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# Inhibitory Potency of Lithocholic Acid Analogs and Other Bile Acids on Glucuronosyltransferase Activity in a Colon Cancer Cell Line[1-3]

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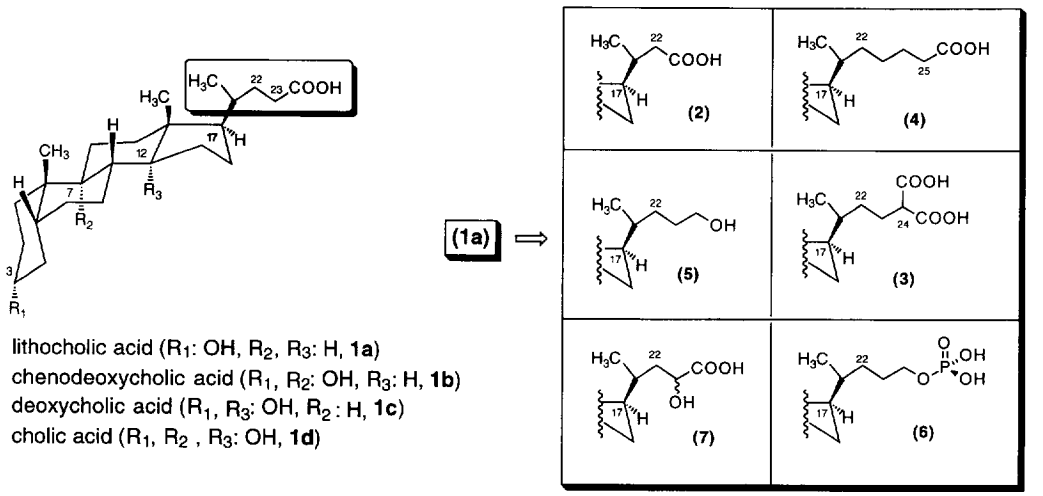
**Summary:** The effectiveness of analogs of lithocholic acid and other bile acids in inhibiting glucuronosyltransferase activity in a colon cancer cell line was measured in order to identify structural features critical for inhibition of the enzyme. Analogs of lithocholic acid with modifications of the side chain (**2-7**) were synthesized, in part, to study their inhibitory effects on glucuronosyltransferase activity. Bishomolithocholic acid (**4**) was synthesized from the iodo derivative (**9**) by a free radical Michael type reaction using *tris*(trimethylsilyl)silane as a free radical mediator.

Colon cancer is thought to arise from the accumulation of mutations in a single cell in the epithelial cell layer of the colon and rectum.[5] High levels of secondary bile acids in the colon caused by high fat diets are thought to play a role in the carcinogenesis process.[6] A hypothesis proposed to account for an increase in the accumulation of mutations caused by colonic bile acids holds that bile acids inhibit xenobiotic-metabolizing enzymes in colonic epithelial cells, with resultant poor defense against mutagenic xenobiotics, and increased mutation frequency.[7] The hypothesis is supported by bile acid inhibition of two enzymes involved in xenobiotic detoxification, glutathione S-transferase[8] and glucuronosyltransferase.[9]

The inhibition studies employed the four major unconjugated fecal bile acids. The order of inhibitory potency for glucuronosyltransferase was lithocholic acid (**1a**) > chenodeoxycholic acid (**1b**) > deoxycholic acid (**1c**) > cholic acid (**1d**).[9] An identical order was obtained for the cytotoxicity of these compounds in colon cancer cell lines,[10] and a similar order was obtained with glutathione S-transferase.[8] However, colonic contents contain additional bile acids, twenty seven in the rat[11, 12] and at least twenty five in humans.[13] Some of these might be more potent inhibitors than lithocholic acid (**1a**), and the identification of such inhibitors would be of interest. Some of the minor bile acids are not readily available. In order to identify structural features that lead to high inhibitory potency, a structure-inhibition study was carried out for glucuronosyltransferase. The study included analogs of lithocholic acid with modified side chains

that have not been synthesized previously. This communication describes the synthesis of analogs of lithocholic acid having modifications in the side chain and their role in the inhibition of glucuronosyltransferase as well as inhibition studies with other commercially available dihydroxy and trihydroxy bile acids.




lithocholic acid ($R_1$: OH, $R_2$, $R_3$: H, **1a**)
chenodeoxycholic acid ($R_1$, $R_2$: OH, $R_3$: H, **1b**)
deoxycholic acid ($R_1$, $R_3$: OH, $R_2$: H, **1c**)
cholic acid ($R_1$, $R_2$ , $R_3$: OH, **1d**)

