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Review paper

Targeting NTCP for liver disease treatment: A promising strategy

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ABSTRACT

The sodium taurocholate co-transporting polypeptide (NTCP), a bile acids transporter, has been identified as a new therapeutic target for the treatment of liver disease. This paper thoroughly investigates the function of NTCP for regulating bile acid regulation, its correlation with hepatitis B and D infections, and its association with various liver diseases. Additionally, in this review we examine recent breakthroughs in creating NTCP inhibitors and their prospective applications in liver disease treatment. While this review emphasizes the promising potential of targeting NTCP, it concurrently underscores the need for broader and more detailed research to fully understand the long-term implications and potential side effects associated with NTCP inhibition.

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1. Introduction

Sodium taurocholate co-transporting polypeptide (NTCP) is one of the seven members of the solute carrier family. The *SLC10A1* gene encodes NTCP, which is expressed as a glycoprotein localized to the cell membrane. In hepatic cells, the NTCP is in charge of the transmembrane transport of bile acids (BAs). In the enterohepatic cycle, NTCP can transfer BAs into hepatocytes and is responsible for approximately 80% of BAs reuptake. To maintain a dynamic balance, NTCP is crucial for the entry of BAs into the liver. By inducing apoptosis in hepatocytes due to high BAs concentrations in the liver and activating the death receptor pathway, BAs are potentially cytotoxic substances that contribute to liver damage, cirrhosis, and liver failure [1]. In addition, among the most important class of amphiphilic molecules generated from cholesterol, BAs control the metabolism of cholesterol and support the dynamic homeostasis of BA synthesis; these molecules are synthesized in the liver and maintained by intestinal microbial metabolism; microbial metabolism; and efficient intestinal reabsorption into the blood (Fig. 1). Several pieces of evidence suggest that elevated BA concentrations during cholestasis induce hepatocyte apoptosis, providing a cellular mechanism for BA-mediated liver injury [1–3]. NTCP mainly transports conjugated BAs, partially unconjugated BAs, estrone-3-sulfate, bromosulfophthalein (BSP), and thyroid hormones [4]. Human NTCP has been shown to be a functional receptor for hepatitis B virus (HBV)/hepatitis D virus (HDV) in addition to its transporter role. In 2012, researchers pinpointed NTCP as a functional receptor responsible for the invasion of hepatitis B virus into cells [5]. This discovery conveys significant assistance for efforts to combat the hepatitis B virus. In this study, we provide an overview of the structure and function of NTCP, as well as its relationship to liver disorders and related therapeutic interventions.

2. Structure and functions of the NTCP

Human NTCP is a dynamic membrane protein that is relatively small and biochemically unstable in nondenaturing detergent solutions. It also lacks soluble folded structural domains. Nine

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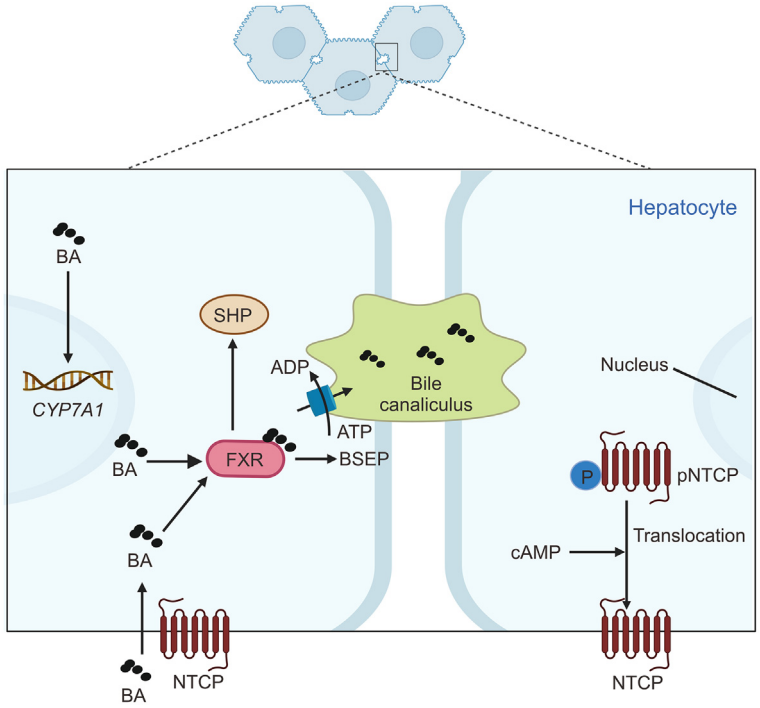


Fig. 1. Schematic representation of the sodium taurocholate co-transporting polypeptide (NTCP)-mediated bile acid transport. This figure illustrates the molecular mechanism by which the NTCP facilitates the uptake of bile acids from the portal blood into hepatocytes. BA: bile acid; *CYP7A1*: cytochrome P450 family 7 subfamily A member 1; SHP: small heterodimer partner; FXR: farnesoid X receptor; ADP: adenosine diphosphate; ATP: adenosine triphosphate; BSEP: bile acid export pump; pNTCP: phosphorylated NTCP; cAMP: cyclic adenosine monophosphate.

transmembrane (TM) helices make up the overall structure of NTCP. These helices can be organized to form two structural domains: a panel and a core. A transmembrane channel forms between the two structural domains and is necessary for substrate transport [6] (Fig. 2A). The structural domain of the membrane panel contains TM1, TM5 and TM6, while the other six helices form the core structural domain, which is the lateral crevice for intracellular and extracellular transport. PreS1, a binding peptide of the high-affinity receptor HBV, is attached to the binding domain of NTCP. The tunnel formed by TM1, TM6, TM8b and TM9 may be associated with preS1 binding. The partial hydrophobic surface regions recognize HBV preS1 through hydrophobic residues. Additionally, NTCP is a sodium-dependent BA cotransporter with two sites, Na1 and Na2, located near the crossover motifs between the discontinuous helices TM3 and TM8. The Na located at these two sites can interact with the oxygen atoms of amino acid residues and drive the transport of bile salts by orienting them from the outer membrane binding site to the cytoplasmic site [7,8] (Fig. 2B).

NTCP is predominantly expressed on the basolateral side of hepatocyte basement membranes (blood sinus side) and is also found apically in pancreatic alveolar cells [9]. It mainly facilitates the uptake of BAs (both conjugated bile salts and unconjugated bile salts) from portal blood and mediates the transport of thyroid hormones, drugs, and toxins [4,10]. NTCP meets all functional needs for a sodium-coupled bile salt transporter in hepatocytes, including: 1) transfer of conjugated bile salts with preference and high affinity; 2) taurocholate transport kinetics that resemble those of individual hepatocytes; 3) the electroproductive nature of sodium ion-taurocholate uptake; 4) specific expression in liver tissues; and 5) similarities between the emergence of NTCP expression and the occurrence of sodium-dependent bile salt uptake during individual development. In addition, NTCP is also a functional receptor for hepatitis B (HBV) and hepatitis D (HDV) [5] and can modulate hepatitis C (HCV) infection [11]. Yan et al. [5] demonstrated in animal studies that NTCP on the cell surface interacts with the pre-S1 structural domain in the hepatitis B virus. Based on studies of NTCP inhibitors, a treatment approach was developed to prevent the hepatitis B virus's entrance into cells.

3. Regulation of NTCP expression

3.1. Regulation of gene-related NTCP expression

The SLC10A1 gene is characterized by single nucleotide polymorphisms (SNPs), and differences in the function of the proteins encoded by the different sequences of nucleotides cause differences in the proteins' abilities to transport bile salts. In 2014, Su et al. [12] studied 933 individuals from the Chinese Han population, and the results showed that the SLC10A1 gene polymorphism may be related to the hepatitis B virus infection's typical progression. The *rs7154439 AA* is associated with hepatitis B virus clearance, and the *rs4646287 AA* is associated with the occurrence of hepatocellular carcinoma. However, given the limited sample size, further large-scale studies are needed for confirmation. Hu et al. [13] reported that the *rs2296651* (*S267F*) variant of *SLC10A1* (NTCP) was associated with the risk of cirrhosis and hepatocellular carcinoma as well as resistance to chronic hepatitis B (CHB) infection. The findings showed that the *GA* or *AA* genotype of the *S267F* variant significantly reduced the potential for hepatocellular carcinoma and cirrhosis and that the *S267F* variant, which is specific to Asian populations, decreased entrance and infection of the hepatitis B virus. The *S267F* mutation of NTCP may reduce the uptake of bile salts into hepatocytes and decrease the potential for cytotoxic bile salts to accumulate in the liver. This could lessen the chances of hepatic inflammation and oxidative stress-mediated tumorigenesis among those suffering from chronic hepatitis B, consequently diminishing the risk of progression to cirrhosis and hepatocellular carcinoma (HCC) [13].

