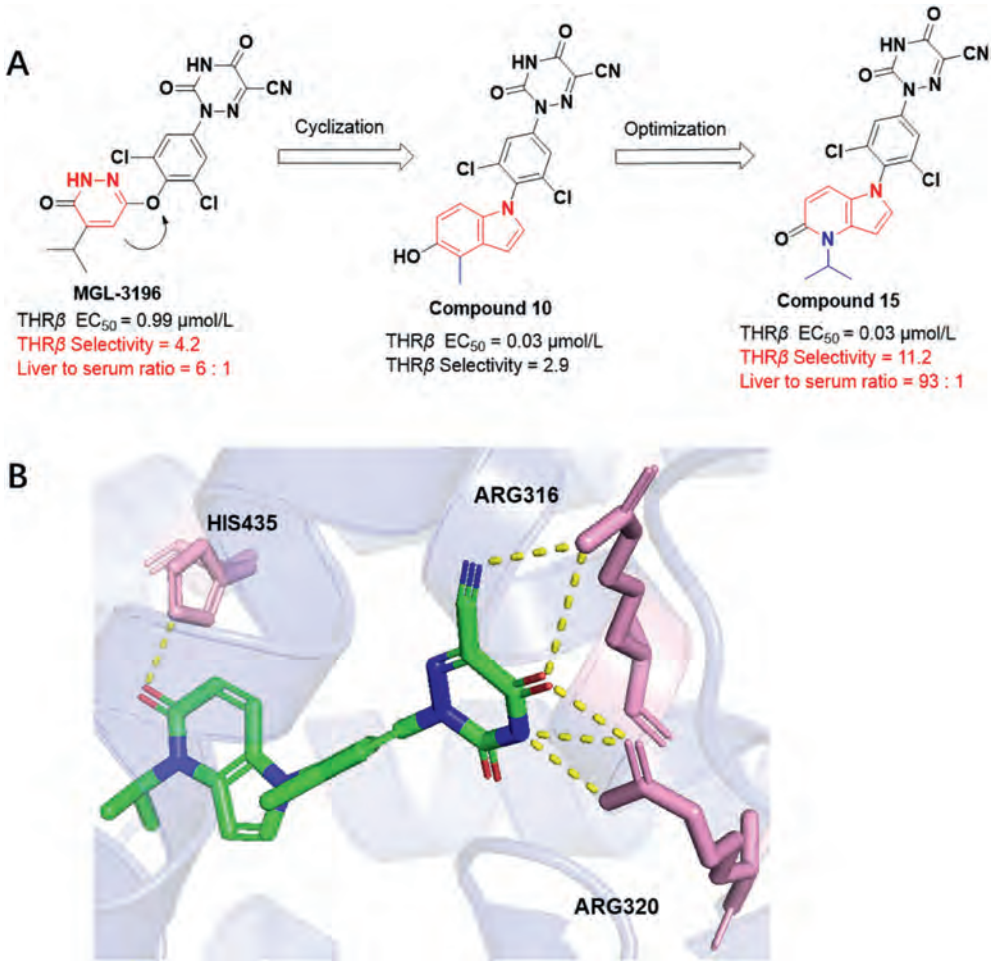


Figure 11 (A) Discovery of compound **15** based on MGL-3196. (B) Binding mode of compound **15** in complex with THRβ pocket. The results were obtained by docking the ligand with the protein crystal complex (PDB: 1N46) using Maestro Schrödinger software.

exclusively for hepatic targeting. As this prodrug is primarily activated in the liver, phosphate prodrugs have been extensively used for the development of drugs for the treatment of liver diseases.

MB07811, also known as (2*R*,4*S*)-4-(3-chlorophenyl)-2-[(3,5-dimethyl-4-(4′-hydroxy-3′-isopropylbenzyl)phenoxy)methyl]-2-oxido-[1,3,2]-dioxaphosphonan(Fig. 12B), is a prodrug form in which the R_4 side chain of GC-1 is replaced by a phosphonic acid group[63]. A series of compounds containing different carboxylic acid groups with phosphonate groups have been designed and synthesized to investigate the binding affinity of THRβ and explore the structure-activity relationship. The findings can be summarized as: (1) For R_1 substituents, such as methyl, ethyl, and isopropyl, there is an increased binding affinity to THRβ as the chain length increases. However, when introducing a rigid benzene ring structure, a significant decrease in binding affinity is observed. (2) Consistent with the prior report, the impact of the R_3 group on activity is I > Br > Cl > CH_3. Subsequently, nine compounds were selected to evaluate their capacity over 24 h to reduce total plasma cholesterol (TPC) in a high-fat cholesterol-fed rat model. At a dosage of 0.2 mg/kg, MB07344 reduced TPC by 37%, while exhibiting almost no distribution in the heart. Interestingly, changing the oxygen substituent at the Y-position to methylene has little effect on the binding affinity of THRβ. However, in the study of lipid-lowering effects in animals, a significant decrease in TPC was observed when the Y-position was oxygen (Fig. 12A). Subsequently, a series of prodrugs of MB07344 were designed and synthesized, evaluating their oral lipid-lowering ability, activation rate, and prodrug residue. Among them, MB07811 emerged as the most promising candidate for further investigation. The pharmacokinetic properties of MB07811 were evaluated in rats at a dosage of 3 mg/kg. The results indicated that it underwent initial clearance by the liver and subsequent activation by tissue-selective enzymes, resulting in the formation of the THRβ-selective active compound (3,5-dimethyl-4-(4′-hydroxy-3-isopropylbenzyl) phenoxy) methylphosphonate, also known as MB07344 (Fig. 12C)[64]. MB07344 exhibited an oral bioavailability of approximately 39%, with minimal distribution in non-hepatic tissues and rapid elimination through the bile system.

