

JOURNAL OF
**MEDICINAL
CHEMISTRY**

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Volume 37, Number 7

April 1, 1994

Communications to the Editor

Specific Inhibitors of Ileal Bile Acid Transport

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Received November 29, 1993

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 sequestrants¹ or surgery² 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 hypocholesterolemic 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

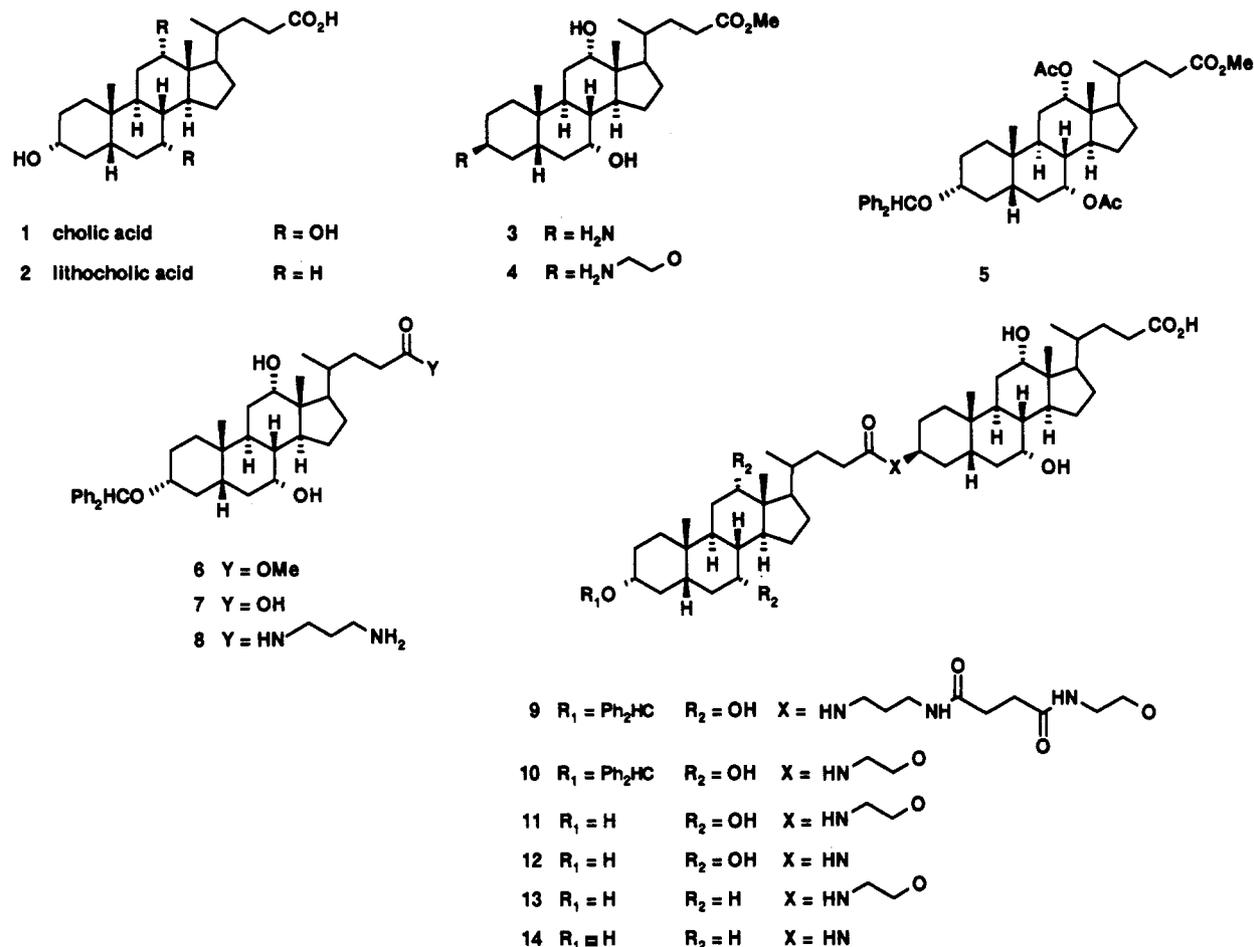
Table 1. Inhibition of Na⁺-Dependent [³H]Taurocholate Uptake into Ileal Brush Border Membrane Vesicles (Rabbit)^{7,8,12}

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 [³H]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 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.

Compounds 9-14 were prepared from readily available starting materials 3, 4,¹⁰ 7, and 8. 3-Mesylocholic 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/CaCO₃ provided 3. For the synthesis of 7 and 8 methyl 7,12-diacetoxycholate¹¹ 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

**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 CaCl₂, 0.51 mmol of MgCl₂, 8.1 mmol of Na₂HPO₄, 2.7 mmol of KCl, 1.47 mmol of KH₂PO₄, 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 [³H]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 [³H]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 [³H]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.

Acknowledgment. We wish to thank F. Girbig, U. Gutjahr, S. Kowalewski, K. Benstein, and H.-J. Thönges for performing biological experiments.

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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