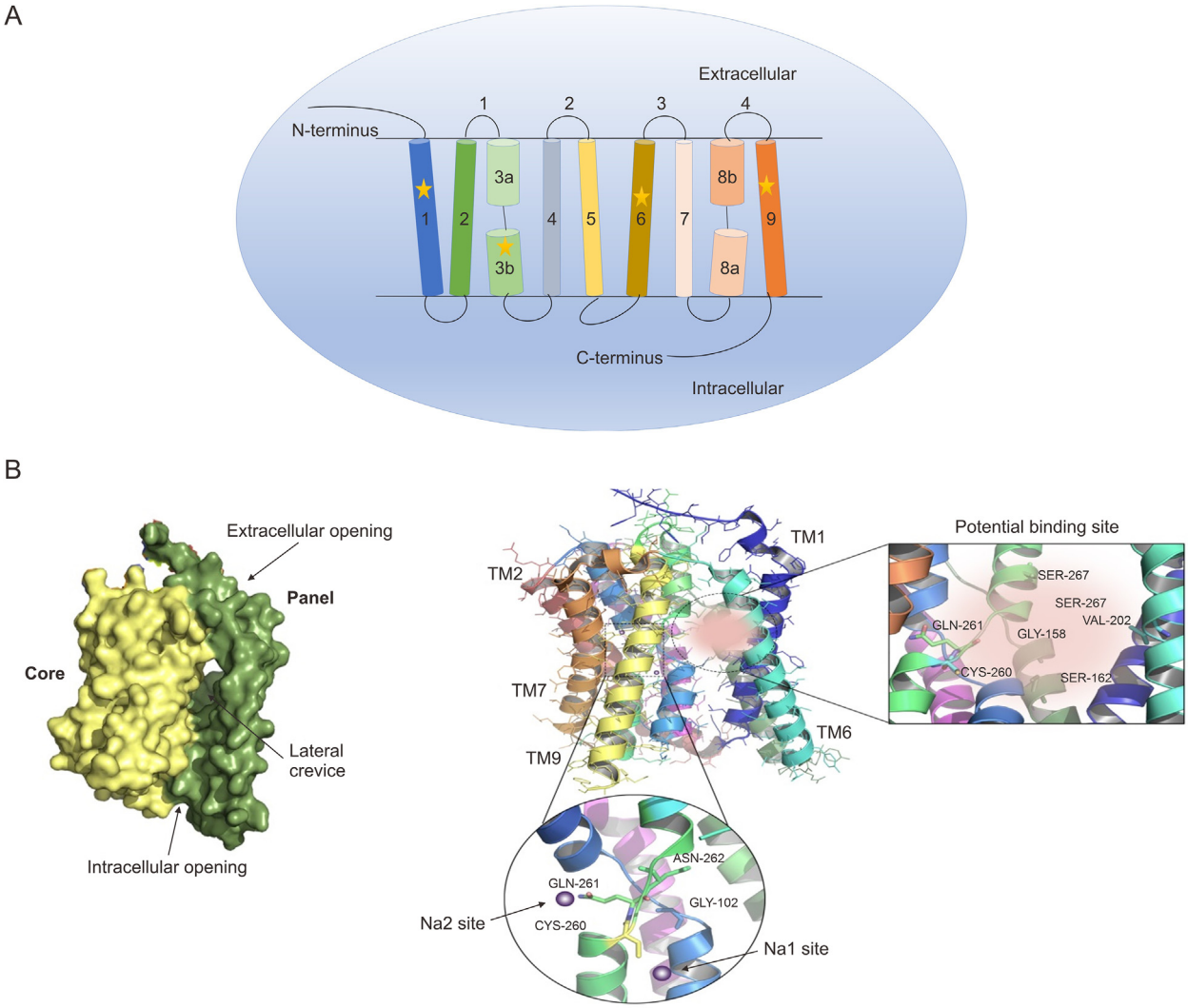


Fig. 2. Structure of the sodium taurocholate co-transporting polypeptide (NTCP). (A) Topology diagrams of NTCP. Helices are colored from blue to orange, from the N- to C-terminus. Core (transmembrane (TM)1, TM5, and TM6) and panel (TM2−4 and TM7−9) domains are indicated; yellow asterisks denote regions that are in contact with the polycyclic scaffold of bound substrate. (B) Binding site map of NTCP (Protein Data Bank (PDB) ID: 7WSI). A lateral crevice is formed between the core and panel binding domains. Some TM helices are numbered. Purple spheres denote the location of bound Na+ ions, which are Na1 site and Na2 site, respectively; the pink region indicates potential sites that may bind to the proteins of hepatitis B virus (HBV).

3.2. Differences in expression between races

A correlation existed between SLC10A1 gene polymorphisms and racial background. NTCP is thought to be critical for maintaining the hepatic-intestinal cycle of BAs and hepatocyte function, and functionally relevant gene polymorphisms in the transporter may have an important impact on BA homeostasis/hepatic function. Ho et al. [14] reported the presence of several SNPs in NTCP in Europeans, Africans, Chinese individuals, and Hispanic Americans. In this study, genomic DNA extracted from peripheral blood lymphocytes of healthy European Americans, African Americans, Chinese Americans and Hispanic Americans was subjected to genomic analysis. Seven SNPs in the NTCP coding region were found, including four nonsynonymous polymorphisms. Even though SNPs change throughout populations, more research is required to comprehend how distinct genotypes' BA transport capacities differ from one another. The data indicate that SNPs in NTCP are generally rare. However, certain SNPs have been identified with relatively high allele frequencies, but this is restricted to specific ethnic populations. In 2016, Su et al. [15] found no association between three SNPs (*rs7154439, rs4646287, and rs2296651*) located in the SLC10A1 gene and their haplotypes or HBV chronicity by examining the Uyghur and Tibetan populations in China, and the NTCP polymorphisms identified in this study tended to be race dependent.

4. Liver diseases associated with NTCP

4.1. NTCP and chronic viral hepatitis

4.1.1. Hepatitis B

HBV infection is a significant global health issue and can result in the development of diverse diseases, spanning from liver fibrosis to hepatocellular carcinoma, through intricate mechanisms. A persistent HBV infection increases the risk of developing liver cirrhosis, fibrosis, and ultimately hepatocellular cancer [16]. Compared to uninfected individuals, HBV carriers have a significantly elevated lifetime risk of developing liver cancer that ranges from 10 to 25 times greater [17]. Moreover, HBV facilitates the progression of hepatocellular carcinoma through intricate mechanisms involving both direct and indirect pathways [18]. HBV is an enveloped virus with a partially double-stranded genome enclosed within a nucleocapsid that is shielded by a lipid bilayer made up of

surface glycoproteins of small (S), medium (M), and large (L) sizes [19]. These three proteins share a common C-terminal S structural domain, but the L and M proteins have different N-terminal structural domain extensions. The N-terminal extensions of the L and M proteins are Pre-S1/S2 and Pre-S2, respectively [20]. The Pre-S1 structural domain of the L protein, especially amino acids 2−48, plays a key role in HBV entry and infection [21,22]. Specific receptors in the S1 structural domain recognize the structure of NTCP, and HBV can then enter hepatocytes, causing HBV infection. The hepatophilic nature of HBV infection is explained by NTCP, a transmembrane protein that is exclusively produced on the basement membrane of developed hepatocytes. Currently, treatment of HBV infection relies on interferon (IFN) and nucleotide analogs (NASs), including entecavir and tenofovir. Interferon causes severe adverse effects, and its acceptance by the population is limited; treatment with nucleotide analogs inhibits HBV replication but has no effective ability to cure chronic infection [23,24] and requires long-term treatment, even if it is consumed for life [6]. Since NTCP has been shown to be a functional receptor for HBV entry, entry inhibitors that specifically target NTCP are available as novel and promising treatment options [17].

4.1.2. Hepatitis C

Around the world, viral hepatitis C is an important risk factor to chronic liver disease [25]. HCV is a single-stranded positive-sense RNA virus of the *Flaviviridae* family. The core of the nucleocapsid encloses a host-derived membrane containing the E1 and E2 glycoproteins, and the viral particles are associated with serum lipoproteins. Like many viral infections, HCV infection is initiated by low-affinity interactions between viral particles and cell-surface proteoglycans containing heparin sulfate proteoglycans (HSPGs) [26,27]. HCV infection requires many host proteins, including cluster of differentiation 81 (CD81), scavenger receptor BI (SR-BI), claudin-1 (CLDN1), occludin (OCLN), and cofactors, such as epidermal growth factor receptor (EGFR), to gain access to hepatocytes, leading to HCV infection [20,28−31]. Although the key host factors mediating HCV entry have been well explained [32], the precise mechanisms by which cellular entry controls viral infection have not been determined. Verrier et al. [11] reported that overexpression of NTCP exacerbates HCV infection in hepatocytes via a mechanism different from that of HBV infection; moreover, NTCP modulates HCV infection by enhancing BA-mediated inhibition of interferon-stimulated genes (ISGs). The regulatory function of NTCP in intrinsic hepatic immunity has been elucidated, providing insight into the intricate interaction between the virus and the host organism. Receptor antagonists, antibodies, peptides, and receptor kinase inhibitors that target viral cell entry present therapeutic potential for the prevention and management of chronic hepatitis B and C [33]. To date, a number of medications classified as cytochrome P450 inhibitors are in clinical development for the treatment of patients infected with HCV [34−36].

4.1.3. Hepatitis D

HDV is an RNA virus that utilizes HBV membrane proteins for assembly, secretion, and hepatocyte-specific entry. HDV can utilize HBV membrane proteins to assemble infectious particles and has been widely used as an alternative model for studying hepatitis B virus entry [37]. Like HBV, HDV infects hepatocytes by binding to cell surfaces through interactions with HSPGs, as well as through specific interactions with NTCP expressed on the basolateral membranes of hepatocytes [5,38]. The NTCP-binding structural domain (75 amino acids) on the PreS1 structural domain of HBV large-HBsAg (L-HBsAg) is essential for HDV and HBV infection. One treatment approach for preventing HBV and HDV infections is targeting the virus's interaction with NTCP [5,39].