MB07811 was observed to increase the mRNA expression levels of liver-associated genes, such as *CYP7A1*, malic enzyme (*ME*), and *LDLR*, while having no discernible effect on the heart and muscle. In DIO mice, MB07811 reduced TC, serum TG, and liver TG without influencing body weight, blood glucose. These results indicate that liver-targeted THR agonists possess the potential to reduce TC and TG while minimizing effects on non-hepatic tissues, thereby potentially enhancing safety for the treatment of hyperlipidemia. Cable et al.[65], employing diverse

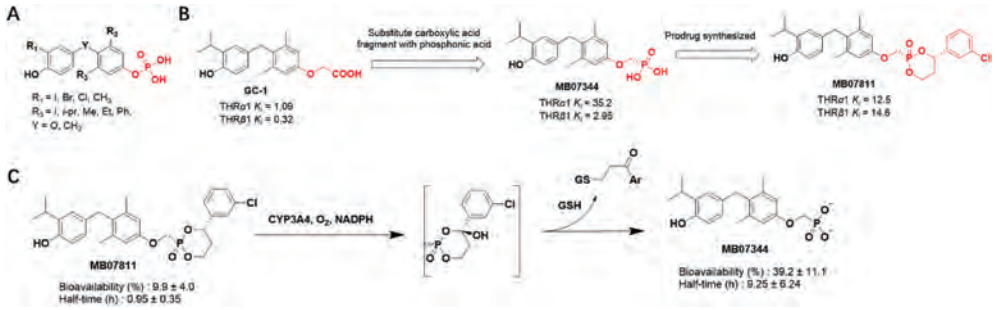


Figure 12 (A) A general formula for the structure of compounds containing phosphonic acid groups. (B) Discovery of MB07811 based on GC-1. (C) Metabolism of prodrug MB07811 to MB07344 *in vivo*.

animal models for evaluating the treatment of MASLD, such as Zucker diabetic fatty (ZDF) rats, *ob/ob* mice, and DIO mice, consistently demonstrated that MB07811 significantly reduced hepatic steatosis, plasma FFA, and TG levels. It was also discovered that MB07811 treatment decreased hepatic TG levels in glycogen storage disease type Ia (GSD Ia) mice by concurrently restoring autophagy, mitochondrial biogenesis, and β-oxidation of fatty acids[66].

In the Phase I clinical trial of MB07811 involving individuals with mild hypercholesterolemia, the multi-dose administration resulted in a substantial reduction in LDL-C, TG, and atherosclerotic proteins. In a Phase II clinical trial targeting MASLD and elevated LDL-C, MB07811 administered at a dosage of 5−10 mg/kg·day, demonstrated a significant reduction in both LDL-C and liver fat content. Importantly, the safety and tolerability profiles were favorable in both clinical trials (NCT02927184). A recent Phase 2b clinical trial for individuals with biopsy-confirmed MASH has been finished. According to the MRI-PDFF evaluations, individuals treated with MB07811 witnessed a significant reduction in hepatic fat content after 12 weeks. Remarkably, an impressive 85% of participants achieved a relative reduction in hepatic fat content at least 30%. Consistent with previous studies, MB07811 treatment let to a significant decrease in LDL-C levels, ranging from 11% to 20%. Additionally, notable reductions were observed in TG and atherosclerotic proteins, such as ApoB, Lp(a), and ApoC-III. Throughout the 12-week study duration, there were no changes in the alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels of the participants (NCT04173065).

4. Potential application of targeting THRβ in other disease indication

While clinical studies on GC-1 and KB2115 for MASH indications have been discontinued, they continue to serve as pivotal tool molecules for investigating the THRβ target. The THR agonists are used clinically for the treatment of multiple indications, including stroke atherosclerosis, liver regeneration and promoting hair growth[67], etc.

4.1. Atherosclerosis

LP(a) is identified as a risk factor for atherosclerosis. In a cynomolgus monkey model, GC-1 was administered at doses of 154 and 924 nmol/kg·day for one week, resulting in a significant reduction of LP(a) levels by approximately 40%, and a decrease in cholesterol levels[38]. Apolipoprotein E (ApoE) plays a pivotal role in intermediate-density lipoprotein particles and interacts with various receptors, including LDLR. Mutations in ApoE hinder the clearance of several lipoproteins, leading to increased plasma cholesterol and the early onset of severe atherosclerosis and cardiovascular disease. ApoE-deficient mice serve as valuable models to investigate the preventive effects of GC-1 against atherosclerosis. GC-1 was administered orally at a dose of 0.12 mg/kg, and biochemical indicators were assessed at 1, 10, and 20 weeks. The results demonstrated that GC-1 could postpone the occurrence of atherosclerosis in ApoE gene-deficient mice[68]. Treatment with GC-1 for 20 weeks resulted in decreased whole aorta content of cholesteryl esters.

4.2. Liver regeneration

The liver exhibits remarkable regenerative capabilities, standing as the only solid organ with the potential for complete self-renewal. Liver transplantation remains the primary treatment for end-stage liver diseases, such as cirrhosis, severe hepatitis, and advanced liver cancer. However, the global scarcity of viable liver donors, coupled with the invasiveness and financial burden of transplantation surgery, underscores the pressing need for alternative therapies. There is an urgent need for safe and effective small-molecule liver regeneration drugs that can serve as alternatives to liver transplantation. Intriguingly, similar to T3, GC-1 demonstrates a significant ability to induce rat liver cell proliferation, suggesting that THRβ agonists might contribute to liver regeneration[69]. The mechanism underlying this effect appears to mirror that observed in normal rats treated with T3, which induces protein kinase (PKA) activation in hepatocytes, leading to downstream Ser675 phosphorylation, activation of β-catenin, and elevated cyclin D1 expression, promoting hepatocyte proliferation. Additionally, T3 can induce extracellular liver endothelial cells to release Wnt protein in a paracrine manner, stimulating the classical Wnt/β-catenin signaling pathway and initiating downstream gene transcription[70−72]. Similar phenomena and results were also observed after the administration of KB2115, IS25, and TG68[73,74]. Specifically, administering TG68 and IS25 to F344 rats for a week can induce significant hepatocyte proliferation, with increased expression of cyclin D1, cyclin A, and proliferating cell nuclear antigen (PCNA) related to mitosis observed in the liver of rats treated with these two THRβ agonists. Notably, hepatocyte proliferation seems to be linked to the activation of THRβ target genes, such as *Dio1* and *Spot14*, suggesting that the mitotic effects of these drugs might arise from the binding and activation of THRβ. Interestingly, while past studies have shown that T3 and GC-1 have a pronounced mitogenic effect on pancreatic acinar

