Bile acids in drug discovery

Alfons Enhsen, Werner Kramer and Günther Wess

Recent advances have provided important new information on the physiological mechanisms of bile acid transport and metabolism. Bile acids, which are essential for the digestion and absorption of lipids and lipid-soluble vitamins, are metabolic products of cholesterol and are a major regulator of cholesterol homeostasis. Bile acids are pharmacologically interesting as potential carriers of liver-specific drugs, absorption enhancers and as new cholesterol-lowering agents. Furthermore, the tools of molecular recognition and combinatorial chemistry have been used to explore the drug discovery possibilities of bile acids. The authors explore current understanding and future prospects for bile acid research.

The human liver produces 600–800 ml of bile per day[1–5]. This bile fluid contains 80% water, and is nearly isotonic with blood. The dissolved components of bile consist of 67% bile acids, 22% phospholipids (mainly lecithin), 4% cholesterol, 0.3% gall pigments and various proteins. Bile is concentrated and stored in the gall bladder during fasting. After food intake and subsequent gall bladder emptying, bile is secreted into the small intestine, where the bile acids perform their essential function in the digestion and resorption of fat, fatty acids and lipid-soluble vitamins. These nutrients, insoluble in water, become dispersed in micelles of bile acids and lipids – it is a prerequisite for their uptake by the mucosal cells of the small intestine. The bile acids are then almost completely reabsorbed by both passive and active mechanisms. Passive transport can occur down the length of the small intestine, whereas active transport is restricted to the terminal ileum. After ileal uptake, the bile acids recirculate to the liver via the portal vein, undergoing this enterohepatic circulation 6–15 times per day (Fig. 1). This represents a very efficient biological recycling; in humans 10–40 g of bile acids are transported daily. Bile acids in the enterohepatic circulation participate in feedback regulation, which is an important factor in serum cholesterol homeostasis. Transport systems for bile acids and the regulation of bile acid metabolism are currently the subject of intense research efforts.

Biosynthesis and structural properties of bile acids

Bile acids are synthesized from cholesterol in hepatocytes. This catabolic pathway represents the major metabolic fate of cholesterol (Fig. 2). Cholic acid and chenodeoxycholic acid are synthesized by the liver via two major pathways utilizing many enzymatic steps. These primary bile acids are conjugated with the amino acids glycine and taurine before secretion into the bile. In the duodenum and the jejunum, bile acids are present almost exclusively as taurine and glycine derivatives, whereas in the colon these amides can be partially cleaved by hydrolases. The resulting free bile acids are then transformed into the secondary bile acids deoxycholate and lithocholate by a bacterial 7α-dehydroxylase. Further oxidases and reductases from the intestinal bacteria, as well as liver enzymes, can attack steroidal positions C-3, C-7 and C-12, leading to a complex pattern of bile acids, depending on the prevailing bacterial population. The human bile acid pool contains about 2.5–5 g of bile acids, consisting mainly (~90%) of cholic acid, chenodeoxycholic acid and deoxycholic acid in a ratio of about 2:2:1. Ursodeoxycholic acid and lithocholic acid are found in the remaining 10% of the pool.

Bile acids do not occur as free 24-carboxylic acids in the gall bladder, but as glycine and taurine amides of these acids in a ratio of about 2:1. To a certain extent they can also be sulphated or glucuronidated. In terms of their chemical structure,

Alfons Enhsen, **Werner Kramer** and **Günther Wess***, Drug Innovation and Approval, Hoechst Marion Roussel Deutschland GmbH, D-65926 Frankfurt, Germany. *tel: +49 69 305 82898, fax: +49 69 305 3221, e-mail: guenther.wess@hmrag.com

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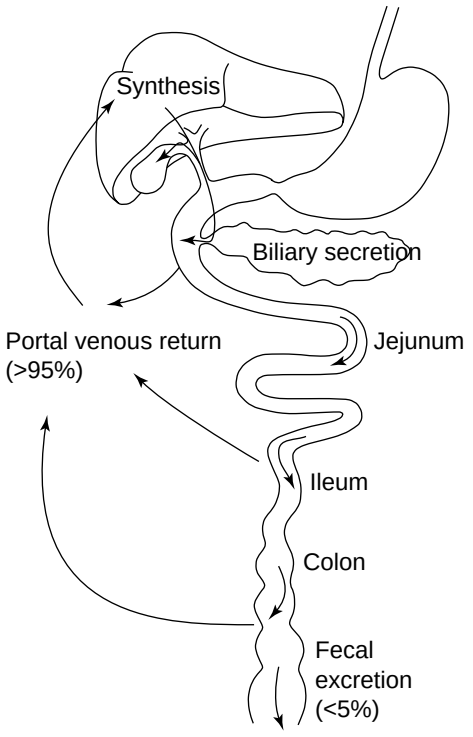


Figure 1. *Enterohepatic circulation of bile acids is >95% efficient.*

bile acids are hydroxy derivatives of cholanoic acid. A common structural feature of all natural bile acids is the *cis* configuration at C-5 between rings A and B of the steroid skeleton, as well as a pentanoic acid side-chain attached to ring D. Their differences are determined by the number, position and stereochemistry of the hydroxyl groups. Because of their unique structure – a convex hydrophobic upper side, a concave hydrophilic α-side and a negatively charged side-chain – bile acids are amphiphilic molecules with detergent properties. Depending on the pH and temperature, bile acids easily form micelles in water at concentrations of 0.6–10 mM; this is of central importance for their physiological function.