4.2. NTCP and nonalcoholic fatty liver disease (NAFLD)

NAFLD, which includes nonalcoholic simple fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH), is the most common liver disease in developed Western countries [40]; the NAFLD activity score includes three indicators, namely, hepatic steatosis, swelling, and inflammation, that distinguish NAFL from NASH [41]. Individuals who have NASH are at a higher risk of developing cirrhosis and its related disorders, such as ascites, variceal hemorrhage, hepatic encephalopathy, hepatocellular carcinoma, and liver failure. The recommended treatments for NASH include weight loss and lifestyle modifications, although the majority of patients do not reach or sustain their dietary objectives or weight loss. Currently, there are no approved medications for the treatment of NASH, and treatments to stop or reverse the disease progression are desperately needed [42]. Bechmann et al. [43] reported that the expression of NTCP and the cholesterol 7 alpha-hydroxylase (CYP7A1) increased in hepatocytes of patients with obesity and hepatocytes stimulated by free fatty acids *in vitro*, and CYP7A1 is the first and rate-limiting enzyme in the bile acid synthesis pathway. Compared with those in NAFL patients, the serum free fatty acid and BA levels in NASH patients were increased. With increasing NAFLD score, the expression of NTCP in liver tissue decreased. Observations indicate that the occurrence of obesity and hyperlipidemia corresponds to an upregulation of NTCP expression in liver tissue. This upregulation facilitates enhanced BA absorption, thus promoting the pathogenesis of NAFL. Persistent and chronic cellular injury fosters the progression of NAFL to NASH. This progression is accompanied by elevated serum BA levels, activation of the farnesoid X receptor (FXR) receptor, and subsequent downregulation of NTCP expression within the liver tissue affected by NASH [43,44]. In summary, there is a substantial connection between viral hepatitis, NAFLD and other related diseases and NTCP (Fig. 3).

4.3. NTCP and liver fibrosis

Liver fibrosis frequently arises from two distinct forms of chronic liver injury: hepatotoxic injury and cholestatic injury. The primary manifestation involves the progressive accumulation of extracellular matrix, primarily driven by the substitution of normal tissue with collagen types I and III. Hepatic stellate cells (HSCs) are essential for this process and actively contribute to the synthesis, secretion, and remodeling of extracellular matrix components, thus promoting liver fibrosis. Normal HSCs are located in the peripheral space of hepatic sinusoids and are generally in a quiescent state, but when activated by hepatic injury signals, they can release a large amount of extracellular matrix, causing liver fibrosis [45]. Svegliati-Baroni et al. [46] demonstrated that BAs can promote the proliferation of HSCs by activating epidermal growth factor receptors on their surface. Recently, Salhab et al. [47] isolated HSCs from hepatic puncture tissues of patients with different fibrosis grades and reported that the NTCP expression in HSCs of patients with F3/F4 grades was significantly greater than that in patients with F0 grades; the results of subsequent treatment with BAs and other treatments suggested that activated HSCs exhibit high NTCP expression and high taurocholic acid uptake, which induces further activation of the cells and leads to hepatic fibrosis. Results from animal experiments further confirmed that the level of NTCP expression in HSCs was linearly correlated with the severity of hepatic fibrosis, that BA transport may be an important step in the development of hepatic fibrosis, and that antagonizing BA uptake may be a strategy for preventing disease progression. This study provides compelling evidence substantiating the potential therapeutic use of NTCP inhibitors for managing liver fibrosis. These findings demonstrated that BAs expedite the proliferation of HSCs

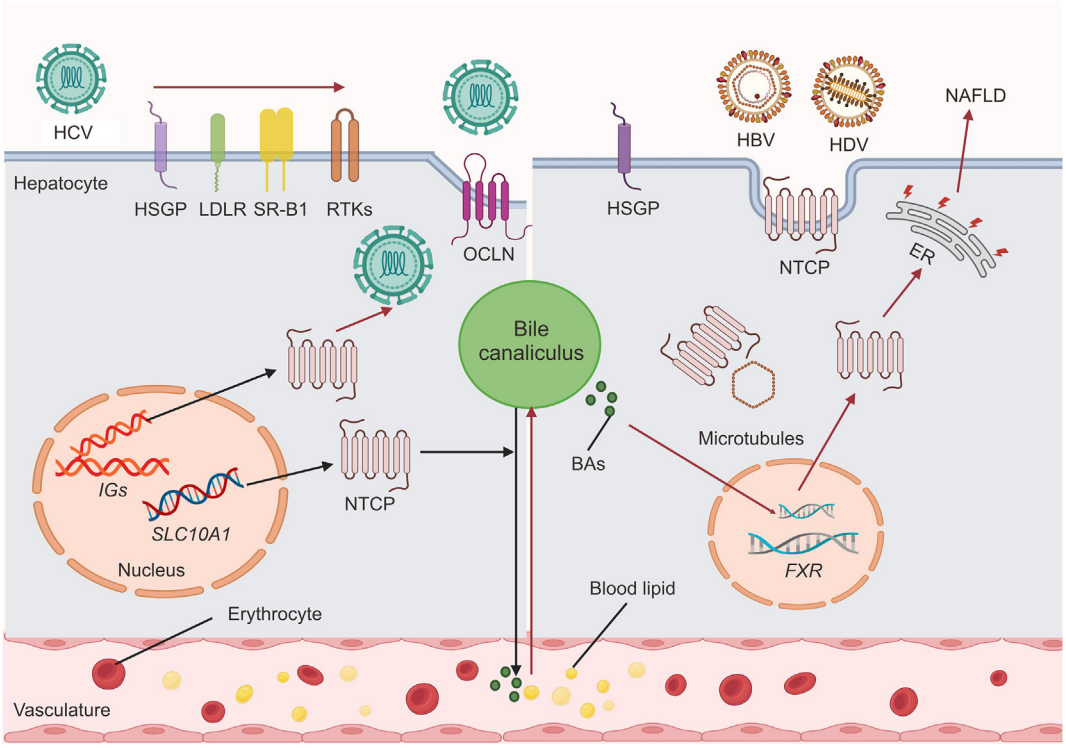


Fig. 3. Relationship between the sodium taurocholate co-transporting polypeptide (NTCP) and diverse diseases. The figure includes a diagrammatic representation of NTCP with connections to different disease states in bile acid-related metabolic disorders and viral hepatitis. Red arrows indicate activation, black arrows indicate inhibition. HCV: hepatis C virus; HSGP: heparan sulfate proteoglycans; LDLR: low density lipoprotein receptor; SR-B1: the scavenger receptor, class B type 1; RTKs: receptor tyrosine kinase; ISGs: interferon stimulated genes; SLC10A1: solute carrier family 10 member 1; OCLN: occludin; HBV: hepatitis B virus; HDV: hepatitis D virus; NAFLD: nonalcoholic fatty liver disease; *FXR*: farnesoid X receptor; ER: endoplasmic reticulum.

by activating epidermal growth factor receptors. Moreover, the prevention or exclusion of BAs safeguards HSCs against apoptosis triggered by BA exposure [47].

Activation of FXR through the accumulation of BAs is a key mechanism of NTCP inhibition. This protein inhibits the retinoid receptor-retinoid receptor heterodimer in rats or the glucocorticoid receptor in humans, causing small heterodimeric chaperones (SHPs), inhibitory factors of hepatic nuclear factor 1 (HNF1) and HNF4α, and interferes with normal NTCP expression (Fig. 4).

4.4. NTCP and primary biliary cholangitis

Primary biliary cholangitis (PBC) is characterized by persistent cholestatic liver disease with considerable regional variation in incidence. However, the etiology of PBC has not been fully elucidated, and PBC is considered an autoimmune disease potentially influenced by various factors, such as environmental triggers, genetic predispositions, the host microbiota, and disturbances in BA metabolism [48]. By investigating the hepatic BA transport system in liver tissue from patients diagnosed with PBC, a notable decrease in the expression of NTCP was observed in those with advanced disease compared to those at an earlier stage. This observation led to the hypothesis that this reduction in NTCP expression may represent a potential compensatory mechanism employed by the body to mitigate the excessive buildup of toxic BAs within the bile ducts [49]. As a first-line treatment option, ursodeoxycholic acid (UDCA) has dramatically slowed the progression of the disease to cirrhosis. In 2013, Honda et al. [50] analyzed 19 early-stage PBC patients who were poorly treated with ursodeoxycholic acid monotherapy, and after 3 months of combined benzofibrate treatment, the patients' serological indices were significantly improved, and the synthesis of BAs was inhibited. Further mechanistic studies confirmed that benzofibrate acted on peroxisome proliferator-activated receptor alpha (PPARα) and FXR simultaneously, downregulated the expression of NTCP and CYP7A1, and inhibited the synthesis and absorption of BAs. Related studies showed that goldenseal, a Chinese herbal medicine extracted from the Goldenseal plant, could improve the prognosis of PBC patients by downregulating inflammation and absorption. Mechanistic studies have confirmed that benzapentane acts on both peroxisome proliferator-activated receptor α and FXR to downregulate the expression of

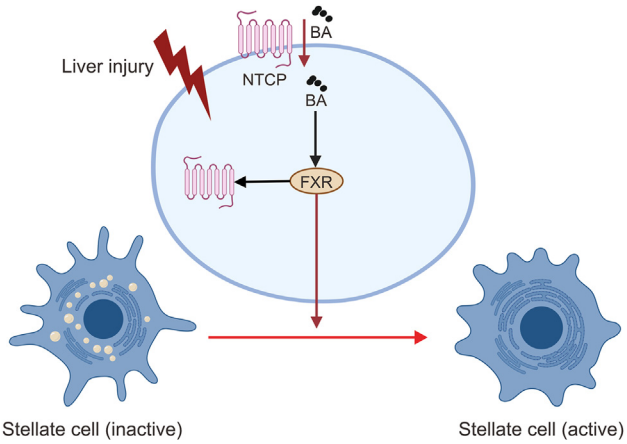


Fig. 4. Relationship between the sodium taurocholate co-transporting polypeptide (NTCP) inhibition and farnesoid X receptor (FXR). Blocking NTCP elevates FXR expression in liver stellate cells and reduces the generation of α-smooth muscle actin (α-SMA) collagen. Red arrows indicate activation, and black arrows indicate inhibition. BA: bile acid.