cells, potentially leading to unknown side effects, no similar significant mitogenic effects were observed in the pancreas of rats treated with TG68 and IS25 at a dose of 50 μg/100 g, indicating higher safety.

4.3. Obesity

Cold-induced adaptive (or nonshivering) thermogenesis in small mammals primarily occurs in brown adipose tissue (BAT)[75], where heightened heat production aids in reducing fat mass. Research indicates that UCP1 expression in BAT is crucial for adaptive thermogenesis and is preferentially stimulated by THRβ[76]. Studies have demonstrated that GC-1, a THRβ agonist, promotes weight loss and lipid reduction in mice and rats by increasing energy expenditure and metabolic rate[77,78]. This suggests the potential of THRβ agonists in facilitating weight loss and lipid reduction.

4.4. Hepatocellular carcinoma (HCC)

Several lines of evidence suggest that conditions characterized by liver damage and enhanced hepatocyte turnover are associated with an increased incidence of HCC in humans as well as experimental animals. And unfortunately, MASLD-related HCC patients have higher tumor stage, lower eligibility for curative treatment, shorter survival time and higher tumor recurrence rate[79]. Notably, T3 is a potent hepatomitogen that induces liver hyperplasia in the absence of cell death[80,81]. Importantly, unlike other proliferative stimuli, it leads to rapid regression of carcinogen-induced hepatic nodules and reduces the incidence of HCC and lung metastasis. The antitumoral property of T3 appears to be specific and not shared by other liver mitogens, such as ciprofibrate, a member of the class of peroxisome proliferators[82]. Short-term treatment with GC-1 (5 mg/kg) in rats significantly reduces the number of precancerous lesions in two different liver cancer experimental models, leading to their reversion to a differentiated phenotype[83]. In a recent study, short-term administration of TG68 (2.8 mg/kg in drinking water for 2 weeks) not only led to liver fat accumulation and decreased serum triglycerides and cholesterol, but also induced the regression of diethylnitrosamine (DEN)-induced precancerous lesions associated with the differentiation process, as evidenced by the loss of tumor markers and the reacquisition of differentiated hepatocyte markers. Although equimolar doses of resmetirom reduced liver fat accumulation, it did not exert any anti-tumor effect[84]. These findings suggest that liver THRβ agonists can reduce the number and size of precancerous liver lesions associated with differentiation procedures, highlighting their positive therapeutic significance for MASLD-related HCC.

4.5. X-linked adrenoleukodystrophy (X-ALD)

Currently, GC-1 is under investigation as an orphan drug for the treatment of the rare disease X-ALD, caused by mutations in the membrane lipid transporter ATP-binding cassette subfamily D member 1 (ABCD1)[85]. This genetic anomaly results in the accumulation of toxic very long-chain fatty acids (VLCFA) in all cells, leading to disorders of the CNS and adrenal glands. Thyroid hormones induce the expression of one of the related genes, ABCD2. Studies have demonstrated that GC-1 can penetrate the CNS and induce ABCD2, thereby increasing VLCFA uptake to alleviate the impact of the disease[86]. In addition, researchers have found that modifying GC-1 to an amide ester prodrug can enhance the ability to cross the blood–brain barrier (BBB)[87–89]. These promising results suggest that the use of THRβ agonists may hold potential for treating neurodegenerative diseases involving demyelination or other oligodendrocyte function abnormalities. This includes conditions such as multiple sclerosis (MS), cerebral palsy, and other leukodystrophies[90,91].

4.6. Multiple sclerosis (MS)

Myelin destruction due to inflammatory damage of oligodendrocytes (OLs) combined with axonal degeneration is one of the major histopathological hallmarks of MS, a common autoimmune disorder affecting the CNS. OLs are responsible for the formation of CNS myelin during development and the renewal and repair of adult myelin. Oligodendrocyte precursor cells (OPCs) can differentiate into OLs, and mature OLs can be wrapped around unmyelinated axons to form myelin sheaths. This repair process, known as myelin regeneration, is considered to be a potential target for the treatment of progressive MS[92]. The gene for kruppel-like factor 9 (*KLF9*), which is rapidly and robustly upregulated by TH, is necessary and sufficient to promote oligodendrocyte differentiation *in vitro*. Moreover, KLF9-induced proteins can activate chemokine (C−X−C motif) ligand 12 (CXCL12) and (C−X−C motif) receptor 4 (CXCR4) which are constitutively expressed in the CNS, and implicated in the pathogenesis of various neurodegenerative and neuroinflammatory diseases. Studies have shown that THs may activate KLF9 gene transcription, up-regulate the KLF9-mediated CXCL12/CXCR4 signaling axis, thereby promoting OPCs, myelination or remyelination, and promoting MS remission[20]. A recent study used a validated cell platform, combined with high-content screening (HCS) imaging technology based on OPCs derived from neural stem cells (NSCs), to reproduce the entire differentiation process and verify the ability of THRβ agonist TG68 to promote myelin regeneration[93].