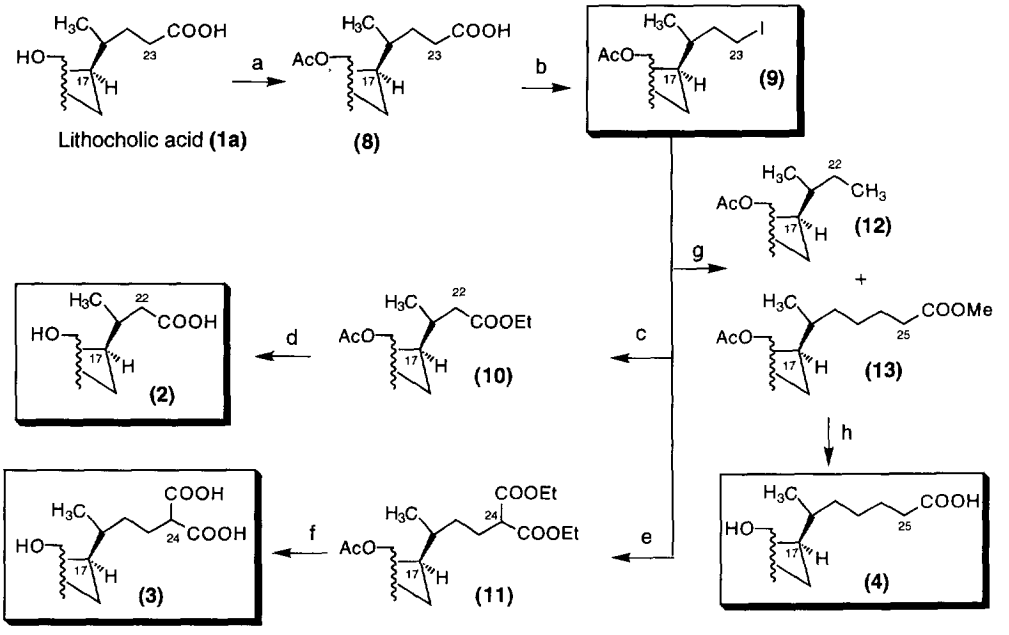
**Figure 1**

*Chain shortening of lithocholic acid (**1a**) by one unit:* The iodo derivative **(9)** was prepared in 84 % isolated yield by the irradiation of a refluxing solution of the acetate derivative of lithocholic acid **(8)**, in the presence of iodosobenzene diacetate (IBDA) and $I_2$ using a 200W tungsten filament lamp followed by the purification over silica gel.[14] The structure of **9** was confirmed by $^1$H-NMR, $^{13}$C-NMR, MS (CI and thermospray).[15] Oxidation of **9** by $RuCl_4/H_5IO_6$ to the carboxylic acid derivative[16] followed by esterification and purification over silica gel gave **10** in 72% yield. Hydrolysis of **10** using 1N NaOH/aq EtOH under refluxing conditions gave norlithocholic acid **(2)** in 90% yield **(Scheme 1)**.

*Chain lengthening of lithocholic acid (**1a**) by one carbon unit to form dicarboxylic acid derivative (**3**)*: The anion of diethyl malonate (NaH, THF) on reaction with the iodo derivative **(9)** under warming conditions (40 °C), followed by purification over silica gel, gave **11** in 60% isolated yield (80% based on the recovered starting material).[15] **11**, on hydrolysis of both the ester groups and the deprotection of the acetate group, was further converted to α-carboxyhomolithocholic acid **(3)** in 85% yield **(Scheme 1).**

*Chain lengthening of lithocholic acid (**1a**) by two carbon units to form bishomolithocholic acid (**4**):* The iodo derivative **(9)** was subjected to free radical Michael type reaction with the methyl ester of acrylic acid.[17-19] Thus, **9** (1.0 eq), methyl acrylate (1.2 eq),