Bile acid transport systems

During enterohepatic circulation, bile acids pass through several cellular membranes via both active and passive transport processes[1–5]. The strict organotropism for uptake in the liver, small intestine and kidney is possible by the presence of active, Na^+-dependent transport systems for bile acids in the sinusoidal membrane of hepatocytes, the brush border membranes of ileocytes and the kidney proximal tubule cells.

The uptake of bile acids from portal blood occurs by the Na^+-dependent bile acid transport system (NTCP, sodium taurocholate cotransporting polypeptide)[6]; additional Na^+-independent transport systems such as OATP (organic anion transport protein) can also mediate bile acid transport[4]. The Na^+-dependent transport system at the hepatocyte sinusoidal membrane has been characterized by photoaffinity labeling with photoreactive bile acid derivatives[7–9], and the hepatic Na^+/bile acid cotransporters from human[6], rat[10] and rabbit liver (S. Stengelin, unpublished) have been identified by expression cloning strategies. Both active and passive systems recognize a variety of amphiphilic organic molecules, including peptides, bilirubin and dyes such as bromosulphophthalein and indocyanine green, in addition to bile acids. These transporters direct bile acids into the liver cells where they are shuttled to the canalicular membrane. Conjugated bile acids are secreted directly into the bile, whereas free bile acids are first conjugated with taurine and glycine. After fulfilling their physiological functions as biological detergents and cofactors, the bile acids are almost completely reabsorbed in the terminal ileum. The proportion of active and passive transport in the ileum depends on the dissociation constants of the individual bile acids and on the intraluminal pH.

By contrast to the broad substrate specificity of the active hepatic bile acid transporter, the ileal active transport system shows a very distinct specificity for bile acids. Active bile acid resorption in the terminal ileum occurs by a Na^+-dependent bile acid cotransport system. The protein components of this cotransport system have been identified[11–14] by photoaffinity labeling. In rabbit, a 93 kDa integral membrane protein and a peripherally attached 14 kDa protein[14] make up the functional oligomeric transporter complex of 451 kDa (Ref. 15). The 14 kDa protein has been identified as membrane-bound, ileal lipid-binding protein (ILBP; gastrotropin) – a cytosolic binding protein for bile acids[16,17]. The cDNAs of the homologous integral transport proteins from several species have been obtained by expression cloning. The ileal Na^+-dependent transporters from human[18], rat[19] and hamster[20] are glycosylated proteins of 348 amino acids with seven putative transmembrane regions, and display 35% homology with their hepatic counterparts[18–20], whereas the rabbit transporter contains 347 amino acids[21].

The interaction between the carrier protein and bile acids is highly specific. SARs suggest certain regions of recognition that interact cooperatively: a region that recognizes the