4.7. Pulmonary fibrosis (IPF) and COVID-19

Remarkably, in 2007, researchers unveiled the pivotal role of thyroxine in the prevention and treatment of pulmonary fibrosis. It was observed that the activity and expression levels of iodothyronine type II deiodinase (*DIO2*) in the lungs of patients with idiopathic pulmonary fibrosis were significantly higher than those in the control group and closely correlated with disease severity. GC-1 has also demonstrated efficacy in attenuating bleomycin-induced pulmonary fibrosis[94]. Furthermore, GC-1 holds promise for preventing and treating acute respiratory distress syndrome (ARDS), a severe condition prevalent in COVID-19 patients, especially the elderly, which can lead to respiratory failure[95]. Research on the efficacy of Sobetirome for these two indications remains in the preclinical stage.

5. Perspectives and conclusions

In the human body, thyroid hormones can enhance the cellular oxidation rate and promote the metabolism of glucose, fat, and protein. Many beneficial effects of thyroid hormones are mediated by THRβ, which has become a prominent target for the treatment of MASLD in recent times. Currently, MASLD is emerging as one of the most prevalent chronic liver diseases globally, and it is a leading cause of increasing cirrhosis and liver cancer. As a chronic disease, there is a high demand for drug safety, and as of now, there is still no approved treatment for this disease. The

endogenous THR ligand T3 exerts a wide range of effects in different cell types, largely due to the cell-specific expression of THR isoforms. THRα, for example, is mainly associated with cardiovascular functions, while THRβ is linked to metabolic regulation. Enhancing the selectivity of THRβ activation could alleviate many side effects caused by the activation of THRα. For MASLD treatment with complex pathogenesis, ideal THRβ agonists should have both selectivity and liver targeting. With advancements in protein crystallography technology, it is evident that the hydrophobic pocket of THRβ is more flexible than THRα. This insight guides pharmaceutical chemists to appropriately increase the steric hindrance of small molecule compounds during development, potentially enhancing the activity and selectivity of THRβ. We observed a significant reduction in the compound's affinity for the THR receptor upon introducing the phosphonic acid group into the hepato-targeted prodrug. Compared with GC-1, the binding affinity of MB07344 to THRβ decreased by about 9 times. How to improve the excitatory activity of hepato-targeted prodrugs is also the bottleneck that needs to be overcome in future research. Resmetirom, distinguished by its remarkable selectivity, circumvents THRα activation outside the liver, thereby avoiding potential effects on organs like the heart and bones. It has become the first drug has been approved for the treatment of MASH. Resmetirom has displayed effectiveness and holds promise for prescription in the near future.

The mechanism underlying the effects of THRβ agonists has been progressively elucidated, revealing their ability to reduce LDL-C and TC, promote fatty acid decomposition, and stimulate mitochondrial biogenesis. These actions contribute to the reduction of lipotoxicity and enhancement of liver function. However, recent studies have uncovered a dose-dependent impact of GC-1 on glycemic control. Lower doses of GC-1 were found to further impair glycemic control, while a higher dose of the same compound resulted in substantially improved glucose tolerance and insulin sensitivity. Intriguingly, all doses were equally effective at reducing hepatic triglyceride levels. Mice treated with KB2115 also exhibited increased fasting glucose, although KB2115 did not elevate fasting insulin levels. These findings suggest that THRβ agonists ameliorate hepatic steatosis but may impact insulin sensitivity *via* discrete pathways, potentially influenced by dosage and duration of treatment[96,97]. Therefore, in evaluating the therapeutic potential of THRβ agonists for MASLD, it is crucial to consider their effects on insulin sensitivity, and a suitable treatment window should be identified.

Endogenous thyroid hormones play a vital physiological role in promoting growth and development. Consequently, THRβ agonists are hypothesized to possess potential benefits in promoting hair growth and liver regeneration. The role of THRβ agonists in promoting liver regeneration has been progressively validated in recent decades, although the specific underlying mechanism is still under investigation. Moreover, the activation of THRβ in organs beyond the liver, including the brain and lungs, suggests potential therapeutic applications for CNS diseases and pulmonary fibrosis. In conclusion, THRβ targets hold significant promise for future research endeavors.

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Author contributions

Kean Wang conceptualized and wrote the manuscript. Feiyang Chen carried out molecular docking and revised the manuscript. Jiang Wang and Hong Liu supervised the whole process and also revised the manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