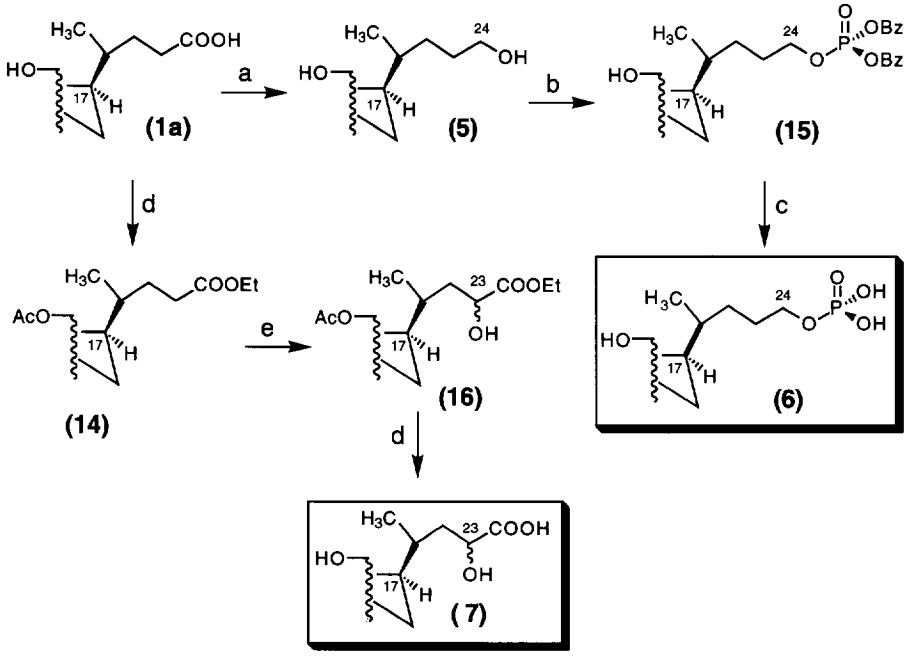
*tris*(trimethylsilyl)silane (TTMSS, 1.2 eq) as a free radical mediator, and AIBN (free radical initiator, 5 mol%) under nitrogen atmosphere at 70 °C in toluene gave **12** (20%) and **13** (64%), respectively, after purification over silica gel.[15] The reaction is very clean and the products are easy to purify over silica gel. Bishomolithocholic acid (**4**) was finally obtained under the deprotection conditions (1N NaOH, aq MeOH, reflux) in 91% yield (**Scheme 1**). *To our knowledge, chain lengthening by free radical Michael type approach and also using silane derivative as a free radical mediator to steroid acids has never been reported earlier.*




**Scheme 1:** (**a**) $Ac_2O$, pyridine, room temperature; (**b**) IBDA, $I_2$, $CCl_4$, 200W tungsten filament lamp, reflux; (**c**) $RuCl_4$, $H_5IO_6$, $CCl_4$, $CH_3CN$, $H_2O$, room temperature; EtOH, *p*TSA, ref;ux; (**d**) 1N NaOH, aq EtOH, reflux, 5h; $H^+$; (**e**) $CH_2(COOEt)_2$ (1.2 eq), NaH (2.0 eq), THF, 40 °C, 3.5 hour; (**f**) 1N NaOH, MeOH, rt, 15 h; $H^+$; (**g**) HC=CHCOOMe (1.2 eq), TTMSS (1.2 eq), AIBN (5 mol %), toluene, 70 °C; (**h**) 1N NaOH, aq MeOH, reflux, 5h; $H^+$.

*Incorporation of the phosphate group in the side chain:* The ethyl ester of lithocholic acid on reduction with lithium aluminum hydride gave the corresponding alcohol (**5**) in 85 % yield. The O-benzylated phosphate derivative of **5** was prepared in 65% isolated yield by the derivatization of the primary alcohol using $(BzO)_2(O)POP(O)(OBz)_2$, NaH, THF, room temperature followed by purification over silica gel (**Scheme 2**). Debenzylation (OBz → OH) on hydrogenation using 10% Pd/C as a catalyst gave **6** in quantitative yield.

*α-Hydroxylation of the side chain of lithocholate ester derivative, 14:* The ethyl ester derivative of O-acetyl-lithocholic acid **(14)** was treated with lithium diisopropylamine (LDA) and *trans*-2-(phenylsulfonyl)-2-phenyloxaziridine (Davis' reagent)[20] to introduce an hydroxyl group α- to the carboxylic ester as a mixture of epimers. The diastereomers were separable by silica gel flash column chromatography (absolute assignment of the stereochemistry has not been made).[15] Each isomer was subjected to deprotection using 1N NaOH in ethanol followed by acidic work-up yielding both of the isomers of α-hydroxylithocholic acid **(7)** **(Scheme 2).**




**Scheme 2**: **(a)** EtOH, *p*TSA, reflux; lithium aluminum hydride, $Et_2O$, room temperature; **(b)** $(BzO)_2(O)POP(O)(OBz)_2$, NaH, THF, room temperature; **(c)** $H_2$, 10% Pd-C, 95% EtOH; **(d)** $Ac_2O$, pyridine, room temperature; EtOH, *p*TSA, reflux; **(e)** LDA, 78 °C, THF, Davis' reagent; **(f)** 1N NaOH, aq MeOH, room temperature.

