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A novel class of apical sodium-dependent bile acid transporter inhibitors: the amphiphilic 4-oxo-1-phenyl-1,4-dihydroquinoline derivatives

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Abstract—A series of 4-oxo-1-phenyl-1,4-dihydroquinolines possessing a linker and an ammonio moiety were synthesized and found to inhibit the apical sodium-dependent bile acid transporter (ASBT). The potency of ASBT inhibition varied with the position and length of the linking tether. Compound **21e** effectively lowered the total serum cholesterol levels in hamsters.

1. Introduction

Bile acid sequestrants (BAS) such as anion exchanging resins have been used to treat hypercholesterolemia and hyperlipidemia with a good record of safety for over 30 years.[1] Even since the introduction of the prevalent therapy with HMG-CoA reductase inhibitors (statins), BASs have remained useful in some patients such as young children and fertile women due to the potential risks posed by systemic exposure to the alternative lipid-altering drugs. Furthermore, the combination therapy with statin and BAS is often required for patients with severe dyslipidemia. In spite of these merits of BAS, several demerits can lead to its therapeutic failure, including poor patient compliance due to high dosage, unpalatability, and gastrointestinal symptoms, and altered absorption of vitamins, minerals, and co-administered drugs.[2] For this reason, physicians have hoped for an alternative method for bile acid sequestration.

The cloning and characterization of apical sodium-dependent bile salt transporter (ASBT/SLC10A2),[3] otherwise known as ileal bile acid transporter (IBAT), greatly accelerated the development of specific inhibitors of bile acid reabsorption, potentially a new class of plasma cholesterol lowering drugs. Several types of compounds have been identified as candidates for ASBT inhibitors.[4] Researchers at Shionogi reported the substituted naphthol **1**(S-8921)[5] as an ASBT inhibitor and showed that **1** was able to lower the total serum cholesterol levels in animals.[6] Evaluation of S-8921 and S-8921 glucuronide **2** (one of the metabolites of **1**[7]) for ASBT inhibitory activity by the hamster ileal ring method revealed that S-8921 glucuronide ($IC_{50} = 2.8$ μM) has higher potency than S-8921 in inhibiting ASBT ($IC_{50} > 185$ μM). Similarly, Searle's ASBT inhibitor **3**[8] ($IC_{50} = 0.89$ μM) was found to be more efficacious than **4** ($IC_{50} > 63$ μM). These findings suggest that the amphiphilic property of these compounds (**2** and **3**) plays an important role in effective ASBT inhibition. As we have been working on the synthesis of 4-oxo-1-phenyl-1,4-dihydroquinolines (e.g., **5**, **6**) as ASBT inhibitors, we decided to prepare 4-oxo-1-phenyl-1,4-dihydroquinolines possessing a linker and an ammonio moiety in order to enhance the ASBT inhibitory activity. We report here the synthesis of a series of novel amphiphilic 4-oxo-1-phenyl-1,4-dihydroquinolines and their biological activities.

2. Chemistry

In the course of our ASBT inhibitor research program at Sankyo, we discovered that a series of 4-oxo-1-phenyl-1,4-dihydroquinolines inhibited ASBT, and further, that the potency of these dihydroquinolines was very sensitive to both the position and nature of the