References

1. Zhang JS, Lazar MA. The mechanism of action of thyroid hormones. *Annu Rev Physiol* 2000;**62**:439−66.
2. Brent GA. Mechanisms of thyroid hormone action. *J Clin Investig* 2012;**122**:3035−43.
3. Mullur R, Liu YY, Brent GA. Thyroid hormone regulation of metabolism. *Physiol Rev* 2014;**94**:355−82.
4. Evans RM. The steroid and thyroid hormone receptor superfamily. *Science* 1988;**240**:889−95.
5. Ohlsson C, Mohan S, Sjogren K, Tivesten A, Isgaard J, Isaksson O, et al. The role of liver-derived insulin-like growth factor-I. *Endocr Rev* 2009;**30**:494−535.
6. Lupu F, Terwilliger JD, Lee K, Segre GV, Efstratiadis A. Roles of growth hormone and insulin-like growth factor 1 in mouse postnatal growth. *Dev Biol* 2001;**229**:141−62.
7. Salvatore D, Simonides WS, Dentice M, Zavacki AM, Larsen PR. Thyroid hormones and skeletal muscle-new insights and potential implications. *Nat Rev Endocrinol* 2014;**10**:206−14.
8. Iniguez MA, De-Lecea L, Guadano-Ferraz A, Morte B, Gerendasy D, Sutcliffe JG, et al. Cell-specific effects of thyroid hormone on RC3/neurogranin expression in rat brain. *Endocrinology* 1996;**137**:103−9.
9. Nedergaard J, Cannon B. UCP1 mRNA does not produce heat. *Biochim Biophys Acta* 2013;**1831**:943−9.
10. Zorzano A, Palacin M, Guma A. Mechanisms regulating GLUT4 glucose transporter expression and glucose transport in skeletal muscle. *Acta Physiol Scand* 2005;**183**:43−58.
11. Zoeller RT, Rovet J. Timing of thyroid hormone action in the developing brain: clinical observations and experimental findings. *J Neuroendocrinol* 2004;**16**:809−18.
12. Ortiga-Carvalho TM, Sidhaye AR, Wondisford FE. Thyroid hormone receptors and resistance to thyroid hormone disorders. *Nat Rev Endocrinol* 2014;**10**:582−91.
13. Dillmann W. Cardiac hypertrophy and thyroid hormone signaling. *Heart Fail Rev* 2010;**15**:125−32.
14. Weiss RE, Murata Y, Cua K, Hayashi Y, Seo H, Refetoff S. Thyroid hormone action on liver, heart, and energy expenditure in thyroid hormone receptor beta-deficient mice. *Endocrinology* 1998;**139**:4945−52.
15. Lazar MA. Thyroid hormone receptors: multiple forms, multiple possibilities. *Endocr Rev* 1993;**14**:184−93.
16. Wagner RL, Huber BR, Shiau AK, Kelly A, Lima STC, Scanlan TS, et al. Hormone selectivity in thyroid hormone receptors. *Mol Endocrinol* 2001;**15**:398−410.
17. Gloss B, Trost S, Bluhm W, Swanson E, Clark R, Winkfein R, et al. Cardiac ion channel expression and contractile function in mice with deletion of thyroid hormone receptor alpha or beta. *Endocrinology* 2001;**142**:544−50.
18. Francavilla A, Carr BI, Azzarone A, Polimeno L, Wang ZQ, Thiel DHV, et al. Hepatocyte proliferation and gene expression

