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Specific Inhibitors of Ileal Bile Acid Transport

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The important physiological role of bile acids in cholesterol homeostasis makes them an attractive target for drug interaction. Bile acids are synthesized from cholesterol in the liver, secreted into the small intestine, and recirculated back to the liver with portal blood. Interruption of the bile acid recirculation by bile acid sequestrants[1] or surgery[2] leads to significant decrease of blood cholesterol. Sequestrants have been used in the treatment of hypercholesterolemia for many years, but there is an increasing demand for new types of nonsystemic hypocholesterolemics that can be used in small quantities.

During enterohepatic circulation, bile acids are transported by specific sodium-dependent transport systems in the sinusoidal membrane of hepatocytes[3,4] and in the brush border membrane of ileocytes.[5-7] Recently, we demonstrated that an integral 93- and a peripheral 14-kDa membrane protein are essential components of the Na^+/bile acid cotransport system in rabbit terminal ileum.[8] Inhibition of the Na^+-dependent ileal transport system should decrease the amount of recirculating bile acids, and thus nonabsorbable specific inhibitors of the ileal bile acid carrier could lead to a new class of nonsystemic antiarteriosclerotic drugs. We report the first specific inhibitors of the ileal bile acid transport system.

Compounds **9–14** were designed as inhibitors based on the following ideas: For optimal recognition of the Na^+-dependent bile acid transport systems in the liver and in the small intestine, a negative charge in the side chain of the bile acid molecule and at least one hydroxy group in position 3, 7, or 12 of the ring system is required. Bile acids lacking the 3-OH group behave in each part of the enterohepatic circulation as analogues of the physiological compounds.[9] Therefore, 3-C has been chosen as a position for an additional moiety. A second bile acid (or derivative) has been coupled at its C-24 via linker to C-3 of the first bile acid. Due to additional recognition sites for the transporter we expected an inhibition of the transporter or at least a decreased absorption rate.

Table 1. Inhibition of Na^+-Dependent [^{3}H]Taurocholate Uptake into Ileal Brush Border Membrane Vesicles (Rabbit)[7,8,12]

compd	IC_{25} (μM)[a]	IC_{50} (μM)[a]	IC_{75} (μM)[a]
1	40 ± 5.6	83 ± 32	152 ± 31
TCDC[b]	13 ± 1.6	28 ± 3.8	61 ± 5.8
9	9 ± 1.7	19 ± 4	30 ± 8
10	125 ± 27	>250	≫250
11	20 ± 4.7	36 ± 5.9	57 ± 11
12	24 ± 4	48 ± 6	84 ± 3
13	154 ± 46	>250	≫250
14	176 ± 33	>250	≫250

[a] The uptake of [^{3}H]taurocholate (50 μM) into ileal brush border membrane vesicles was measured for 60 s in the presence of 0, 25, 50, 100, 150, 200, and 250 μmol of the indicated compounds both in the presence and the absence of an inwardly directed Na^+ gradient. Inhibition values are expressed as the difference of uptake in the presence of Na^+ minus the absence of Na^+. The IC_x values are the mean ± SD of 3–10 independent experiments using different membrane preparations. [b] TCDC = taurochenodeoxycholic acid.

Compounds **9–14** were prepared from readily available starting materials **3**, **4**,[10] **7**, and **8**. 3-Mesylcholic acid was transformed to the 3-azide using sodium azide in DMF at 130 °C. After esterification with methanol/HCl, 23 °C, catalytic hydrogenation with $Pd/CaCO_3$ provided **3**. For the synthesis of **7** and **8** methyl 7,12-diacetoxycholate[11] was treated with 1.4 equiv of bromodiphenylmethane in *N,N*-diisopropylethylamine at reflux for 8 h. Removal of acetyl groups at C-7 and C-12 providing **6** occurred using 6.5 equiv of sodium methoxide in methanol at reflux for 3 h. Compound **6** was hydrolyzed to **7** with aqueous sodium hydroxide in ethanol at 23 °C for 2.5 h. Reflux of **6** in 1,3-diaminopropane for 3 h provided amine **8**. For the synthesis of inhibitors **10–14** the corresponding carboxylic acids **1**, **2**, and **7** were activated with 1.1 equiv of ethyl chloroformate in the presence of 4.5 equiv of triethylamine in THF at 0 °C. Subsequent reaction of the intermediate mixed anhydrides with 1.0 equiv of the respective amines **3** and **4** furnished the desired dimers. Hydrolysis with aqueous sodium hydroxide in ethanol at 23 °C provided inhibitors **10–14**. For the preparation of **9** amine **4** was