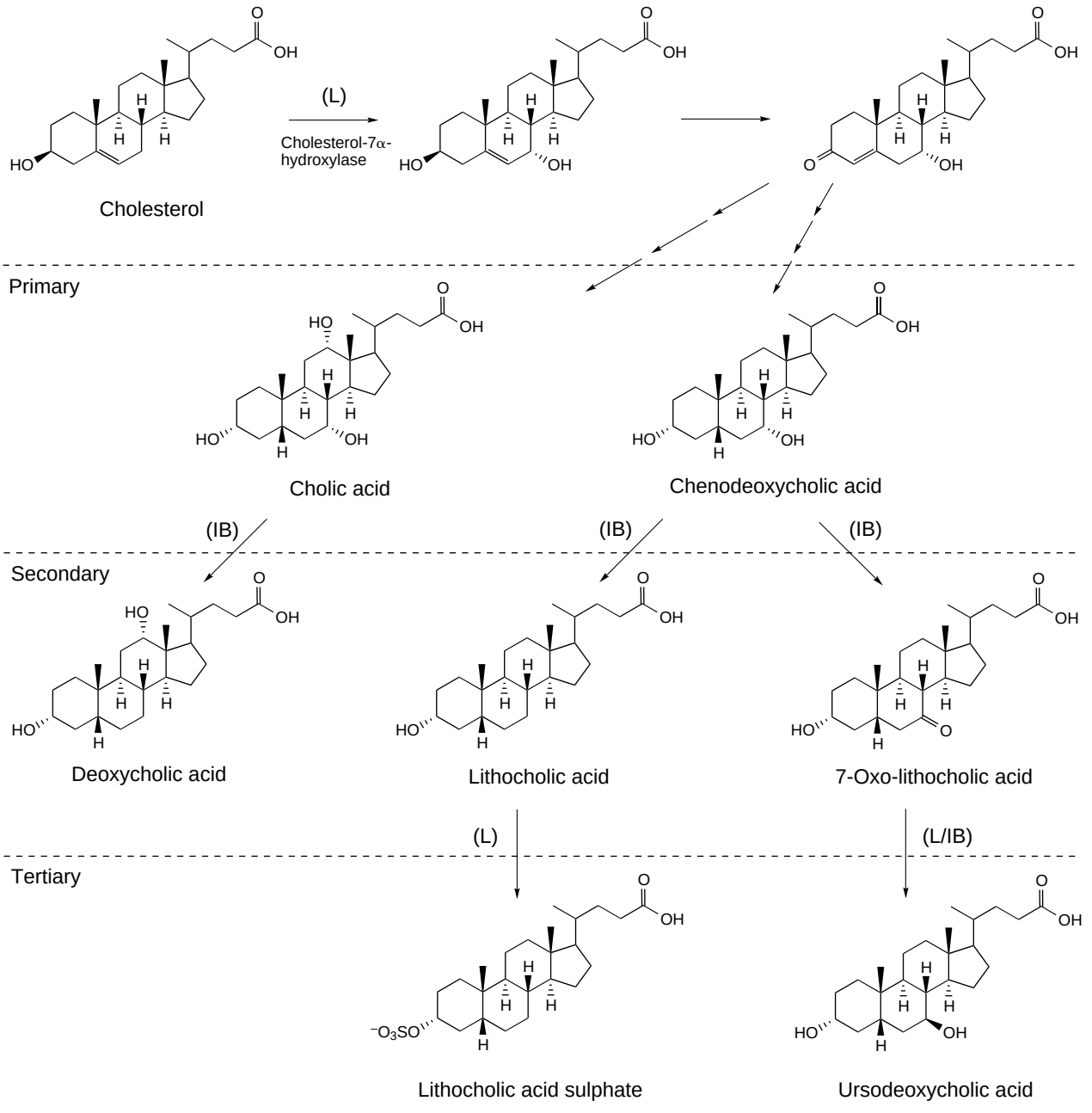


Figure 2. *Bile acid metabolism – production of primary, secondary and tertiary bile acids occurs in the liver (L) and by intestinal bacteria (IB).*

steroid skeleton, a region that interacts with the acidic side-chain, and a binding site for Na^+ close to the region interacting with the carboxylate groups. Vicinal cysteine and lysine side-chains are believed to be essential for transport activity in the Na^+-dependent carriers[13].

Because of the specificity and high capacity of the ileal and hepatic transport systems for bile acids in the entero-hepatic circulation, attempts have been made to take advantage of these properties and the organotropism of bile acids for pharmaceutical applications.

Drug targeting and resorption

The treatment of chronic diseases in most cases involves long-term or even life-long use of drugs. Two aspects of

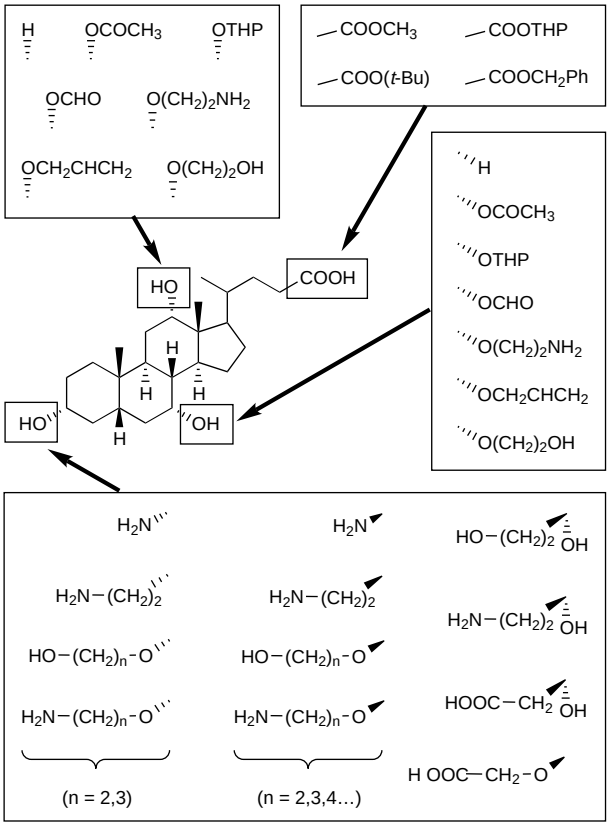


Figure 3. *Bile acid building blocks for the synthesis of drug–bile acid conjugates.*

long-term drug therapy are of major importance for patients and physicians: a site-specific drug action without adverse side effects, and noninvasive, preferably oral, administration of the drug(s). The physiology of bile acid transport described above, with the exclusive involvement of the liver and the small intestine, should therefore be ideally suited for the use of bile acids as putative shuttles of pharmaceuticals.

Current research efforts are focused on specific drug targeting to the liver, and on improving the intestinal absorption of poorly or non-absorbed drugs (e.g. peptides) by bile acids. Drug–bile acid conjugates have therefore been synthesized. After specific uptake of the pharmacologically inactive prodrug by the liver and small intestine, metabolism inside the target cell can liberate the active molecule to exert its pharmacological action. For successful design of drug–bile acid conjugates, the SARs of bile acids towards their natural transporters must be known and carefully adhered to[11–13,22,23]. The following crucial structural properties for recognition should be preserved: the negatively charged side-chain of the natural bile acids, the *cis* configuration of rings A and B of the steroid skeleton, and at least one axial hydroxyl group on the steroid nucleus at position 3, 7 or 12.

The various drugs investigated were attached via a linker moiety to the steroid nucleus at position 3, 7 or 12, with a defined stereochemistry of the linker in these positions. As the functional groups of drugs suitable for attachment to a modified bile acid molecule differ depending on the particular drug in question, it was necessary to develop a new chemical methodology for the synthesis of the required bile acid building blocks. A variety of modified bile acids were prepared carrying linkers of different functionality, length and polarity, attached in defined positions on the steroid molecules[24–27] (Fig. 3).

Chlorambucil–bile acid conjugates

To demonstrate the potential of liver-specific drug targeting by coupling to bile acids, three different drugs for different medical indications were chosen. Chlorambucil is an alkylating cytostatic agent used for the treatment of various cancers (chronic lymphoblastic leukemia and Hodgkin's lymphoma). Chlorambucil and its metabolites are predominantly excreted by the kidney. It was chosen as a model drug to evaluate whether the normal route of renal elimination could be changed to a hepatobiliary one. The drug conjugate **1** was synthesized by amide bond formation