induced by triiodothyronine *in vivo* and in vitro. *Hepatology* 1994;**20**: 1237−41.
19. Kowalik MA, Perra A, Pibiri M, Cocco MT, Samarut J, Plateroti M, et al. TRβ is the critical thyroid hormone receptor isoform in T3-induced proliferation of hepatocytes and pancreatic acinar cells. *J Hepatol* 2010;**53**:686−92.
20. Zhang M, Ma ZY, Qin HC, Yao ZX. Thyroid hormone potentially benefits multiple sclerosis *via* facilitating remyelination. *Mol Neurobiol* 2016;**53**:4406−16.
21. Ness GC, Lopez D. Transcriptional regulation of rat hepatic low-density lipoprotein receptor and cholesterol 7α hydroxylase by thyroid hormone. *Arch Biochem Biophys* 1995;**323**:404−8.
22. Baxter JD, Webb P. Thyroid hormone mimetics: potential applications in atherosclerosis, obesity and type 2 diabetes. *Nat Rev Drug Discov* 2009;**8**:308−20.
23. Sinha R, You SH, Zhou J, Siddique MM, Bay BH, Zhu XG, et al. Thyroid hormone stimulates hepatic lipid catabolism *via* activation of autophagy. *J Clin Investig* 2012;**122**:2428−38.
24. Weitzel JM, Alexander IK. Coordination of mitochondrial biogenesis by thyroid hormone. *Mol Cell Endocrinol* 2011;**342**:1−7.
25. Wrutniak CC, Casas F, Cabello G. The direct tri-iodothyronine mitochondrial pathway: science or mythology?. *Thyroid* 2000;**10**: 965−9.
26. Djouadi F, Riveau B, Merlet BC, Bastin J. Tissue-specific regulation of medium-chain acyl-CoA dehydrogenase gene by thyroid hormones in the developing rat. *Biochem J* 1997;**324**:289−94.
27. Holness MJ, Bulmer K, Smith ND, Sugden MC. Investigation of potential mechanisms regulating protein expression of hepatic pyruvate dehydrogenase kinase isoforms 2 and 4 by fatty acids and thyroid hormone. *Biochem J* 2003;**369**:687−95.
28. Jekabsons MB, Gregoire FM, Schonfeld-Warden NA, Warden CH, Horwitz BA. T_3 stimulates resting metabolism and UCP-2 and UCP-3 mRNA but not nonphosphorylating mitochondrial respiration in mice. *Am J Physiol* 1999;**277**:E380−9.
29. Chiellini G, Apriletti JW, Yoshihara H, Baxter JD, Ribeiro RCJ, Scanlan TS. A high-affinity subtype-selective agonist ligand for the thyroid hormone receptor. *Chem Biol* 1998;**5**:299−306.
30. Underwood AH, Emmett JC, Ellis D, Flynn SB, Leeson PD, Benson GM, et al. A thyromimetic that decreases plasma cholesterol levels without increasing cardiac activity. *Nature* 1986;**324**:425−9.
31. Leeson PD, Ellis D, Emmett JC, Shah VP, Showell GA, Underwood AH. Thyroid hormone analogs. Synthesis of 3′-substituted 3,5-diiodo-L-thyronines and quantitative structure-activity studies of in vitro and *in vivo* thyromimetic activities in rat liver and heart. *J Med Chem* 1988;**31**:37−54.
32. Leeson PD, Emmett JC, Shah VP, Showell GA, Novelli R, Prain HD, et al. Selective thyromimetics. Cardiac-sparing thyroid hormone analogs containing 3′-arylmethyl substituents. *J Med Chem* 1989;**32**: 320−36.
33. Yokoyama N, Walker GN, Main AJ, Stanton JL, Morrissey MM, Boehm C, et al. Synthesis and structure-activity relationships of oxamic acid and acetic acid derivatives related to L-thyronine. *J Med Chem* 1995;**38**:695−707.
34. Taylor AH, Stephan ZF, Steele RE, Wong NCW. Beneficial effects of a novel thyromimetic on lipoprotein metabolism. *Mol Pharmacol* 1997; **52**:542−7.
35. Bleicher L, Aparicio R, Nunes FM, Martinez L, Dias SMG, Figueira ACM, et al. Structural basis of GC-1 selectivity for thyroid hormone receptor isoforms. *BMC Struct Biol* 2008;**8**:8.
36. Trost SU, Swanson E, Gloss B, Wang-Iverson DB, Zhang HJ, Volodarsky T, et al. The thyroid hormone receptor-β-selective agonist GC-1 differentially affects plasma lipids and cardiac activity. *Endocrinology* 2000;**141**:3057−64.
37. Lammel LJ, Webb P. Sobetirome: the past, present and questions about the future. *Expert Opin Ther Tar* 2016;**20**:145−9.
38. Grover GJ, Egan DM, Sleph PG, Beehler BC, Chiellini G, Nguyen N, et al. Effects of the thyroid hormone receptor agonist GC-1 on metabolic rate and cholesterol in rats and primates: selective actions relative to 3,5,3′-triiodo-L-thyronine. *Endocrinology* 2004;**145**: 1656−61.
39. Freitas FRS, Moriscot AS, Jorgetti V, Soares AG, Passarelli M, Scanlan TS, et al. Spared bone mass in rats treated with thyroid hormone receptor TRβ-selective compound GC-1. *Am J Physiol* 2003; **285**:E1135−41.
40. Perra A, Simbula G, Simbula M, Pibiri M, Kowalik MA, Sulas P, et al. Thyroid hormone (T3) and TRβ agonist GC-1 inhibit/reverse nonalcoholic fatty liver in rats. *FASEB J* 2008;**22**:2981−9.
41. Ye L, Li YL, Mellstroem K, Mellin C, Bladh L, Koehler K, et al. Thyroid receptor ligands. 1. agonist ligands selective for the thyroid receptor β1. *J Med Chem* 2003;**46**:1580−8.
42. Grover GJ, Mellstroem K, Ye L, Malm J, Li YL, Bladh L, et al. Selective thyroid hormone receptor-β activation: a strategy for reduction of weight, cholesterol, and lipoprotein (a) with reduced cardiovascular liability. *Proc Natl Acad Sci U S A* 2003;**100**:10067−72.
43. Bryzgalova G, Effendic S, Khan A, Rehnmark S, Barbounis P, Boulet J, et al. Anti-obesity, anti-diabetic, and lipid lowering effects of the thyroid receptor β subtype selective agonist KB-141. *J Steroid Biochem* 2008;**111**:262−7.
44. Berkenstam A, Kristensen J, Mellstroem K, Carlsson B, Malm J, Rehnmark S, et al. The thyroid hormone mimetic compound KB2115 lowers plasma LDL cholesterol and stimulates bile acid synthesis without cardiac effects in humans. *Proc Natl Acad Sci U S A* 2008; **105**:663−7.
45. Ladenson PW, Kristensen JD, Ridgway EC, Olsson AG, Carlsson B, Klein I, et al. Use of the thyroid hormone analogue eprotirome in statin-treated dyslipidemia. *Obstet Gynecol Surv* 2010;**362**:906−16.
46. Sjouke B, Langslet G, Ceska R, Nicholls SJ, Nissen SE, Ohlander M, et al. Eprotirome in patients with familial hypercholesterolaemia (the AKKA trial): a randomized, double-blind, placebo-controlled phase 3 study. *Lancet Diabetes Endo* 2014;**2**:455−63.
47. Runfola M, Sestito S, Bellusci L, La-Pietra V, D'Amore VM, Kowalik MA, et al. Design, synthesis and biological evaluation of novel TRβ selective agonists sustained by ADME-toxicity analysis. *Eur J Med Chem* 2020;**188**:112006.
48. Runfola M, Sestito S, Gul S, Chiellini G, Rapposelli S. Collecting data through high throughput in vitro early toxicity and off-target liability assays to rapidly identify limitations of novel thyromimetics. *Data Brief* 2020;**29**:105206.
49. Caddeo A, Kowalik MA, Serra M, Runfola M, Bacci A, Rapposelli S. TG68, a novel thyroid hormone receptor-β agonist for the treatment of NAFLD. *Int J Mol Sci* 2021;**22**:13105.
50. Beck-Peccoz P, Chatterjee VK. The variable clinical phenotype in thyroid hormone resistance syndrome. *Thyroid* 1994;**4**:225−32.
51. Collingwood TN, Adams M, Tone Y, Chatterjee VKK. Spectrum of transcriptional, dimerization, and dominant negative properties of twenty different mutant thyroid hormone β-receptors in thyroid hormone resistance syndrome. *Mol Endocrinol* 1994;**8**:1262−77.
52. Borngraeber S, Budny M, Chiellini G, Cunha-lima ST, Togashi M, Webb Paul, et al. Ligand selectivity by seeking hydrophobicity in thyroid hormone receptor. *Proc Natl Acad Sci U S A* 2003;**100**: 15358−63.
53. Li Q, Yao BQ, Zhao ST, Lu Z, Zhang Y, Xiang QP, et al. Discovery of a highly selective and H435R-sensitive thyroid hormone receptor β agonist. *J Med Chem* 2022;**65**:7193−211.
54. Kelly MJ, Pietranico-Cole S, Larigan JD, Haynes N, Reynolds CH, Scott N, et al. Discovery of 2-[3,5-dichloro-4-(5-isopropyl-6-oxo-1,6-dihydropyridazin-3-yloxy)phenyl]-3,5-dioxo-2,3,4,5-tetrahydro[1,2,4] triazine-6-carbonitrile (MGL-3196), a highly selective thyroid hormone receptor β agonist in clinical trials for the treatment of dyslipidemia. *J Med Chem* 2014;**57**:3912−23.
55. Hoenes GS, Sivakumar RG, Hoppe C, Koenig J, Fuehrer D, Moeller LC. Cell-specific transport and thyroid hormone receptor isoform selectivity account for hepatocyte-targeted thyromimetic action of MGL-3196. *Int J Mol Sci* 2022;**23**:13714.
56. Kannt A, Wohlfart P, Madsen AN, Veidal SS, Feigh M, Schmoll D. Activation of thyroid hormone receptor-β improved disease activity