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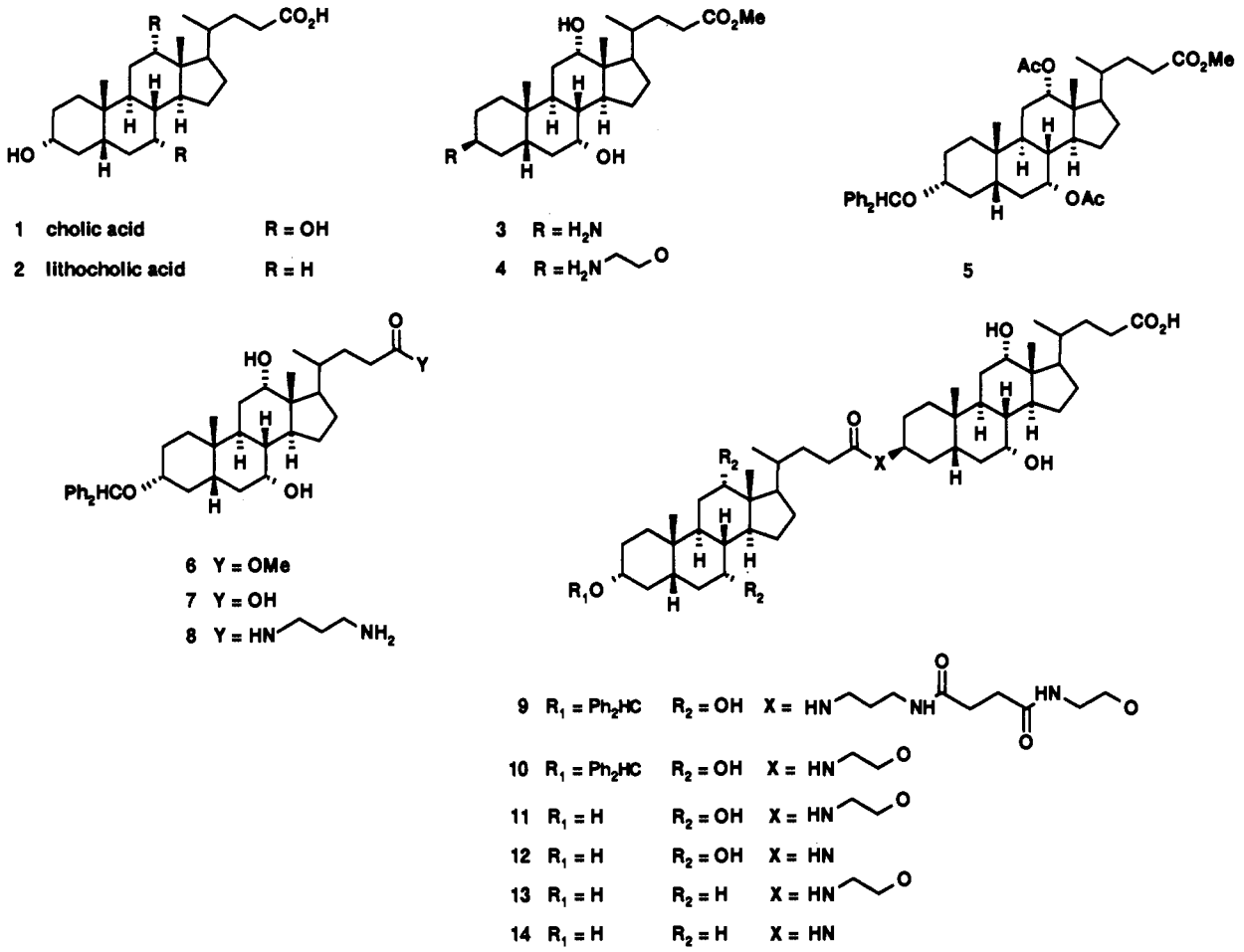