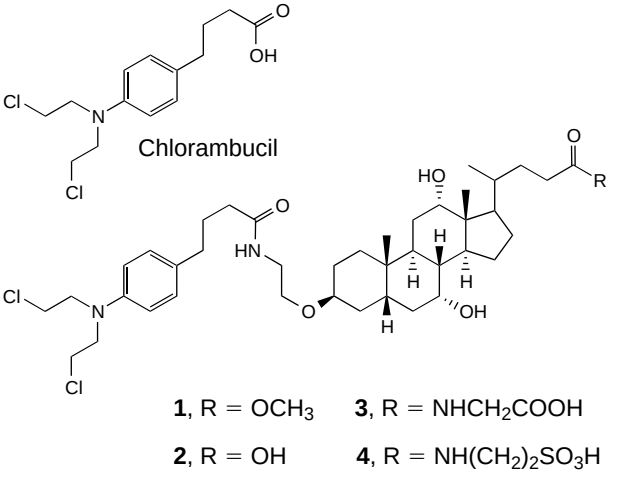


Figure 4. *Chlorambucil–bile acid conjugates; they have potential as chemotherapeutics in hepatocellular carcinoma.*

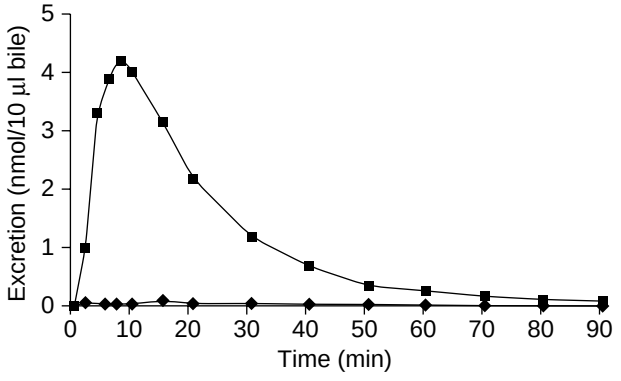


Figure 5. *Biliary excretion of chlorambucil (diamonds) and its bile acid conjugate **4** (squares) after injection into peripheral mesenteric veins of anesthetized rats.*

between chlorambucil and a linker-modified cholic acid ester. Saponification gave the free 24-carboxylic acid derivative **2**, which served for the synthesis of the glycine and taurine conjugates **3** and **4**, respectively[14,28,29] (Fig. 4).

Chlorambucil itself had only a small effect on the Na^+-dependent uptake of bile acids in freshly isolated rat hepatocytes, whereas the corresponding bile acid conjugates strongly inhibited hepatic taurocholate transport with the ranking: methyl ester **1** < carboxylic acid **2** < glycine conjugate **3** < taurine conjugate **4**. IC_{50} values were about 100 µM for chlorambucil and 3–5 µM for conjugate **4**. This specific interaction was also found for intestinal brush border membrane vesicles. The parent drug and methyl ester **1** did not inhibit taurocholate uptake, whereas the conjugates **2**, **3** and **4** strongly inhibited Na^+-dependent taurocholate transport.

Nevertheless, the suitability of modified bile acids as shuttles was demonstrated by *in situ* perfusion experiments of rat liver and ileal segments. The chlorambucil conjugates are absorbed from the ileum, and their bile acid character is reflected by the fact that these conjugates are secreted by the liver, by contrast to the renal clearance of chlorambucil itself (Fig. 5). The characteristic pharmacological activity of chlorambucil as an alkylating anticancer agent was also preserved in the intact drug–bile acid conjugates, as demonstrated by their ability to alkylate proteins after incubation with hepatocytes[14]. Transport of the chlorambucil–taurocholate conjugate **4** by the transport systems NTCP and OATP, which are still present in hepatocellular carcinomas, could be demonstrated, suggesting that intra-arterial application of cytostatic agents coupled to bile acids could be a chemotherapeutic approach to the treatment of hepatocellular carcinomas in humans[30].

Peptide drug–bile acid conjugates

The importance of peptide drugs would increase tremendously if their major drawbacks could be overcome. Two of the most important drawbacks are susceptibility to enzymatic hydrolysis and relative impermeability at biological membranes. For example, oxaprolylpeptides are very effective inhibitors of prolyl-4-hydroxylase, a key enzyme in collagen biosynthesis. However, for the effective therapy of liver fibrosis, these drugs must act exclusively in the liver. To evaluate the pharmacokinetic behavior of oxaprolylpeptides, 7-[7-nitrobenzo(1,2,5) oxadiazol-4-yl]-β-Ala-Phe-Opr-Gly-OH **5**, its *t*-butyl ester **6** and the related cholic acid conjugate **7** were synthesized[14,29,31] (Fig. 6). All three compounds are inhibitors of isolated prolyl-4-hydroxylase. To measure their intestinal absorption *in vivo*, ileum perfusion experiments were performed: neither the oxaprolylpeptide **5** nor its lipophilic *t*-butyl ester were absorbed by the small intestine. The conjugate **7**, however,

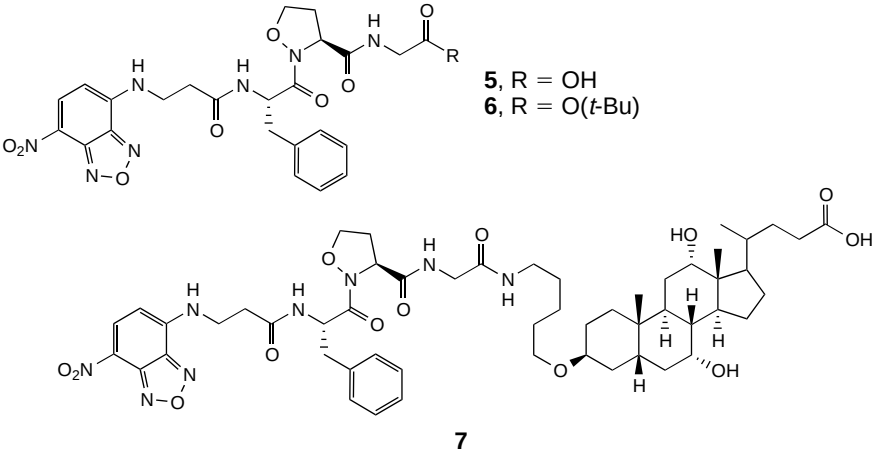


Figure 6. *Oxaprolylpeptide–bile acid conjugate has greatly improved ileal absorption over the parent drug.*