and metabolism independent of body weight in a mouse model of non-alcoholic steatohepatitis and fibrosis. *Br J Pharmacol* 2021;**178**: 2412−23.
57. Taub R, Chiang E, Chabot-Blanchet M, Kelly MJ, Reeves RA, Guertin M, et al. Lipid lowering in healthy volunteers treated with multiple doses of MGL-3196, a liver-targeted thyroid hormone receptor-β agonist. *Atherosclerosis* 2013;**230**:373−80.
58. Harrison SA, Bashir MR, Guy CD, Zhou R, Moylan CA, Frias JP, et al. Resmetirom (MGL-3196) for the treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet* 2019;**394**:2012−24.
59. Hovingh GK, Klausen IC, Heggen E, McCarty K, Zhou R, Isaac BF, et al. Resmetirom (MGL-3196) in patients with heterozygous familial hypercholesterolemia. *J Am Coll Cardiol* 2022;**79**:1220−2.
60. Harrison SA, Taub R, Neff GW, Lucas KJ, Labriola D, Moussa SE, et al. Resmetirom for nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled phase 3 trial. *Nat Med* 2023;**29**: 2919−28.
61. Hu LY, Gu YP, Liang J, Ning MM, Yang JL, Zhang Y, et al. Discovery of highly potent and selective thyroid hormone receptor β agonists for the treatment of nonalcoholic steatohepatitis. *J Med Chem* 2023;**66**: 3284−300.
62. Erion MD, Reddy KR, Boyer SH, Matelich MC, Gomez-Galeno J, Lemus RH, et al. Design, synthesis, and characterization of a series of cytochrome P_{450} 3A-activated prodrugs (hepdirect prodrugs) useful for targeting phosph(on)ate-based drugs to the liver. *J Am Chem Soc* 2004; **126**:5154−63.
63. Boyer SH, Jiang H-J, Jacintho JD, Reddy MV, Li HQ, Li WY, et al. Synthesis and biological evaluation of a series of liver-selective phosphonic acid thyroid hormone receptor agonists and their prodrugs. *J Med Chem* 2008;**51**:7075−93.
64. Erion MD, Cable EE, Ito BR, Jiang HJ, Fujitaki JM, Finn PD, et al. Targeting thyroid hormone receptor-β agonists to the liver reduces cholesterol and triglycerides and improves the therapeutic index. *Proc Natl Acad Sci U S A* 2007;**104**:15490−5.
65. Cable EE, Finn PD, Stebbins JW, Hou JZ, Ito BR, van-Poelje PD, et al. Reduction of hepatic steatosis in rats and mice after treatment with liver-targeted thyroid hormone receptor agonist. *Hepatology* 2009;**49**: 407−17.
66. Zhou J, Waskowicz LR, Lim A, Liao XH, Lian B, Masamune H, et al. A liver-specific thyromimetic, VK2809, decreases hepatosteatosis in glycogen storage disease type Ia. *Thyroid* 2019;**29**:1158−67.
67. Olah A, Gherardini J, Bertolini M, Cheret J, Ponce L, Kloepper J, et al. The thyroid hormone analogue KB2115 (Eprotirome) prolongs human hair growth (anagen) *ex vivo*. *J Investig Dermatol* 2016;**136**: 1711−4.
68. Kannisto K, Rehnmark S, Slaetis K, Webb P, Larsson L, Gaafvels M, et al. The thyroid receptor β modulator GC-1 reduces atherosclerosis in ApoE-deficient mice. *Atherosclerosis* 2014;**237**:544−54.
69. Columbano A, Pibiri M, Deidda M, Cossu C, Scanlan TS, Chiellini G, et al. The thyroid hormone receptor-β agonist GC-1 induces cell proliferation in rat liver and pancreas. *Endocrinology* 2006;**147**: 3211−8.
70. Pibiri M, Ledda-Columbano GM, Cossu C, Simbula G, Menegazzi M, Shinozuka H, et al. Cyclin D1 is an early target in hepatocyte proliferation induced by thyroid hormone (T3). *FASEB J* 2001;**15**: 1006−13.
71. Fanti M, Singh S, Ledda-Columbano GM, Columbano A, Monga SP. Tri-iodothyronine induces hepatocyte proliferation by protein kinase A-dependent β-catenin activation in rodents. *Hepatology* 2014;**59**: 2309−20.
72. Alvarado TF, Puliga E, Preziosi M, Poddar M, Singh S, Columbano A, et al. Thyroid hormone receptor β agonist induces β-catenin-dependent hepatocyte proliferation in mice: implications in hepatic regeneration. *Gene Expr* 2016;**17**:19−34.
73. Szydlowska M, Pibiri M, Perra A, Puliga E, Mattu S, Ledda-Columbano GM, et al. The thyromimetic KB2115 (Eprotirome) induces rat hepatocyte proliferation. *Gene Expr* 2017;**17**:207−18.
74. Perra A, Kowalik MA, Cabras L, Runfola M, Sestito S, Migliore C, et al. Potential role of two novel agonists of thyroid hormone receptor-β on liver regeneration. *Cell Prolif* 2020;**53**:e12808.
75. Ribeiro MO, Carvalho SD, Schultz JJ, Chiellini G, Scanlan TS, Bianco AC, et al. Thyroid hormone-sympathetic interaction and adaptive thermogenesis are thyroid hormone receptor isoform-specific. *J Clin Investig* 2001;**108**:97−105.
76. Ribeiro MO, Bianco SDC, Kaneshige M, Schultz JJ, Cheng SY, Bianco AC, et al. Expression of uncoupling protein 1 in mouse brown adipose tissue is thyroid hormone receptor-β isoform-specific and required for adaptive thermogenesis. *Endocrinology* 2010;**151**: 432−40.
77. Baxter JD, Webb P, Grover G, Scanlan TS. Selective activation of thyroid hormone signaling pathways by GC-1: a new approach to controlling cholesterol and body weight. *Trends Endocr Met* 2004;**15**: 154−7.
78. Villicev CM, Freitas FRS, Aoki MS, Taffarel C, Scanlan TS, Moriscot AS, et al. Thyroid hormone receptor β-specific agonist GC-1 increases energy expenditure and prevents fat-mass accumulation in rats. *J Endocrinol* 2007;**193**:21−9.
79. Mohamad B, Shah V, Onyshchenko M, Elshamy M, Aucejo F, Lopez R, et al. Characterization of hepatocellular carcinoma (HCC) in non-alcoholic fatty liver disease (NAFLD) patients without cirrhosis. *Hepatol Int* 2016;**10**:632−9.
80. Ledda-Columbano GM, Perra A, Piga R, Pibiri M, Loi R, Shinozuka H, et al. Cell proliferation induced by 3,3′,5-triiodo-L-thyronine is associated with a reduction in the number of preneoplastic hepatic lesions. *Carcinogenesis* 1999;**20**:2299−304.
81. Nejak-Bowen KN, Monga SPS. Beta-catenin signaling, liver regeneration and hepatocellular cancer: sorting the good from the bad. *Semin Cancer Biol* 2011;**21**:44−58.
82. Ledda-Columbano GM, Perra A, Concas D, Cossu C, Molotzu F, Sartori C, et al. Effects of the liver mitogens triiodo-thyronine and ciprofibrate on the development of rat hepatocellular carcinoma. *Toxicol Pathol* 2003;**31**:113−20.
83. Perra A, Kowalik MA, Pibiri M, Ledda-Columbano GM, Columbano A. Thyroid hormone receptor ligands induce regression of rat preneoplastic liver lesions causing their reversion to a differentiated phenotype. *Hepatology* 2009;**49**:1287−96.
84. Caddeo A, Serra M, Sedda F, Bacci A, Manera C, Rapposelli S, et al. Potential use of TG68−a novel thyromimetic−for the treatment of non-alcoholic fatty liver (NAFLD)-associated hepatocarcinogenesis. *Front Oncol* 2023;**13**:1127517.
85. Hartley MD, Kirkemo LL, Banerji T, Scanlan TS. A thyroid hormone-based strategy for correcting the biochemical abnormality in X-linked adrenoleukodystrophy. *Endocrinology* 2017;**158**:1328−38.
86. Genin EC, Gondcaille C, Trompier D, Savary S. Induction of the adrenoleukodystrophy-related gene (ABCD2) by thyromimetics. *J Steroid Biochem* 2009;**116**:37−43.
87. Placzek AT, Ferrara SJ, Hartley MD, Sanford-Crane HS, Meinig JM, Scanlan TS. Sobetirome prodrug esters with enhanced blood−brain barrier permeability. *Bioorganic Med Chem* 2016;**24**:5842−54.
88. Ferrara SJ, Meinig JM, Placzek AT, Banerji T, McTigue P, Hartley MD, et al. Ester-to-amide rearrangement of ethanolamine-derived prodrugs of sobetirome with increased blood−brain barrier penetration. *Bioorganic Med Chem* 2017;**25**:2743−53.
89. Barez-Lopez S, Hartley MD, Grijota-Martinez C, Scanlan TS, Guadano-Ferraz A. Sobetirome and its amide prodrug Sob-AM2 exert thyromimetic actions in mct8-deficient brain. *Thyroid* 2018;**28**: 1211−20.
90. Meinig JM, Ferrara SJ, Banerji T, Sanford-Crane HS, Bourdette D, Scanlan TS. Targeting fatty-acid amide hydrolase with prodrugs for CNS-selective therapy. *ACS Chem Neurosci* 2017;**8**: 2468−76.
91. Saponaro F, Sestito S, Runfola M, Rapposelli S, Chiellini G. Selective Thyroid hormone receptor-beta (TRβ) agonists: new perspectives for the treatment of metabolic and neurodegenerative disorders. *Front Med* 2020;**7**:331.