Figure 1. Chemical structures of intermediates and inhibitors.

Table 2. In Situ Ileal Perfusion of Anesthetized Wistar Rats[a]

compd	% inhibition of ileal taurocholate transport[b]		
	12 min	30 min	60 min
9	26 ± 14.5	16 ± 3.7	10 ± 5.7
11	23 ± 18.8	23 ± 13.6	18 ± 8.5
12	51 ± 3.8	59 ± 2.5	54 ± 2.3

[a] Compounds, dissolved at 100 μM in a buffer of 137 mmol of NaCl, 0.9 mmol of $CaCl_2$, 0.51 mmol of $MgCl_2$, 8.1 mmol of Na_2HPO_4, 2.7 mmol of KCl, 1.47 mmol of KH_2PO_4, 1% DMSO, and 1% ethanol were instilled (2 mL) into an 8-cm ileal segment of urethane anesthetized male Wistar rats with bile fistula and recirculated for 60 min at a flow rate of 1 mL·min^{-1}. Bile samples were taken at 12, 30, and 60 min after addition of sodium taurocholate, 3 mmol/L, with a trace amount of [^{3}H]taurocholic acid, and radioactivity was analyzed. [b] Percent inhibition is expressed as

$$\frac{[(\text{recovery of } [^3\text{H}]\text{TCA in bile})\text{control} - (\text{recovery of } [^3\text{H}]\text{TCA in bile})\text{compound}] \times 100}{(\text{recovery of } [^3\text{H}]\text{TCA in bile})\text{control}} \pm \text{SD} \quad n = 3$$

reacted with succinic anhydride in THF in the presence of triethylamine, coupled with amine 8 and hydrolyzed as described for **10–14**.

Interaction of **9–14** 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 of the rabbit[7,8,12] (Table 1). Compounds **9**, **11**, and **12** showed strong interaction with the transporter stronger than cholic acid (**1**) and comparable to taurochenodeoxycholic acid (TCDC), the most potent physiological compound. Examples **10**, **13**, and **14** showed weaker or no interaction. Shortening of the linker in **9** led to **10** with dramatic loss of inhibitory activity. Replacement of diphenylmethyl in **10** by hydrogen gave **11** with considerably increased activity similar to **9**. Replacement of the left-hand cholic acid moiety in **11** and **12** leading to **13** and **14** resulted again in a strongly decreased activity. From these data it can be concluded that the linker moiety between the two bile acids as well as the left-hand bile acid play an important role in the recognition and binding process at the ileal transporter in brush border membrane vesicles.

Compounds **9**, **11**, and **12** were further characterized pharmacologically by in situ ileal perfusion of anesthetized Wistar rats (Table 2). Therefore, an ileal segment was perfused in a closed loop with [^{3}H]taurocholate. The common bile duct was cannulated and radioactivity of bile samples was determined in the presence of the inhibitors. Compounds **9** and **11** exhibit moderate, compound **12** strong, inhibitory activity.

In conclusion, compounds **9**, **11**, and **12** represent the first specific inhibitors of ileal bile acid transporters.

Currently, extensive animal studies in various species are underway to evaluate in vivo the pharmacological profile of this new type of inhibitors on metabolism of bile acids and cholesterol.

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Supplementary Material Available: The procedures for the compounds **9–14** and corresponding analytical data (10 pages). Ordering information is given on any current masthead page.

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