NTCP and BA synthetase, inhibiting BA synthesis and absorption, and improving the prognosis of patients with PBC. Other studies have shown that ginkgolide, a traditional Chinese medicine extracted from *Ginkgolides*, can inhibit ethinylestradiol-mediated hepatic inflammation through the downregulation of inflammatory factors and can also improve cholestasis through the action of FXR, which can downregulate the expression of NTCP and inhibit the synthesis of bile acids [51]. The effect of NTCP on PBC can be observed during the development of PBC. Therefore, the development and occurrence of PBC are significantly influenced by NTCP.

4.5. NTCP and hepatocellular carcinoma

Hepatocellular carcinoma (HCC) ranks as the fourth-most-common cause of cancer-related mortality worldwide, making it the second-leading contributor to reduced life expectancy resulting from cancer on a global scale. These findings underscore the considerable disease burden associated with liver cancer [52,53]. Among noncirrhotic individuals, those with NASH have a greater risk of HCC than patients with other etiologies of liver disease [54]. Dysregulation of the gut microbiota and reduced immune surveillance are two emerging mechanisms of hepatocarcinogenesis in NAFLD patients [40]. A meta-analysis of 19 studies and 168,571 NASH patients in a systematic review showed that the prevalence of NAFLD-associated HCC in NASH patients without cirrhosis was approximately 38%, whereas it was 14% in patients without cirrhosis with other etiologies of liver disease (i.e., liver disease associated with alcohol consumption or HBV or HCV infection) ($P < 0.001$) [54]. This study revealed a very high prevalence of HCC in patients with NASH. Huang et al. [40] showed that the prevalence of NAFLD varies in different countries and regions, especially in China and the United States, where the prevalence is increasing. Patients diagnosed with NAFLD and cirrhosis exhibited the most substantial risk for developing HCC. Additionally, advanced age, male sex, and potential variants of the patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) gene were identified as other significant risk factors associated with HCC. Hepatocellular carcinoma development is significantly influenced by diabetes and obesity as risk factors. The researchers concluded that patients with NAFLD should undergo intensive lifestyle modifications to reduce the risk of diabetes, obesity, and subsequent hepatocellular carcinoma. In patients with advanced liver fibrosis and cirrhosis, proactive consideration should be given to HCC screening. Recent study indicates that dysregulated gut ecology, elevated intestinal inflammation, and lowered immune surveillance may be important stages in the development of tumors [40]. Based on the current evidence, the use of pharmacologic prophylaxis cannot be routinely recommended for the primary prevention of HCC, but metformin, statins, and possibly aspirin may play a preventive role.

4.6. Other diseases associated with NTCP

NTCP has been identified as a significant factor in the pathogenesis of several other diseases. In 2019, Mao et al. [55] reported multiple phenotypic abnormalities in the gallbladders of mice caused by knockout of the *SLC10A1* gene: thickening of the gallbladder wall, enlargement of the gallbladder, dark green coloration of the bile, deformity of the gallbladder base, a small rod-shaped gallbladder, and thickening of the gallbladder wall. As part of an investigation into the potential correlation between the loss of NTCP function and the development of gallbladder abnormalities in humans, a retrospective analysis was conducted on 33 individuals who carried the NTCP p.Ser267Phe variant. The findings from this study revealed a heightened risk of gallbladder abnormalities among individuals with the NTCP p.Ser267Phe variant [55]. In conclusion, the study suggested that hepatocellular NTCP deficiency leads to gallbladder abnormalities in mice and humans. In 2014, the first instance of NTCP deficiency resulting in hyperbilirubinemia without itchiness or jaundice and with normal levels of autotaxin and bilirubin in the serum was reported by Vaz et al. [56]. A little girl with hypotonia, motor delay, and growth delay was the index patient [56]. According to another study, the presence of NTCP deficiency should be included in the differential diagnosis for the following clinical scenarios: unexplained dysplasia, cholestasis without jaundice, fetal death, or unexplained histologic hepatic abnormalities linked to hypercholesterolemia [57].

5. NTCP inhibitors

5.1. Myrcludex B (MyrB)

MyrB is a myristoylated synthetic lipopeptide consisting of 47 amino acids (Fig. S1). It is derived from the preS1 structural domain of the hepatitis B virus large surface protein [58]. This drug represents a pioneering member of a novel drug class that operates by modulating NTCP function to hinder the entry of HBV and HDV into hepatocytes, thereby effectively preventing viral infections (Tables 1 [59–65] and 2). MyrB binds to human NTCP [66] and can strongly inhibit HBV infection [67]. Zhao et al. [68] found by generating humanized HBV-infected uPA/SCID mice that MyrB may block intrahepatic virus transmission in HBV-infected urokinasetype plasminogen activator-severe combined immune deficency (uPA/SCID) mice. MyrB not only affects the entry of HBV virus into hepatocytes but also has an inhibitory effect on the replication of the virus *in vivo*. MyrB was effective at blocking de novo HBV and HDV infections both *in vitro* [69] and *in vivo* [70]. MyrB has been shown to be well tolerated in healthy volunteers [71], with antiviral effects against HBV and HDV infections demonstrated in recent research [72]. However, substantial increases in plasma BA levels require adverse event monitoring in patients receiving long-term MyrB therapy, with a focus on potential interactions of BAs with membrane-bound receptors (e.g., Takeda G protein-coupled receptor 5 (TGR5)) and nuclear receptors (e.g., FXRs) [73]. Frequently observed adverse effects include injection site reactions, heightened levels of total bile salts, elevated alanine aminotransferase (ALT) levels, and augmented BA secretion. One group reported that elevated total bile salt levels were the most common adverse reaction, with bile salt levels returning to baseline levels at the week 1 follow-up after cessation of treatment [74]. In 2020, the European Union granted a license to MyrB to treat adult patients with chronic HDV infection who had compensated liver damage induced by HDV RNA [75]. The drug is also being studied in phase I clinical trials for the treatment of dyslipidemia, as well as in the preclinical phase of study for nonalcoholic steatohepatitis and primary cholangitis [76].

5.2. Hepalatide

Hepalatide, an NTCP-specific inhibitor, has promising potential for mitigating the progression of NAFLD. It is a peptide that is very similar to MyrB. Its therapeutic effects include ameliorating hepatic steatosis and insulin resistance, as well as inhibiting hepatic fibrosis. These benefits likely arise from hepalatide's ability to hinder BA uptake, facilitate an increase in serum-bound BAs [77], alleviate endoplasmic reticulum stress, and attenuate hepatocyte damage [78]. A common adverse effect of Hepalatide is elevated BA levels due to its inhibition of NTCP-mediated BA transport; when NTCP is blocked by hepatic phospholipids, more BA in portal plasma escapes first-pass extraction from the hepatic sinusoids, which in turn leaks into the peripheral circulation, leading to elevated serum BA levels and inhibition of NAFLD progression [59].

Table 1
Preclinical studies of drugs for the sodium taurocholate co-transporting polypeptide (NTCP).

Medicine	Subjects	Dose	Target/mechanism (mode of delivery)	Therapy	Refs.
Hepalatide	Mouse	20 or 60 mg/kg	Inhibits bile acid synthesis and absorption	NAFLD	[59]
Ergosterol peroxide	dHuS-E/2 cells	10 or 20 μM	Inhibits viral entry into cells	HBV	[60]
Exophillic acid	HepG2-hNTCP-C4	10 μM	Inhibits viral entry into cells	HBV/HDV	[61]
Vanitaracin A	HepG2-hNTCP-C4 and HepG2 cells	6.25−100 μM	Inhibits viral entry into cells	HBV/HDV	[62]
Bexarotene	HepG2-hNTCP cell	10 μM	Inhibits viral entry into cells	HBV/HDV	[63]
DBA-41	Mouse	50 mg/kg	Inhibits viral entry into cells	HBV	[64]
Myrcludex B	Mouse	2.5 μg/g	Inhibits bile acid synthesis and absorption	NAFLD and PBC	[65]

NAFLD: Non-alcoholic fatty liver disease; HBV: hepatitis B virus; HDV: hepatitis D virus; PBC: primary biliary cirrhosis.

The medication is currently being studied in clinical trials as a possible therapeutic intervention for the treatment of NAFLD [79].