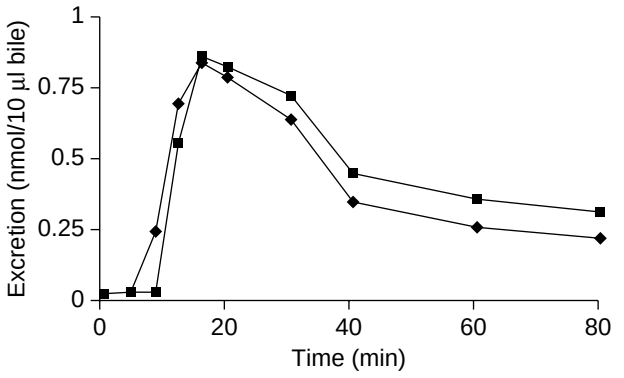


Figure 7. *Biliary excretion of bile acid conjugate* **7** *(diamonds) and a metabolite (squares) after ileal perfusion with compound* **7** *in anesthetized rats.*

together with a metabolite that is formed during passage through the liver, was secreted into the bile (Fig. 7).

Under the experimental conditions used, an overall ileal absorption of about 7% was achieved, compared with less than 0.5% for the unconjugated parent drug. Intestinal uptake occurred via the specific ileal bile acid transport system. Inhibition of hepatic prolyl-4-hydroxylase and inhibition of collagen biosynthesis in the liver were demonstrated with conjugate **7**. From these results it can be concluded that, by coupling peptides to modified bile acids, it is possible to partially avoid the usual metabolism of peptides and allow their access to tissues served by bile acid transport systems.

HMG-CoA reductase inhibitor–bile acid conjugates

Ideally, 3-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase inhibitors should be active exclusively in the liver. To achieve organ-selective actions, HMG-CoA reductase inhibitors should be taken up into hepatocytes by a mechanism that is unique to parenchymal liver cells. Following an approach that combines the essential structural elements of HMG-CoA reductase inhibitors with those of specific uptake by bile acid transport systems, the 3,5-dihydroxyheptanoic acid moiety of HMG-CoA reductase inhibitor **8** was covalently linked by an amide bond to α-aminoalkoxy-substituted bile acids[25,32,33] (Fig. 8). A variety of linkers of different structure, length and stereochemistry were investigated because the active drug must be released from such conjugates for pharmacological activity. Prodrugs such as compounds **9**, **10** and **11** were not expected to inhibit the target enzyme as long as their dihydroxyheptanoic acid was blocked by linkage to the bile acids. In fact, they showed inhibition on isolated rat liver microsomal HMG-CoA reductase three orders of magnitude less than the parent drug **8**.

The uptake of natural bile acids by rat hepatocytes was specifically inhibited by these conjugates, whereas the carrier-mediated uptake of serine remained unaffected. Compared with the parent drug **8**, compounds **9**, **10** and **11** showed a strongly increased affinity for the hepatocyte bile acid transport systems. The uptake of **9** itself was energy dependent, saturable and carrier mediated. A very similar behavior was observed for the intestinal transport system in brush border membrane vesicles[33,34]. Liver perfusion experiments *in vivo* showed that the secretion profile of these compounds into bile was very similar to that of natural bile acids, by contrast to the parent drug (Fig. 9). A major polar metabolite was formed during liver passage from both **8** and conjugate **9**, indicating intracellular release of the active inhibitor **8** from the inactive bile acid prodrug. Moreover, after intravenous application of the conjugates to rats, the concentration of the conjugated drug in extrahepatic organs was considerably lower than that in the liver, indicating a high degree of selectivity.

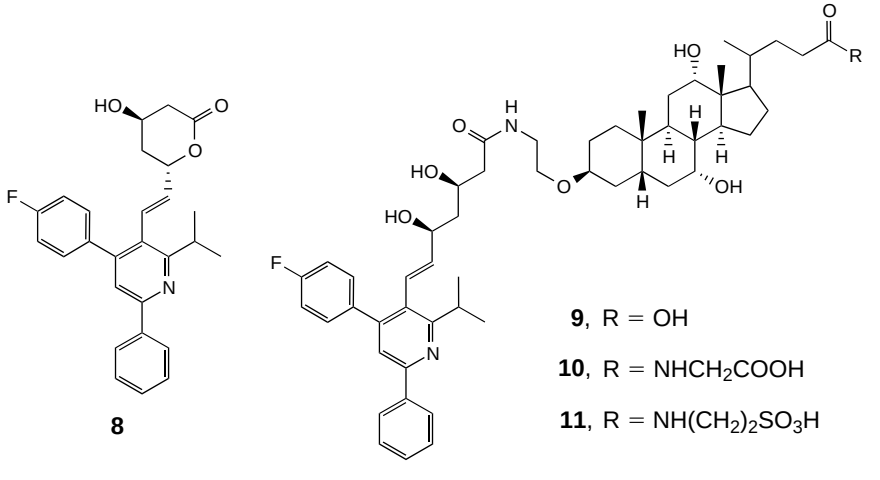


Figure 8. *HMG-CoA reductase inhibitor–bile acid conjugates become pharmacologically active after hepatic cleavage of the prodrug.*