92. Zhao XD, Jacob C. Mechanisms of demyelination and remyelination strategies for multiple sclerosis. *Int J Mol Sci* 2023;**24**: 6373.
93. Baldassarro VA, Quadalti C, Runfola M, Manera C, Rapposelli S, Calza L. Synthetic thyroid hormone receptor-beta agonists promote oligodendrocyte precursor cell differentiation in the presence of inflammatory challenges. *Pharmaceuticals* 2023;**16**:1207.
94. Yu GY, Tzouvelekis A, Wang R, Herazo-Maya JD, Ibarra GH, Srivastava A, et al. Thyroid hormone inhibits lung fibrosis in mice by improving epithelial mitochondrial function. *Nat Med* 2018;**24**: 39−49.
95. Fröhlich E, Wahl R. Physiological role and use of thyroid hormone metabolites−potential utility in COVID-19 patients. *Front Endocrinol* 2021;**12**:587518.
96. Vatner DF, Weismann D, Beddow SA, Kumashiro N, Erion DM, Liao XH, et al. Thyroid hormone receptor-β agonists prevent hepatic steatosis in fat-fed rats but impair insulin sensitivity *via* discrete pathways. *Am J Physiol* 2013;**305**:E89−100.
97. Martagon AJ, Lin JZ, Cimini SL, Webb P, Phillips KJ. The amelioration of hepatic steatosis by thyroid hormone receptor agonists is insufficient to restore insulin sensitivity in *ob/ob* mice. *PLoS One* 2015;**10**:e0122987/1−0122987/15.