5.3. Immunosuppressants

5.3.1. Cyclosporin A (CsA)

CsA is an immunosuppressive agent extensively used in clinical settings to effectively manage immune rejection in xenograft tissues. Calmodulin neurophosphatase dephosphorylates nuclear factor of activated binding of HBV LHBs to NTCP, thus allowing nuclear translocation and downstream gene translation. Cyclosporine mainly suppresses the immunological response by attaching to CN and blocking its ability to bind LHBs to NTCP. This CN inhibition contributes to the suppression of the immune response. In addition, CsA inhibits the transporter protein activity of membrane transporter proteins, including those of the multidrug resistance (MDR) and multidrug resistance-associated protein (MRP) families. It has been reported that CsA can inhibit the transporter protein activity of NTCP and block the binding of NTCP to large envelope proteins *in vitro*. Nkongolo et al. [80] showed that CsA inhibited the activity of the NTCP transporter and disrupted the binding of LHBs to NTCP *in vitro*. In addition, the inhibition of HBV infection was also observed when patients were treated with other compounds that can inhibit NTCP. Moreover, the inhibition of viral entry may be an effective strategy for preventing HBV infection through clinical outcomes such as postexposure prophylaxis, blocking vertical transmission and preventing HBV recurrence after liver transplantation [80]. Furthermore, investigators demonstrated that CsA and its nonimmunosuppressive derivatives inhibit HCV replication and that anti-HCV activity is mediated by the inhibition of cell cycle proteins [81,82].

CsA, isolated from the fungus *Tolypocladium inflatum*, is a cyclic peptide consisting of 11 amino acids. Due to the potent antiviral activity of CsA, CsA has undergone structural modifications in a number of studies to synthesize analogs that are utilized for treating diseases. However, previous modifications of the cyclic peptide residues did not improve efficacy. Subsequently, Liu et al. [83] modified the unusual amino acid sequence butenyl-methylthreonine (BMT) in the structure and obtained a series of derivatives, among which compound 27A exhibited enhanced selectivity for NTCP and antiviral activity compared to CsA. These derivatives were synthesized by opening the double bond of BMT to form a five-membered ring, followed by the addition of various side chains. Compounds with a sulfur atom connecting the five-membered ring and the benzene ring showed excellent inhibitory activity; the presence of a methyl group on the benzene ring was also necessary (Fig. 5). The attachment of other moieties (such as pyrimidines, methylpiperazines, and morpholines) at the 4-position of the benzene ring altered the activity, possibly due to novel interactions between the extending side chain and the binding protein, resulting in reduced activity. However, compared to those of the 4-position substitutions, the 3-*O*-substituted compounds exhibited significantly greater inhibitory potency, with the benzyloxy derivative displaying the strongest inhibition (the half maximal inhibitory concentration (IC_{50}) = 0.110 μM). Nonetheless, further structural modifications based on this indication did not markedly improve potency. Subsequently, *in vivo* and *in vitro* studies revealed that compound 27A demonstrated favorable pharmacokinetic properties and anti-HBV activity, likely through the inhibition of the interaction between the HBV Pre-S1 peptide and NTCP. Additionally, compound 27A showed excellent oral bioavailability and on-target selectivity, rendering it a potential anti-HBV/HDV drug candidate.

5.3.2. Everolimus

Everolimus was previously recognized as a drug that induces cholestasis in humans, possibly through the potent inhibition of the human bile salt efflux pump (BSEP), even in the presence of impaired NTCP-mediated BA uptake. In 2023, Saran et al. [84] screened different kinase inhibitors for the inhibition of NTCP function and taurocholic acid (TCA) uptake in Huh-7 cells transfected with the SLC10A1 gene and determined that the macrocyclic immunosuppressant everolimus were moderately potent NTCP inhibitors (IC_{50} = 6.7−8.0 μM) [84] (Fig. S2). NTCP-mediated BA uptake may be inhibited by macrocyclic peptides, which may be further exploited for the development of new drugs against HBV/HDV.

5.4. Angiotensin II receptor blockers

5.4.1. Irbesartan

Irbesartan belongs to a class of drugs that exert their effects on the final component of the renin-angiotensin system. It is clinically used for antihypertensive therapy and for mitigating renal damage in patients diagnosed with type 2 diabetes [85]. In recent studies, angiotensin II receptor antagonists have been identified as inhibitors of NTCP in humans and exhibit varying degrees of

Table 2
Clinical trials of drugs for the sodium taurocholate co-transporting (NTCP).

Medicine	Target/mechanism (mode of delivery)	Therapy	Phase	Duration	Clinical trial number
Myrcludex B	Inhibits entry of HBV/HDV virus into cells and inhibits viral replication	HBV and HDV	Phase II	February 16, 2016 to January 31, 2018	NCT03546621
Hepalatide	Inhibits the entry of HBV/HDV virus into hepatocytes; improves the process of liver fibrosis	HBV and HDV	Phase II	June 1, 2023 to March 1, 2024	NCT05827146

HBV: hepatitis B virus; HDV: hepatitis D virus.

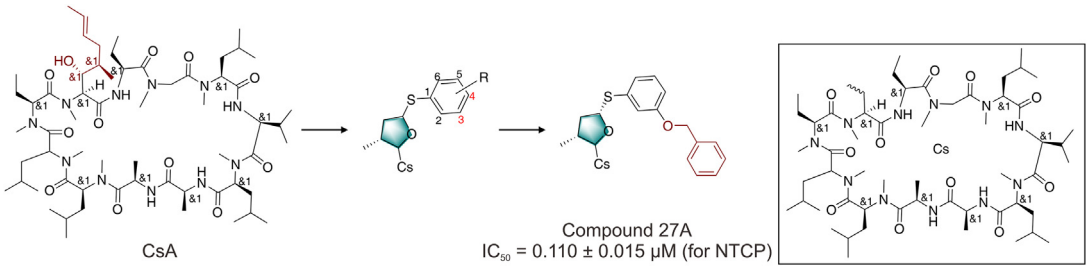


Fig. 5. Structure of cyclosporin A (CsA) and the derivative process of Compound 27A. IC_{50}: half maximal inhibitory concentration; NTCP: the sodium taurocholate co-transporting polypeptide.

inhibitory potency. Among these antagonists, irbesartan has demonstrated the highest efficacy as an NTCP inhibitor. The mechanism of action of irbesartan is as follows: it has no direct binding or solubilizing effect on the HBV envelope and has an inhibitory effect on HBV uptake at physiological temperatures; irbesartan effectively inhibits the formation of HBV covalently closed circular DNA (cccDNA), but it has no significant effect on the regulation of HBV expression [86]. Ko et al. [87] conducted a study revealing the inhibitory effect of irbesartan on the entry of HBV into the human hepatocellular carcinoma cell line HepG2-NTCP. Notably, the inhibitory effect of irbesartan on hepatitis B virus infection is associated with the BA transporter NTCP [88]. These findings suggest that irbesartan inhibits HBV infection by inhibiting the BA transporter function of NTCP. Irbesartan also inhibited hepatic fibrosis to some extent. To achieve an anti-HBV effect, the blood concentration of irbesartan needed to be approximately 1.4 μg/mL, corresponding to an IC_{50} value of 3.3 μM. When up to 300 mg of irbesartan was dosed daily (the highest dose usually recommended), the peak concentration Cmax was approximately 2.6 μg/mL in healthy volunteers [89]. In accordance with these results, irbesartan has become an appropriate therapy for HBV patients, potentially providing therapeutic advantages through the achievement of two key goals: impeding HBV entry and ameliorating liver fibrosis [86]. However, whether irbesartan can be used to treat HBV still needs verification.

5.5. Novel thiazolidinedione derivatives (TZD)

Rosiglitazone and troglitazone have been formulated as clinical agents for the management of type 2 diabetes mellitus. These compounds are ligands for peroxisome proliferator-activated receptor gamma (PPAR-γ), which is used to improve blood glucose levels in patients with type 2 diabetes by enhancing insulin secretion from pancreatic β-cells and insulin sensitivity in adipose tissues and the liver. In 2019, Fukano et al. [73] investigated the effects of TZD derivatives on HBV infection and reported that troglitazone and selegiline acted as HBV entry blockers, hindering the formation of cell surface NTCP dimers and blocking HBV internalization after viral attachment. In contrast to other HBV entry inhibitors, troglitazone interferes with the internalization of HBV from the cell surface to the intracellular compartment [73]. Interestingly, the combination of troglitazone with MyrB significantly increased anti-HBV activity. HBV entry inhibitors are expected to be used for the prevention of vertical transmission and HBV recurrence after liver transplantation, as well as for postexposure prophylaxis.

5.5.1. Rosiglitazone

Rosiglitazone is a thiazolidinedione hypoglycemic agent, but it is rarely used for hypoglycemic therapy in diabetic patients because it causes cardiovascular disease and increases the risk of obesity [90]. PPAR is a ligand-activated transcription factor that plays a role in glycolipid metabolism, the immune response, and inflammation. Wakui et al. [91] showed that rosiglitazone was able to inhibit HBV replication *in vitro* and that the combination of rosiglitazone with nucleoside analogs or interferon may be a therapeutic option for chronic HBV infection [91].