The release of a drug from the inactive prodrug can be influenced by the

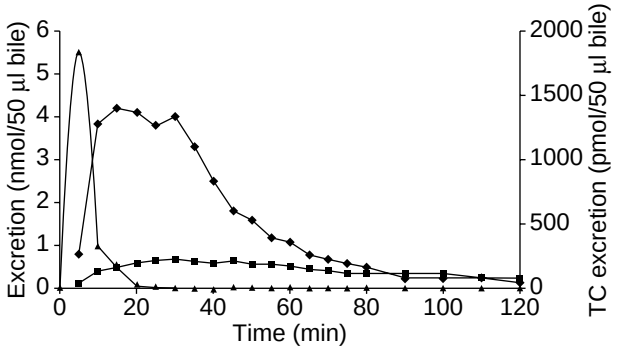


Figure 9. *Biliary excretion of HMG-CoA reductase inhibitor **8** (squares), its bile acid conjugate **9** (diamonds) and taurocholate (TC; triangles) after injection into the jejunal veins of anesthetized rats.*

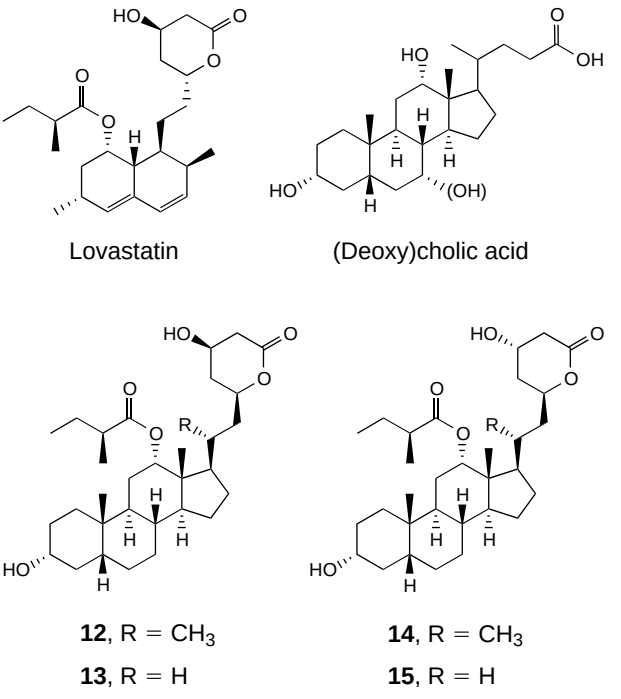


Figure 10. *HMG-CoA reductase inhibitor–bile acid hybrids possess both inhibitory activity of HMG-CoA reductase and specificity for bile acid transport systems.*

linker chosen between the drug and the bile acid moiety[35]. By contrast to the isolated enzyme, HMG-CoA reductase inhibitor prodrugs were able to inhibit HMG-CoA reductase and thereby cholesterol biosynthesis in HepG2 cells. After oral application to hamsters and rats, the prodrug **9** inhibited cholesterol biosynthesis in the liver only, with a slow onset of action caused by the passage time along the small intestine.

HMG-CoA reductase inhibitor–bile acid hybrids

Besides the principle of conjugating drugs to linker-modified bile acids, a second very interesting means of increasing the hepatoselectivity of HMG-CoA reductase inhibitors was described by three different groups[32,36,37]. By contrast to prodrugs, this concept combines in one molecule the structural requirements necessary for the inhibition of HMG-CoA reductase and for specific recognition by the bile acid transport systems. The hexahydronaphthalene moiety of Lovastatin™ was replaced by a modified steroid system derived from bile acids (Fig. 10). The lactone moiety of HMG-CoA reductase inhibitors is very sensitive to structural variation, thus the 21-methyl group plays an important role in these hybrid molecules. Starting from natural bile acids, several HMG-CoA reductase inhibitor–bile acid hybrids were synthesized and their properties investigated with respect to enzyme inhibition and specific interaction with bile acid transporters. Compounds **12** and **13**, containing the side-chain configuration of Lovastatin, showed higher inhibition on HMG-CoA reductase and cholesterol biosynthesis than their diastereomers **14** and **15**, whereas the demethylated compound **13** was even more active than **12**. Different observations were made for recognition by the specific intestinal bile acid transporter. The stereochemistry of the side-chains at C-3 and C-5 was less important, but the 21-methyl group of hybrids **12** and **14** contributed significantly to recognition by the transport system.

Inhibition of bile acid reabsorption

Serum cholesterol is regulated by the liver. The enterohepatic circulation of bile acids and their excretion play decisive roles within a complex regulatory system. An average Western diet provides a cholesterol uptake of 0.3–0.5 g per day, and *de novo* synthesis contributes another 0.8 g per day. With a bile acid pool size of 2.5–5 g, the daily throughput of the enterohepatic circulation is 10–40 g of bile acids and about 2.5 g of cholesterol. By fecal excretion, 0.2–0.6 g of bile acids and 0.6–0.8 g of cholesterol are excreted per day. The liver can compensate for imbalances of the cholesterol level by various mechanisms. An increase of hepatic cholesterol can be achieved either by low-density lipoprotein (LDL) receptor induction and higher uptake from plasma, or by the stimulation of HMG-CoA

reductase and increased *de novo* synthesis. A higher excretion is reached by increased conversion of cholesterol into bile acids or by elevated biliary cholesterol secretion. In humans, two different approaches have demonstrated that interruption of the enterohepatic circulation results in a significant decrease in serum cholesterol levels.

