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A Novel Class of Apical Sodium Co-Dependent Bile Acid Transporter Inhibitors: The 2,3-Disubstituted-4-Phenylquinolines

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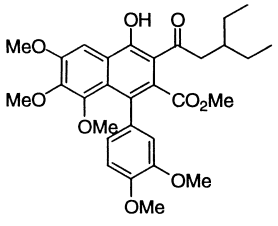
Abstract—A series of 2,3-disubstituted-4-phenylquinolines were prepared and were found to inhibit the apical sodium co-dependent bile acid transporter (ASBT). Alkyl and ester substitution at the 3-position showed comparable activities while substitution at the 2-position was much more sensitive to the nature of the substituent. The synthesis and in vitro potency data are presented for this novel class of compounds.

Bile acids are synthesized from cholesterol in the liver and are excreted in the bile. The apical sodium co-dependent bile acid transporter (ASBT) promotes re-absorption of bile acids from the intestinal tract into the enterohepatic circulation. Prevention of bile acid re-absorption has been implicated in the lowering of cholesterol levels. Elevated plasma low density lipoprotein (LDL) cholesterol levels are a major risk factor for arteriosclerosis and coronary heart disease.[1,2]

Current therapies to treat hyperlipidemia include the use of bile acid sequestrants to prevent absorption of bile acids into the circulation and statins acting as HMG-CoA reductase inhibitors. Sequestrants suffer from poor patient compliance due to their large doses and non-palatability.[3]

Partial surgical removal of the ileum was reported to cause a lowering of plasma LDL cholesterol levels indicating an ASBT inhibitor may be an effective therapy to reduce serum cholesterol levels.[4] An ASBT inhibitor would lower plasma cholesterol levels by raising the amount of bile acids excreted in the feces creating a deficiency of bile acids causing the further conversion of cholesterol to bile acids. An ASBT inhibitor would have a physiological response similar to a bile acid sequestrant while eliminating its non-palatability.

A number of substituted aryl naphthols have been reported to inhibit ASBT. Hara and co-workers reported the keto ester substituted naphthol **1** as an ASBT inhibitor.[5,6] Kondo and co-workers reported the structurally similar diester naphthol **2** was effective at lowering total serum cholesterol levels.[7,8] Based upon these reports we sought to prepare the isoelectronic quinolines **3** and quinoline *N*-oxides **4** in an effort to prepare a more potent therapeutically useful ASBT inhibitor.



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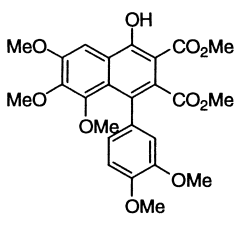

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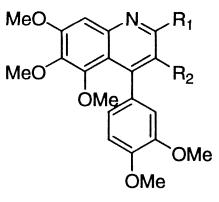

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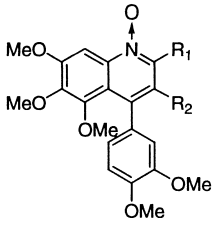

4

The benzophenone **6** was used as a common intermediate for all analogues prepared below (Scheme 1). 3,4,5-Trimethoxyaniline was protected as the carbamate **5**. Treatment of carbamate **5** with 2 equiv of *t*-butyl lithium in THF followed by trapping with the appropriate ester followed by removal of the Boc group with trifluoroacetic acid afforded benzophenone **6.**

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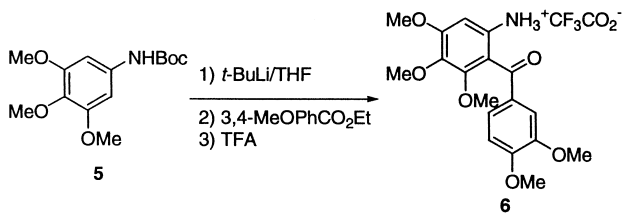
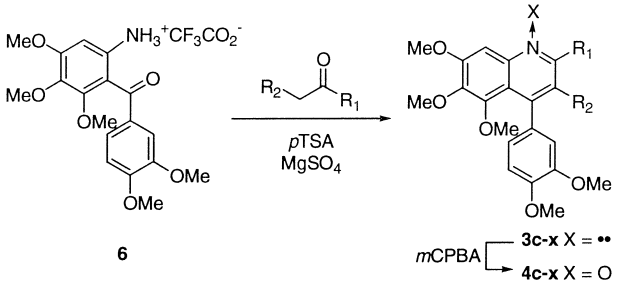


Scheme 1. Preparation of ketone **6**.