5.5.2. Troglitazone

Troglitazone is a TZD derivative. It has been found to block HBV internalization by the dissociation of NTCP dimers on the plasma membrane, but not HBV adhesion to the cell surface [73]. Studies have also shown that troglitazone disrupted NTCP oligomerization, suggesting that this oligomerization may play a role in HBV-NTCP internalization. Troglitazone differs from previously identified entry inhibitors in that it represents a new class of anti-HBV entry inhibitors that are thought to interfere with the internalization of HBV from the cell surface into the intracellular compartment. Unfortunately, troglitazone has been withdrawn from commercial use due to an FDA warning on potential hepatic toxicity [92].

5.6. Cholesterol absorption inhibitors

Currently, ezetimibe is the only cholesterol absorption inhibitor used for the treatment of hypercholesterolemia; it selectively inhibits small intestinal cholesterol transport proteins, effectively decreases cholesterol absorption in the intestinal tract, and lowers plasma cholesterol levels and hepatic cholesterol stores. Ezetimibe, a cholesterol-lowering drug characterized by a pharmacophore that inhibits NTCP, was shown to block HBV infection in HepaRG cells [93]. Ezetimibe may be more effective at inhibiting HCV infection than at inhibiting HBV infection because it has a much greater IC_{50} for HBV infection than for HCV infection [93,94]. A phase II clinical trial evaluated the efficacy and safety of 10 mg/day ezetimibe in patients with HDV (those who were already receiving interferon therapy or were not suitable for interferon therapy) [95]. A total of 44 patients with chronic hepatitis D were enrolled in this study and completed 12 weeks of treatment for chronic hepatitis D. Forty-three percent (18/42) of the patients treated with ezetimibe had a 100% reduction in viral load, suggesting that ezetimibe is able to attenuate viral replication in patients with chronic hepatitis D. Ezetimibe can also be used to treat liver fibrosis [96].

5.7. FXR agonists

Previous studies have shown that inhibition of NTCP increases FXR expression in hepatic stellate cells, thereby decreasing α-SMA and collagen synthesis [47]. Obeticholic acid (OCA) is a modified BA and FXR agonist derived from the human primary bile acid — chenodeoxycholic acid. OCA is a selective FXR agonist and is currently the only approved second-line therapy for patients with PBC who cannot tolerate or do not respond adequately to UDCA [97]. The key mechanisms of action of OCA, including its choleretic, anti-inflammatory, and antifibrotic properties, underlie its hepatoprotective effects and lead to reduced injury and improved liver

function in cholestatic liver disease (e.g., PBC). However, because its effectiveness rate is only 50% and because it is associated with problems such as skin rashes and other adverse reactions, we consider it problematic for practical application and promotion. In September 2017, the FDA issued a warning that the use of OCAs in PBC patients with decompensated cirrhosis (Child-Pugh-Turcotte classes B and C) was associated with clinical deterioration and even death [48]. A phase III clinical trial demonstrated the long-term efficacy and safety of acetylcholic acid in patients with primary cholangitis who were intolerant or inadequate responders to UDCA by studying 217 patients with primary cholangitis who were intolerant or inadequate responders to obeticholic acid [98].

5.8. Benzamide analogs

At present, a variety of previously developed drugs have been recognized as NTCP inhibitors and subsequently incorporated into NTCP studies. Studies have shown that drugs such as rosiglitazone and fasiglifam can inhibit NTCP, affect HBV infection [99], and cause severe hepatotoxicity [100]. Despite their inhibitory effect on NTCP and antitumor properties in hepatocellular carcinoma, these inhibitors have been associated with many side effects [101,102]. Therefore, the development of effective NTCP inhibitors is a primary goal in the treatment of hepatocellular carcinoma. In 2019, researchers discovered a new NTCP inhibitor (compound 35) that inhibits NTCP expression [103]. HBV DNA levels were also found to be significantly reduced after compound 35 treatment of HepG2.2.15 cells. Western blot analysis revealed that compound 35 inhibited NTCP expression while concurrently upregulating Bax, downregulating Bcl-2, and inducing the cleavage of caspase-3 and poly adenosine diphosphate (ADP)-ribose polymerase (PARP). Furthermore, compound 35 demonstrated substantial antiproliferative activity and the ability to induce apoptosis in HepG2 cells [91]. Molecular docking diagrams revealed that compound 35 could form three hydrogen bonds with Ser109, Asn54 and Tyr108; the benzene ring of compound 35 could interact with Phe44 to form π−π interactions; and the benzyl group at the other end of compound 35 could penetrate the hydrophobic cavity of NTCP, showing that the compound 35-NTCP complexes have a certain degree of stability [103]. Compound 35 is a benzamide derivative obtained by structural modification of the lead compound AO-081/40926746 and was identified from the ZINC database using the CDOCKER protocol. The *N*-(2-(2-benzylindenyl-2-oxoethyl) acetamide chain of the lead compound remained unchanged, while only portions A and B were structurally altered. The introduction of a chlorine-containing heterocycle at the para position of the benzene ring in the main chain, along with retention of the benzene ring on the left side of the amide linkage, resulted in a compound exhibiting potent antiproliferative activity against HepG2.15 cells and significantly reduced DNA levels of HBV within the cells. According to the binding model, the benzylic moiety of compound 35 could be inserted into the hydrophobic pocket of NTCP, and form π−π interactions with the benzene ring, while other structures could form hydrogen bonds, ultimately enabling high-affinity binding to NTCP. Compound 35, an NTCP inhibitor, was mentioned in the study as a potential therapy option for HCC in the future (Fig. 6). In summary, there are many drugs that have been marketed that have the effect of inhibiting NTCP. These drugs are listed in Table 3.

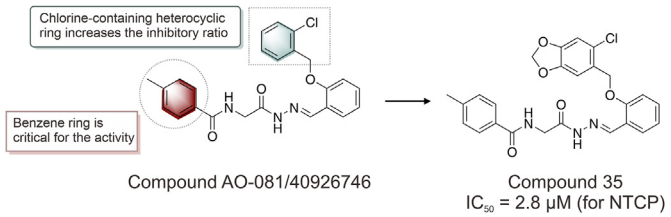


Fig. 6. Structure and structure-activity relationship (SAR) of compound 35. IC_{50}: half maximal inhibitory concentration; NTCP: the sodium taurocholate co-transporting polypeptide.

5.9. Natural products

5.9.1. Epigallocatechin gallate (EGCG)

EGCG is a flavonoid found in green tea extract and a member of the catechin subclass. As early as 2005, several researchers found that it has antiviral and antioxidant properties and can reduce HBV entry and help reduce HBV replication in the body [104]. Huang et al. [105] found that EGCG induced lattice protein-dependent endocytosis of NTCP from the plasma membrane, followed by protein degradation. In addition, EGCG was able to inhibit lattice protein-mediated endocytosis of transferrin. These results suggest that green tea-derived EGCG molecules can effectively inhibit HBV entry and can be used to prevent HBV reinfection. EGCG also possesses antiangiogenic, antioxidant, and antifibrotic properties and may have therapeutic potential for HCV-induced cirrhosis [106]. Yu et al. [107] showed that EGCG had antifibrotic effects on bile duct-ligated rats and transforming growth factor-beta 1 (TGF-β1) stimulated LX-2 cells *in vitro* by inhibiting the phosphatidylinositol-3 kinase (PI3K)/Akt/Smad pathway.

5.9.2. Junceellolide B

Briarane-type diterpenoids [108] have a significant inhibitory effect on HBV replication, and junceellolide B was identified as one of the compounds with significant activity. Junceellolide B selectively reduced viral marker levels in both HBV-infected HepG2-NTCP cells and HBV-replicating HepAD38 cells [109]. Further studies revealed that junceellolide B may act as a transcriptional repressor to downregulate cccDNA transcription by inhibiting the expression of RNA polymerase II-related transcription factors. In 2020, the experimental results of Li et al. [109] reported that junceellolide B mainly inhibited HBV cccDNA replenishment rather than inducing the degradation of existing HBV cccDNA. These findings suggest that junceellolide B is a transcriptional inhibitor of HBV cccDNA through a mechanism different from that of clinically used anti-HBV drugs. It is speculated that with subsequent studies, junceellolide B can be used for future HBV treatment.

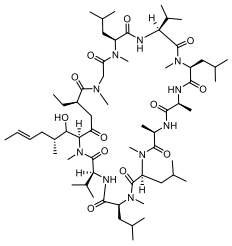
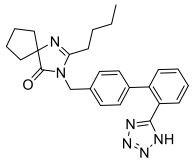
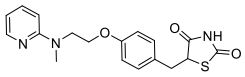
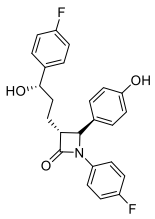
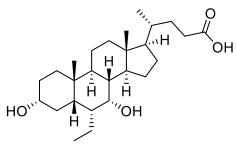
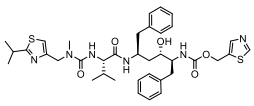
5.9.3. Curcumin (CCM)

Curcumin is a major phytochemical found in the rhizomes of Curcuma longa. Curcumin-like derivatives consist of curcumin, demethoxycurcumin and bisdemethoxycurcumin. CCM has anti-inflammatory, antimicrobial, antioxidant, antitumor and antiviral effects [110−112]. Curcumin reduces the HBV viral load by decreasing the histone acetylation of cccDNA infected with HepG2.2.15 cells [113]. CCM inhibits HCV, dengue virus and influenza virus [114−117]. Thongsri et al. [118] found that CCM inhibits HBV entry into stem cells through the mediation of NTCP. CCM reduces HBV entry through NTCP binding. This process results in a decrease in the expression of all the HBV markers in the infected hepatocytes.