Bile acid sequestrants have been used to treat hypercholesterolemia for over 20 years[38]. Anion exchange resins bind bile acids, thereby removing them from the enterohepatic circulation. This loss is compensated by resynthesis of bile acids from cholesterol. Much more dramatic were results obtained from the POSCH (Program on the Surgical Control of the Hyperlipidemics) study[39]: partial ileal bypass surgery in 421 patients led to a mean decrease of 23% total cholesterol and 37.7% LDL-cholesterol.

The disadvantages of sequestrants are related to the high dosages required (15–30 g per day), and side effects include constipation, maldigestion, malabsorption and compliance problems. Thus, a more effective way of interrupting the enterohepatic circulation might be the development of highly specific, nonabsorbable inhibitors of the intestinal bile acid transport systems. Such bile acid reabsorption inhibitors would have several advantages: high specific mode of action, no systemic drug load and consequently no systemic toxicity, no malabsorption and maldigestion, low dosage and high compliance. Therefore, modified bile acids were chosen as putative inhibitors. For their design, the structural features that are necessary for specific and high-affinity binding to the ileal transport were taken into account. The attachment of bulky groups linked via spacers of different length and structure to the bile acid moiety should prevent transmembrane transport of the inhibitor itself across the ileal brush border membrane. As the ileal bile acid transport system is an oligomeric protein complex containing several transporter units, we developed the concept of linking bile acid molecules together to obtain dimers, trimers or tetramers. These molecules should inhibit ileal bile acid uptake by the simultaneous occupation of more than one transporter site, resulting in an efficient and specific inhibition of the ileal bile acid transport system without significant uptake of the inhibitor itself.

Dimeric and trimeric bile acids

Many bile acid dimers with the individual bile acid moieties linked together via the steroid rings A, B, C or the side-chain of ring D, as well as trimers, were synthesized[40–44] (Fig. 11). The A–D dimers **16–19** were prepared from readily available starting materials. For example, compound **19** was synthesized from methyl cholate by converting the 3α-hydroxy group into a 3α-amino group (selective mesylation of 3-OH, azide exchange, catalytic hydrogenation). Acylation of this amino function with cholic acid and subsequent saponification of the methyl ester provided the dimer **19** in high yield[40]. The trimeric derivative **20** was prepared from Kemp's triacid as a trivalent core unit and three molecules of the corresponding linker-modified bile acid.

The interaction of compounds **16–20** with the specific ileal bile acid transport system was studied by inhibition of Na^+-dependent [^{3}H]taurocholate uptake into ileal brush border membrane vesicles. Compounds **16**, **18**, **19** and **20** showed strong inhibition. Shortening of the linker in **16** led to **17**, which had a dramatic loss of inhibitory activity. However, replacement of the diphenylmethyl in **17** by hydrogen gave **18**, which had an inhibitory activity similar to that of **16**. From these data it was concluded that the linker moiety and the left-hand bile acid play an important role in the recognition and binding process. Compounds **16**, **18**, **19** and **20** were further characterized pharmacologically

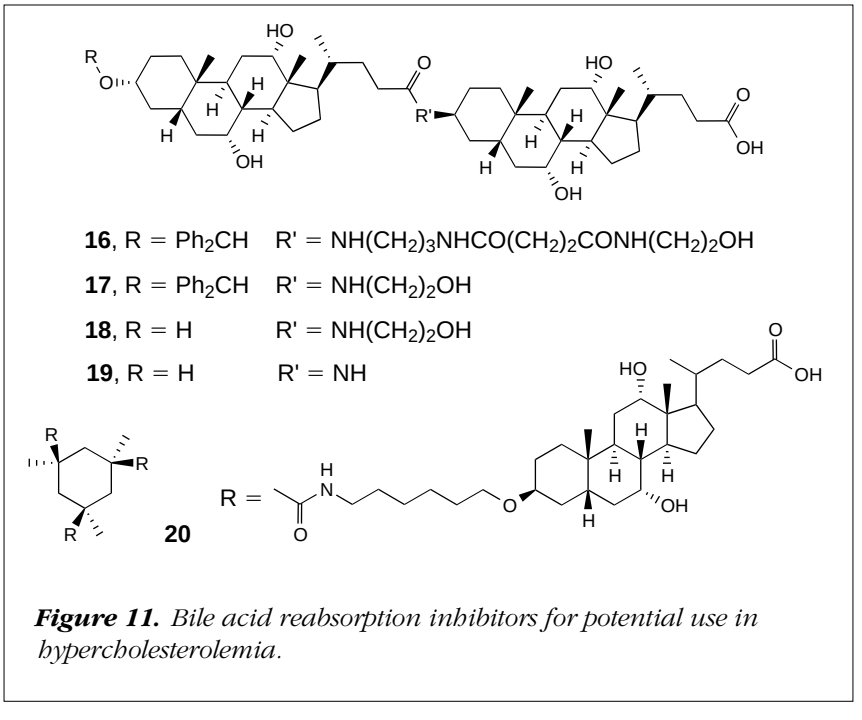


Figure 11. *Bile acid reabsorption inhibitors for potential use in hypercholesterolemia.*

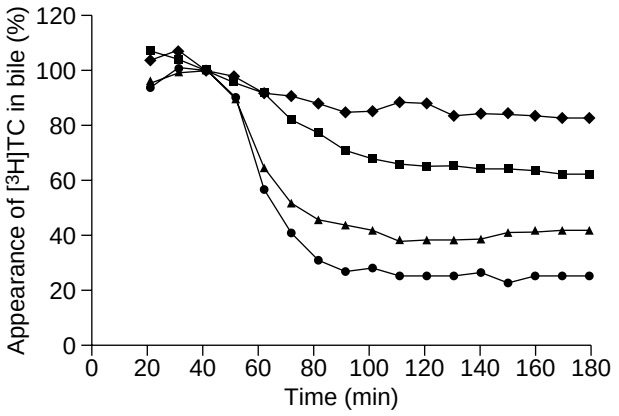
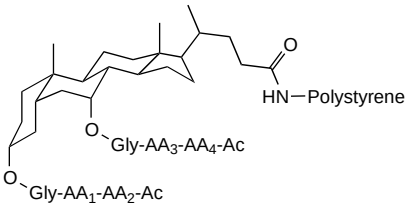