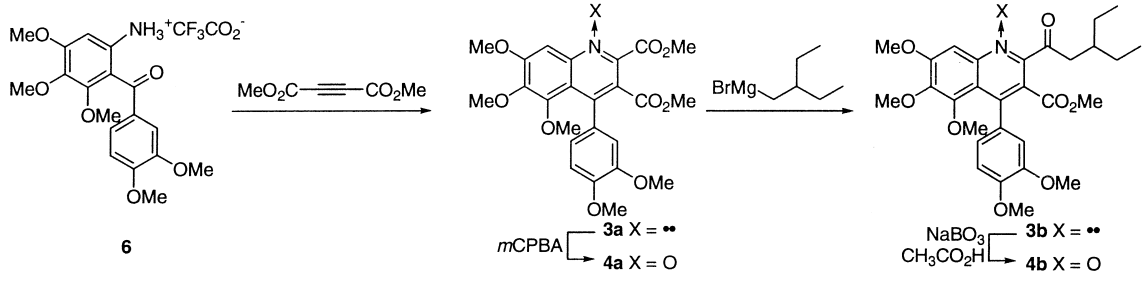
Scheme 3. Preparation of quinolines **3** and quinoline *N*-oxides **4**.

It was first sought to synthesize compounds **4a** and **4b** as the quinoline *N*-oxide isoelectronic equivalents to the aryl naphthols **1** and **2** (Scheme 2). Reaction of **6** with dimethyl acetylenedicarboxylate afforded the diester quinoline **3a**. Oxidation of **3a** with *m*-CPBA afforded the diester quinoline *N*-oxide **4a**. To prepare the quinoline similar to **1** we treated **3a** with the appropriate Grignard and obtained selective addition to the more electron deficient ester to afford the quinoline keto ester **3b**. Treatment of **3b** with *m*CPBA was not successful; however, the use of sodium perborate in acetic acid afforded the quinoline *N*-oxide **4b**.

The in vitro activity was determined by measuring the uptake of [^{14}C]-taurocholate in baby hamster kidney cells transfected with the cDNA of human ASBT (H14 cells).[9] Naphthol **1** had an $IC_{50} = 2.55$ μM. The isoelectronic quinolines **3a**, **3b**, **4a** and **4b** showed little to no inhibition of ASBT (see Table 1).

It was decided to make a series of ester, ketone, alcohol, alkyl and aryl substituted quinolines. Preparation of the remaining quinoline analogues (**3**) involved the straightforward condensation of a number of ketones, keto esters and diesters with the keto aniline **6** (Scheme 3). Treatment of **3** with *m*-CPBA or sodium perborate afforded the respective quinoline *N*-oxides (**4**).

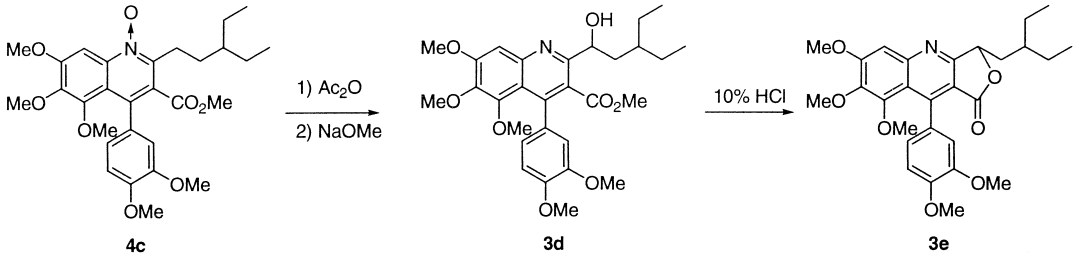
The α-hydroxy quinoline **3d** and lactone **3e** were prepared from the quinoline *N*-oxide **4c** (Scheme 4). Treatment of **4c** with acetic anhydride transfers the *N*-oxide



Scheme 2. Preparation of diester **3a** and **4a** and ketoester **3b** and **4b**.