5.9.4. Triterpene acids in Poria cocos

Poria cocos is the dried mycelium of the fungus *P. cocos* and is a commonly used Chinese herbal medicine that has significant inhibitory effects on the function of NTCP. The active ingredients in *P. cocos* that exert inhibitory effects on NTCP are porcine acid A, porcine acid B, and polydienoic acid C [119]. It has been reported that *P. cocos* may exert its lipid-lowering effect by inhibiting the BA

Table 3
Marketed drugs with inhibitory effects on the sodium taurocholate co-transporting (NTCP).

Medicine	Class of drugs	Structure	Indication	Role for NTCP	Progress
Cyclosporin A	Calcium-modulated phosphatase inhibitor		Anti-inflammatory, anti-fungal, and anti-tumor	Inhibits NTCP activity, thereby inhibiting HBV entry into hepatocytes	Treatment of HBV
Irbesartan	Angiotensin II receptor blockers		Treatment of essential hypertension, combined with hypertension in type 2 diabetic nephropathy.	Inhibition of bile acid transporter function of NTCP	Treatment of HBV
Rosiglitazone	Novel thiazolidinedione derivatives		For patients with type 2 diabetes who are unable to achieve glycemic control goals with other hypoglycemic agents	Inhibits HBV entry into liver cells	Prevention of vertical transmission and HBV recurrence after liver transplantation
Ezetimibe	Cholesterol absorption inhibitors		hypercholesterolemia	Attenuates HDV virus replication in the body	Treatment of HBV and HDV
Obeticholic acid	Farnesoid X agonist		Treatment of primary biliary cirrhosis and non-alcoholic fatty liver disease	Inhibition of bile acid transport by NTCP	Treatment of liver fibrosis
Ritonavir	Antiviral drug		Treatment of patients with advanced or non-progressive AIDS	Inhibition of bile acid transporter function of NTCP	Treatment of HBV and HDV

HBV: hepatitis B virus; HDV: hepatitis D virus; AIDS: acquired immunodeficiency syndrome.

uptake transporter in the enterohepatic circulation and reducing the return of BAs to the liver, thereby increasing the conversion of cholesterol to Bas [119]. However, its application in the treatment of other liver diseases requires further research.

5.9.5. Ergosterol peroxide (EP)

EP, a steroid that occurs naturally in medicinal mushrooms, lichens, and sponges, has been reported to have antitumor, inflammatory, and oxidative bioactivities [120,121]. Relevant research has demonstrated that EP prevents HBV infection in immortalized primary human hepatocytes (dHuS-E/2 cells) via interfering with the fusion/endocytosis stage of HBV entry. A study by Huang et al. [60] showed that the transgene activity of EP against the HBV genotypes A-D highlights its potential for the treatment of HBV infection. In conclusion, EP not only shows therapeutic promise in the treatment of viral hepatitis by disrupting the association of NTCP with HBsAg-enveloped viral particles but also presents a structural backbone that can be used to develop novel HBV entry inhibitors.

5.9.6. Exophillic acid

The fungal secondary metabolite exophillic acid inhibits HBV and HDV infection but not HCV infection by acting on NTCP. Kobayashi et al. [61] showed that exocytotic acid has strong anti-HBV/HDV activity, with an IC_{50} of 1.1 μM against HBV in primary human hepatocytes (PHHs). The mode of action was also investigated, and the compound was found to interact with the HBV/HDV receptor NTCP and inhibit viral attachment to host cells. The exogenous acid analogs TPI-1 and TPI-2 showed similar antiviral activity, suggesting that the 2,4-dihydroxyalkylbenzoic acid portion is essential for activity. This compound can be used to create new antiviral medications to treat chronic hepatitis B and D, prevent HBV infection during vertical transmission, prevent HBV reactivation following liver transplantation, or provide postexposure prophylaxis [122].

5.9.7. Vanitaracin A

Vanitaracin A, a structurally unique tricyclic polyketide, has been discovered by researchers to particularly prevent HBV infection [62]. Thus, we identified a fungal metabolite, vanitaracin A,

which is a potent, well-tolerated, and broadly active inhibitor of HBV and HDV entry. Kaneko et al. [62] demonstrated that vanitaracin A interacted with NTCP to inhibit HBV attachment to host hepatocytes. As the effects of these compounds were pre-S1- and NTCP dependent, vanitaracin A also inhibited infection by HDV, which requires the same envelope-receptor involvement [5]. We hope to investigate the use of vanitaracin A for developing a novel class of antiviral agents against both HBV and HDV.

5.9.8. Betulin derivatives

The pentacyclic lupanetype triterpenoid betulin, produced from birch, has been shown to exhibit distinct inhibitory potency and selectivity towards the viral receptor function of NTCP. Researchers have shown that betulinic acid might have additive antiviral effects on HBV/HDV through the targeting of different cellular targets involved in cell entry (NTCP) and replication superoxide dismutase 2 (SOD2) [123].

5.9.9. Proanthocyanidin (PAC)

PAC is an oligomeric flavonoid that inhibits HBV entry into host cells by targeting the large surface proteins (LHBs) of HBV. PAC prevents the attachment of the preS1 region in LHBs to its cellular receptor, NTCP. PAC was shown to target HBV particles and impair their infectivity but did not affect NTCP-mediated BA transport activity [124]. A novel family of anti-HBV drugs known as PAC and its analogs specifically targets the preS1 region of the HBV large surface protein. These compounds could help in the development of potent tolerant, and broadly active HBV infection inhibitors.

5.10. Azelastine hydrochloride (N4)

In 2014, Fu et al. [125] demonstrated that compound N4 (azelastine hydrochloride) was validated as a new lead "best-match" compound for targeting NTCP, which provides new clues for hepatitis B therapy against NTCP. In this study, 30 small molecules were screened to identify the five inhibitors with the highest degree of binding to NTCP, and *in vitro* virology and cytotoxicity studies of the target compounds allowed the investigators to identify the most active compound, N4. In contrast to previously FDA-approved drugs for the treatment of HBV (which only affects the HBV polymerase due to relatively few available targets for antiviral development), azelastine hydrochloride is a direct antagonist of NTCP in the hepatitis B infection process.

5.11. Bexarotene

Bexarotene, a retinoic acid X receptor (RXR) agonist, was found to inhibit HBV infection RXR signaling through the induction of phospholipase A2 group IIA (PLA2G2A), which activates the arachidonic acid/eicosanoid biosynthesis pathway [126]. Kinesin family member 4 (KIF4) is a highly conserved member of the kinase family [127,128]. KIF4 is known to move to the nucleus during mitosis, where it interacts with chromatin to alter spindle length and control cytoplasmic division [129]. Interestingly, HBV upregulated KIF4 expression in HepG2 hepatocellular carcinoma cells, which has been reported to be markedly increased in HBV-associated liver malignancies [130]. Based on the screening results, the investigators determined that KIF4 is a positive regulator of the early stage of HBV/HDV infection. Further studies have shown that human KIF4 is a critical regulator of NTCP surface transport and localization and is required for NTCP to function as a receptor for HBV/HDV entry [63]. KIF4 regulates NTCP-mediated entry of HBV/HDV. Bexarotene and other RXR agonists reduce KIF4 expression and HBV/HDV infection by targeting forkhead box M1 (FOXM1). This is the first study to show that KIF4 plays an important role in HBV/HDV entry and that it can be used to construct potent anti-HBV entry inhibitors.

5.12. Dimeric BA derivatives (DBADs)

A previous study identified the anti-HBV and anti-HDV activities of DBADs as NTCP inhibitors [131]. DBADs showed strong and long-lasting potency for NTCP inhibition, whereas different ligands and structures exhibited different inhibitory effects. Investigators have shown that some DBADs exhibit an IC_{50} of less than 50 nM and long-lasting inhibitory potency. Liu et al. [64] found that DBADs effectively inhibited HBV infection by interfering with the HBV Pre-S1-NTCP interaction without interfering with the membrane localization of NTCP. Researchers developed a potent and high-affinity human NTCP-targeted compound, DBA-41, and investigated its effects in human NTCP knock-in mice; the results showed that DBA-41 has a strong affinity for human NTCP and displayed efficacy in mice. The use of DBAD compounds in drug development for inhibiting NTCP-mediated HBV entry and substrate transport is a novel design strategy, and these compounds may also serve as useful tools for characterizing the molecular mechanisms of NTCP-mediated viral entry and substrate transport (Fig. 7). DBADs are compounds obtained by combining the C-3 hydroxyl moiety and C-24 side chain of dimeric BAs in various configurations, including tauroursodeoxycholic acid (TUDCA) and ursodeoxycholic acid (UDCA), and TUDCA enhances antiviral activity. Compared to monomers, these dimeric compounds exhibit greater binding affinity for NTCP and more favorable interactions. Among these, DBA-41 demonstrated the highest specificity in inhibiting NTCP and excellent biosafety *in vivo*. DBA-41 contains a cyclic structure linking two BA monomers via a rigid triazole heterocycle, which not only improves inhibitory potency but also confers structural and metabolic stability. Additionally, the cyclic scaffold and taurine conjugation in the linker are beneficial for sustained NTCP inhibitory activity. The taurine moiety may also contribute to the improved serum protein binding of DBA-41, although it displays increased affinity for NTCP; moreover, DBA-41 exhibits suboptimal oral bioavailability (Table 1).