**Results and Discussion:** The inhibition of glucuronosyltransferase activity in intact cells of the colon cancer cell line HT-29 by the bile acids under study was measured as described earlier.[9] Lithocholic acid was the most potent inhibitor **(Table 1)**. All changes in structure decreased the inhibitory potency. Among the monosubstituted compounds with normal side chains, orientation of A and B rings, unsaturation, in the A ring or replacement of the hydroxyl groups by a ketone had relatively small effects on inhibitory potency. A factor in the poor inhibitory potency of 5β-cholanic acid might be its low solubility; the compound precipitated at the nominal 5μM concentration used with all of the bile acids.

Among the disubstituted acids, keto derivatives were more inhibitory than the hydroxy analogs (5β-cholanic acid-3α-ol,6-one, 5β-cholanic acid-3α,6α-diol and 5β-cholanic acid-3α–ol,7-one, 5β-cholanic acid-3α,7α-diol. Modification of the lithocholic acid side chain invariably decreased inhibitory potency. Relatively small changes were caused by introducing a hydroxyl group in the side chain. Notably, the α-carboxyhomolithocholic acid **(3)** was a less potent inhibitor than norlithocholic acid **(2)** and bishomocholic acid **(4)**.

The greater inhibitory potency of lithocholic acid, as well as previous studies,[9] are in accord with the view that lithocholic acid plays an important role in the pathological effects associated with inhibition of glucuronosyltransferase by bile acids in the colon.

**Table 1:** Effect of bile acids (5 μM) on the inhibition of glucuronosyltransferase activity in the colon cancer line HT-29

| **Bile acid type** | **Bile acid** | **Activity remaining (%) and S. D. (N = 3)** |
|---|---|---|
| *monosubstituted, normal side chain* | 5β-cholenic acid-3β-ol | 39.1 ± 1.2 |
| | 5β-cholenic acid-3α-ol | 41.4 ± 0.9 |
| | 5β-cholanic acid-3α-ol **(1a)** | 25.3 ± 2.2 |
| | 5β-cholanic acid-3β-ol | 86.9 ± 7.7 |
| | 5α-cholanic acid-3β-ol | 46.8 ± 1.0 |
| | 5β-cholanic acid-3-one | 46.0 ± 4.0 |
| | 5β-cholanic acid | 92.01 ± 6.25 |
| *disubstituted, normal side chain* | 5β-cholanic acid-3β,12α-diol | 46.4 ± 9.2 |
| | 5β-cholanic acid-3α,7α-diol **(1b)** | 38.4 ± 1.1 |
| | 5β-cholanic acid-3α,12α-diol **(1c)** | 48.0 ± 2.3 |
| | 5β-cholanic acid-3α,6α-diol (hyodeoxycholic acid) | 75.2 ± 4.2 |
| | 5β-cholanic acid-3α,7β-diol (ursodeoxycholic acid) | 73.3 ± 5.0 |
| | 5β-cholanic acid-3α,7α,12α-triol **(1d)** | 98.3 ± 2.1 |
| | 5β-cholanic acid-3α,6α,7α-triol | 100.0 ± 0 |
| | 5β-cholanic acid-3α,6β,7β-triol | 78.5 ± 3.0 |
| | 5β-cholanic acid-3α,6β,7α triol | 73.9 ± 5.9 |
| | 5β-cholanic acid-3α-ol-6-one | 28.1 ± 5.5 |
| | 5β-cholanic acid-3α-ol-7-one | 51.1 ± 7.9 |
| | 5β-cholanic acid-3α-ol-12 one | 36.1 ± 7.1 |
| | 5β-cholanic acid-3α-ol -7,12- dione | 100.0 ± 0 |

| | | |
|---|---|---|
| *monosubstituted,* | norlithocholic acid **(2)** | 43.1 ± 2.7 |
| *modified side-chain* | α-Carboxyhomocholic acid **(3)** | 74.3 ± 6.5 |
| | bishomolithocholic acid **(4)** | 41.9 ± 6.3 |
| | **(5)** | 72.8 ± 5.5 |
| | **(6)** | 74.0 ± 9.2 |
| | **(7,** separate diastereomers) | 34.9 ± 6.7 (55.4 ± 5.4) |
| | **(7,** mixture) | 40.0 ± 5.2 |

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