Table 1. In vitro assay of quinolines that inhibit ASBT-mediated uptake of [^{14}C]-taurocholate

Compound	R_1	R_2	X	IC_{50} (μM)	% Inhibition @ 50 μM
3a	CO_2Me	CO_2Me	••		0
4a	CO_2Me	CO_2Me	O		22
3b	$C(O)CH_2CHEt_2$	CO_2Me	••		35
4b	$C(O)CH_2CHEt_2$	CO_2Me	O		0
3c	$CH_2CH_2CHEt_2$	CO_2Et	••	13.3	
4c	$CH_2CH_2CHEt_2$	CO_2Et	O		32
3d	$CH(OH)CH_2CHEt_2$	CO_2Et	••		0
3e	$-CH(CH_2CHEt_2)O_2C-$		••		34
3f	*i*-Pr	CO_2Et	••	4.4	
3g	Et	Me	••	7.1	
4g	Et	Me	O		36
3h	$CH_2CH_2CHMe_2$	H	••	9.2	
3i	*i*-Pr	H	••	10.8	
3j	*i*-Pr	Me	••	21.4	
3k	$-CH_2CH_2CH_2CH_2-$		••		50
3l	*n*-Bu	Me	••		37
3m	Me	CH_2CHMe_2	••		31
3n	Ph	Me	••	3.5	
3o	*p*-NO_2Ph	CO_2Et	••		12
3p	*p*-NH_2Ph	CO_2Et	••	24.7	
3q	*p*-FPh	CO_2Me	••	9.3	
3r	*p*-FPh	H	••		49



4c 3d 3e

Scheme 4. Preparation of lacton **3e**.

oxygen to the α-position on the alkyl side chain. The acetate group was removed by treatment with sodium methoxide to produce **3d**. The lactone **3e** can be formed by subsequent treatment of **3d** with 10% hydrochloric acid.

The quinoline *N*-oxides (**4c** and **4g**) exhibited a slight loss of activity when compared to the corresponding quinolines (**3c** and **3g**). Placement of an oxygen at the α-position, as in the alcohol **3d** and the lactone **3e,** also resulted in the reduction of in vitro activity.

Alkyl and phenyl substituents are well tolerated at the R_1 position. Good activities were observed with *i*-propyl (**3f**, **i**, **j**), ethyl (**3g**) and *i*-pentyl (**3h**)**.** The 2-phenyl substituted quinoline **3n** was found to be the most potent compound with an $IC_{50} = 3.5$ μM. Some electronic effects are apparent on the 2-phenyl ring where the electron deficient nitro group reduces potency with respect to an amino substitution (**3o** versus **3p**).

Substitution at the R_2 group tolerated an ester, a methyl or a proton. When $R_1 = i$-Pr the order of activity at R_2 was **3f**(CO_2Et) >**3i**(H) >**3j**(Me) and when $R_1 = p$-FPh it was **3q**(CO_2Me) >**3r**(H) indicating that an electron deficient R_2 substituent may be beneficial.

It is evident that a number of substitutions at the R_1 and R_2 positions for this class of quinolines are well tolerated for producing a potent ASBT inhibitor. The oxygen substitution at the quinoline nitrogen or α-carbon of the 2-alkyl group is not tolerated and resulted in a loss of activity. A variety of alkyl and aryl substituents at the R_1 position while substituting R_2 with a hydrogen, methyl or ester produces 4-arylquinolines with good in vitro potencies. This work is a positive step toward the goal of producing a novel ASBT inhibitor to lower plasma cholesterol levels and more work needs to be done.

References and Notes

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9. H-14 cells expressing functional human ASBT (H-14 cells) were grown in T150 tissue culture flasks in DMEM (HG) media. The cells were trypsinized and plated into opaque white 96-well tissue culture plates at a density of 6×10^4 cells per well. Naïve BHK cells were seeded at the same density and run in the assay as a background control. After attaching and growing for 24 h, the culture media was decanted and the cells washed with 200 μL/well assay buffer consisting of Hanks' balanced salt solution containing 25 mM HEPES, pH 7.4, and 0.1% bovine serum albumin (BSA). Test compounds were prepared as a 50 mM stock solution in dimethyl sulfoxide (DMSO) and diluted to the required concentration in assay buffer. A 100 μL of assay buffer containing 5 μM [14C]taurocholic acid and the indicated concentration of test compound was added to each well. Each concentration was tested in triplicate wells. Control wells contained 0.1% DMSO in assay buffer, the final concentration of DMSO in the wells containing test compounds. The 96-well plate was incubated at 37 °C for 2 h in a humidified incubator of 7.5% CO_2. After the incubation period, the plates were decanted and each well washed twice with ice-cold phosphate-buffered saline (PBS) containing 0.1% fatty-acid free BSA followed by one time with straight PBS. Each well received 200 μL Microscint-20 and a clear plastic heat seal was placed over the top of the plate. The radioactivity in each well was counted in a Packard TopCount scintillation counter.