5.13. Ritonavir

Ritonavir is a protease inhibitor antiretroviral drug used to treat HIV infection. However, due to its strong inhibitory effect on the metabolic function of NTCP, it was approved by the FDA as an NTCP inhibitor. It is also useful for the treatment of hepatitis B and D viruses [88]. In 2022, Yurdaydin et al. [132] followed up with 55 patients with chronic HDV with four weeks of combination therapy with different doses of lonafarnib and ritonavir and found that several patients became HDV-RNA-negative and had normalized alanine aminotransferase levels; however, the proportions of gastrointestinal adverse events in the high-dose and low-dose groups were 49% and 22%, respectively [132].

5.14. Ro41-5253

Investigators used the HepaRG-based HBV infection system to screen for small molecules that reduce HBV infection and found that pretreatment of host cells with Ro41-5253 reduced HBV infection [122]. Cyclosporin A and its derivatives, as well as BAs, including UDCA and TCA, inhibit HBV entry by blocking the interaction between NTCP and the large surface protein of HBV [80,133]. Different from these medicines, Ro41-5253 has been shown to

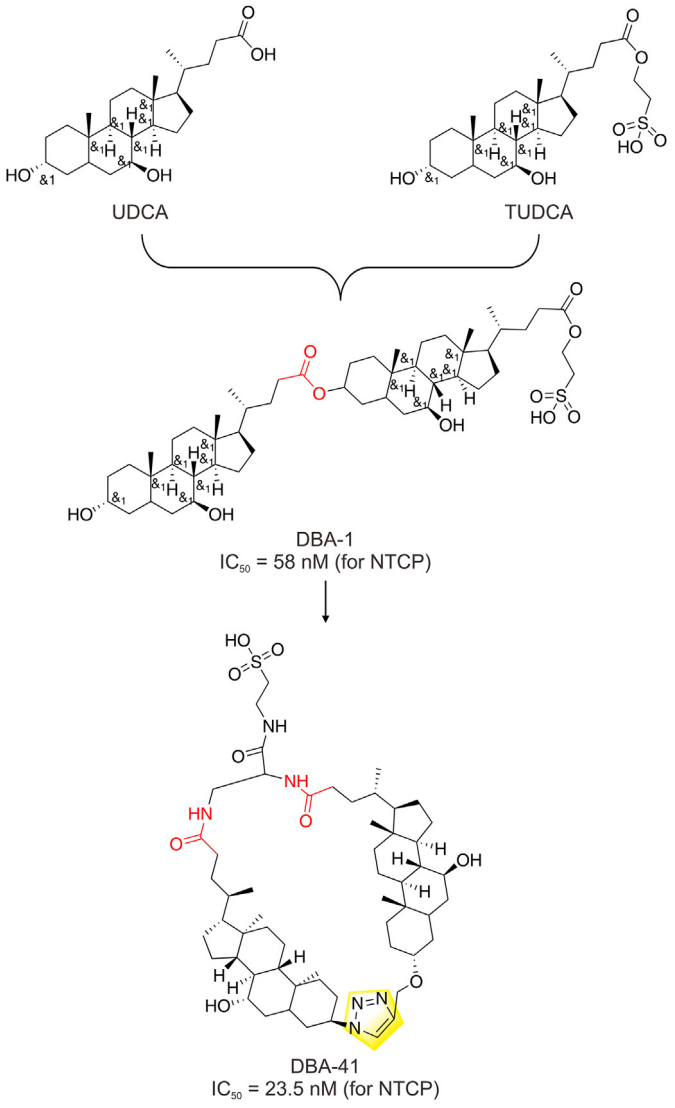


Fig. 7. Structure and the derivative process of DBA-41. The combination of UDCA and TUDCA obtains DBA-1, followed by structural modification to obtain DBA-41. UDCA: ursodeoxycholic acid; TUDCA: tauroursodeoxycholic acid; IC_{50}: half maximal inhibitory concentration; NTCP: the sodium taurocholate co-transporting polypeptide.

reduce host vulnerability to HBV infection through NTCP expression modulation.

5.15. N6HB426-20

In 2022, Takemori et al. [134] established a monoclonal antibody (mAb), N6HB426-20, that recognizes the extracellular domain of human NTCP and blocks HBV entry *in vitro* into human liver cells but has a markedly decreased inhibitory effect on BA uptake [135]. Studies using mouse models have revealed that N6HB426-20 requires a higher dose than MyrCludex to achieve comparable HBV inhibition, but N6HB426-20 maintains inhibition long after administration, proportional to the half-life of the IgG monoclonal antibody [134]. Potential clinical applications of N6HB426-20 include postexposure prophylaxis or preventing vertical transmission or reinfection after liver transplantation in HBV-infected individuals. In summary, we speculate that N6HB426-20, a murine mAb, is a promising option for the treatment of chronic hepatitis B.

6. NTCP inhibitors in combination with other drugs for the treatment of liver disease

At present, for the treatment of HBV/HDV, the commonly used drugs are interferon and antiviral nucleoside analogs. Interferon, when used for HBV infection treatment, has uncertain efficacy and a poor safety profile. The nucleoside analogs tenofovir disoproxil fumarate (TDF), tenofovir alfa-amino acid and entecavir are currently the most effective drugs used for HBV suppression. These drugs are also virtually drug resistant, easy to administer orally, have few side effects, and do not require monitoring. However, these agents require lifelong administration because they do not eliminate the viral genome present in infected hepatocytes [39], and long-term treatment is accompanied by severe side effects or resistance. Therefore, there is an urgent need to discover and develop new therapeutic agents. Researchers have explored the use of NTCP inhibitors in combination with other drugs for the treatment of liver diseases.

In a phase II clinical trial (NCT03546621) [136], the efficacy and safety of MyrB combined with tenofovir or tenofovir alone for inhibiting viral replication in HBV/HDV patients were evaluated. Monotherapy for 24 weeks resulted in a decrease in the serum HDV RNA concentration, but this treatment had no effect on patients with hepatitis B virus and HDV coinfection. The results of this trial showed the strong synergistic effect of combination therapy on decreasing HDV RNA levels, with many patients experiencing a greater decrease in HBs Ag. Low-dose MyrB may have greater efficacy when combined with tenofovir because it works synergistically with tenofovir, and high-dose tenofovir is more effective at stimulating gene induction than is high-dose MyrB. This study provides the first evidence that MyrB in combination with tenofovir has therapeutic potential for chronic HDV and HBV infections. The use of such inhibitors may protect hepatocytes from new HBV viral infections [137]. A combination of antiviral nucleoside analogs and NTCP inhibitors may be used to improve treatment efficacy in patients with hepatitis B. The occurrence of clinically significant liver injury is a well-known adverse effect with the use of isoniazid and rifampicin combination therapy. It has been shown that hepatic injury is caused by the involvement of NTCP and the bile salt export pump (BSEP; ABCB11) in antituberculosis drugs and that the combination of isoniazid (INH) and rifampicin (RFP) has a stronger effect on NTCP expression than INH or RFP alone [137]. These findings suggested that the downregulation of hepatic NTCP and BSEP expression may play an important role in isoniazid (INH)- and rifampicin (RFP)-induced liver injury. As a potential inhibitor, INH may exacerbate RFP-induced cholestasis by inhibiting key BA transporters, such as NTCP and BSEP. Monoammonium glycyrrhizinate is commonly used for hepatoprotection, and its mechanism of action may be related to its modulation of the expression of hepatobiliary membrane transporter proteins [138]. Zhou et al. [138] comprehensively characterized for the first time the significant changes in the expression of the hepatobiliary transporter proteins multidrug resistance-associated protein 2 (Mrp2), NTCP, and organic anion transporting polypeptide 1a4 (Oatp1a4) in the hepatoprotective effects of monoammonium glycyrrhizin (MAG) against RFP- and INH-induced hepatotoxicity.

7. Conclusions and perspectives

It has been reported that the most common phenotypic feature of NTCP deficiency in adults was hypercholanemia, as determined by a comprehensive medical evaluation of 10 adults with NTCP deficiency, vitamin D deficiency, bone loss, and cholestasis. Osteoporosis and vitamin D insufficiency are associated with hypercholanemia. Since the inhibition of NTCP is a pharmacological

approach for the treatment of hepatitis B and D virus infections, hepatic fibrosis, and hepatic bone disease, monitoring total BAs and vitamin D levels, as well as bone mineral density, should also be considered during the development of NTCP inhibitors. Currently, NTCP inhibitors are mainly used to treat HBV and HDV, and the main mechanism is to inhibit viral entry into liver cells; however, this is not the only mechanism involved, and additional research is needed to explore the potential of NTCP inhibitors for the treatment of various other diseases. For the treatment of NAFLD, NTCP inhibitors can slow the development of NAFLD by inhibiting BA uptake, reducing hepatocellular damage, improving hepatic steatosis and insulin resistance and inhibiting hepatic fibrosis, thus achieving therapeutic effects. Some drugs for the treatment of metabolic diseases have also been found to have some inhibitory effect on NTCP and may be considered for development as NTCP inhibitors in the future for the treatment of hepatitis B, NAFLD and cirrhosis.

CRediT author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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