Figure 12. *Effect of bile acid dimer* ***19*** *on the absorption of [3H]taurocholate (TC) in rat ileum perfusion experiments. Diamonds show the control absorption profile (i.e. buffer + [3H]TC), and the squares, triangles and circles show the effect of different concentrations of compound* ***19*** *(i.e. 1, 10 and 100 μM, respectively).*

by *in situ* ileal perfusion experiments in rats. Thus, an ileal segment was perfused with [3H]taurocholate, the common bile duct was cannulated and the level of radioactivity in bile samples was determined in the presence of the inhibitors. Compounds **16** and **18** exhibited moderate inhibitory activity, while compounds **19** (Fig. 12) and **20** showed strong inhibition of bile acid uptake.

Further indications of a pharmacological action were obtained by measuring fecal bile acid output. For this experiment, the endogenous bile acid pool was radiolabeled. Then the inhibitors **19**, **20**, or cholestyramine (a bile acid sequestrant) were administered orally. Determination of the daily fecal excretion of radioactive bile acids showed that the dimer **19** and the trimer **20** led to increased fecal bile acid output. The amounts of drug for equipotent activity were more than one order of magnitude less with **19** and **20** than with cholestyramine. The inhibitors themselves remain within the intestinal lumen and are not absorbed to a significant extent. These new nonabsorbable bile acid transport inhibitors are highly efficient and may become a new class of nonsystemically active lipid-lowering drugs. Photoreactive analogs of these nonabsorbable bile acid transport inhibitors carrying a photoreactive diazirino- or azido function in the 'left' or 'right' bile acid moiety were successfully used to investigate the topology of the ileal Na^+/bile acid cotransport system[43,45].



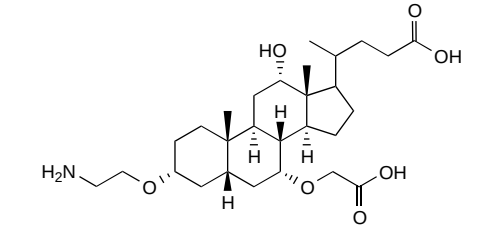
21

Figure 13. *Synthetic peptidosteroid receptor library.*

Bile acids in combinatorial chemistry

In the field of molecular recognition, synthetic receptors, usually of cage-like shape, have been individually tailored to bind simple organic or biological substrates. Natural receptors (e.g. antibodies) can bind larger structures with high affinity. Still and coworkers[46] reported a practical synthetic method for creating new receptor molecules, which greatly accelerates the search for the desired substrate specificity. A library of 10^4 different peptidosteroids, **21**, based on chenodeoxycholic acid as a scaffold was synthesized by encoded combinatorial chemistry on solid phase (Fig. 13). The library of potential receptor molecules was screened with four enkephalin-like compounds in a solid-phase assay. For each of the substrates a few selective receptors were found that showed sequence-specific binding and distinguished between these substrates.

Combinatorial chemistry opens new opportunities in drug discovery for lead structure acquisition and optimization. The design of new nonpeptide scaffolds is crucial to cover broad structural diversity. Bile acids have been chosen as scaffolds to generate combinatorial libraries because of their unique structural properties[47,48]. Conformationally constrained amino acid building blocks are used to replace peptide sequences and to preorganize pharmacophores and other groups. The amino acid equivalent **22** formally represents a tetrapeptide mimetic. The *cis*-decalin part of this bile acid skeleton carries an amino function in position C-3 and a carboxylate group in position C-7, both attached via a linker moiety to the steroid nucleus. The 40 member library **23** (derived from compound **22**) has been synthesized. By comparison with known peptide structures, members of this library that allow chain extensions in the N- and C-terminal directions have been shown to represent complete β-turn mimetics (Fig. 14). The bile acid template **22** thus provides a building block for the design of combinatorial libraries, extending the scope of known β-turn



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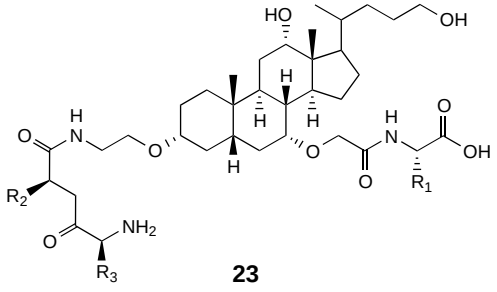

23

Figure 14. *The bile acid template (**22**) represents a tetrapeptide mimetic from which the construction of a combinatorial library (**23**) is possible.*

mimetics. Incorporation of this compact hydrophobic surface into peptide drugs may improve their pharmacological properties.

Conclusion

The examples outlined in this review clearly demonstrate the potential of bile acids and their derivatives in drug discovery. By taking advantage of specific transport systems, the combination of bile acids and drugs can lead to liver-specific pharmaceuticals, or result in improved resorption of poorly or non-absorbable drugs. Understanding the central role of bile acids in cholesterol catabolism enables new approaches for the treatment of hypercholesterolemia. Bile acids have become important tools in medicinal chemistry. Furthermore, their unique molecular properties provide a wealth of possibilities for application in combinatorial chemistry approaches and for molecular recognition.

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