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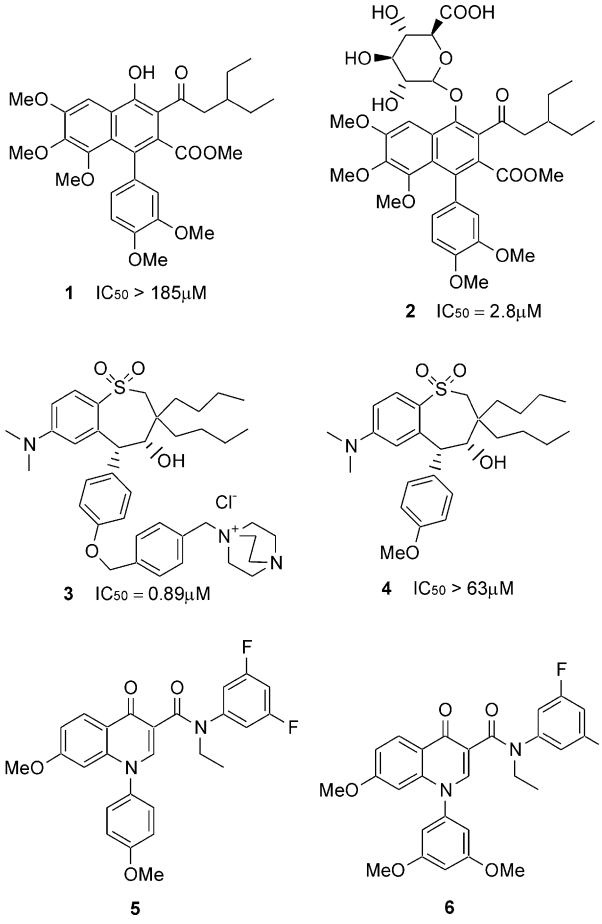
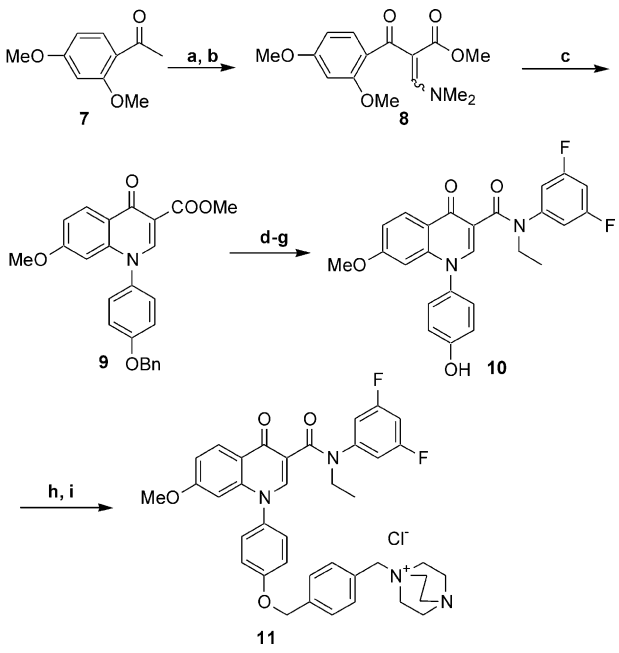


substituent (data not shown). As a result of optimization of the substituents, compounds such as **5** and **6** were found to exhibit potent ASBT inhibitory activity in vitro. Consequently, we first sought to replace the methyl of the methoxy group in **5** and **6** with a linker and an ammonio moiety in order to preserve the optimized substituents.

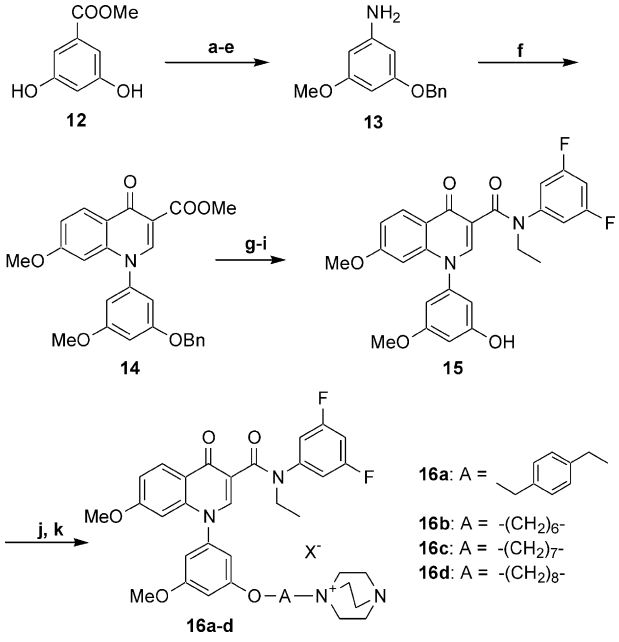
Based on the structures of **3** and **5**, we designed **11** and synthesized it by the process shown in Scheme 1.[9]

Methoxycarbonylation of **7** and subsequent condensation with *N*,*N*-dimethylformamide dimethyl acetal were carried out to give **8**, which was treated with 4-benzyloxyaniline and cyclized to furnish **9**. In this cyclization reaction, the methoxy group was found to work as a good leaving group, and this methodology greatly facilitated preparation of 4-oxo-1-phenyl-1,4-dihydroquinoline derivatives. *N*-Ethyl *N*-(3,5-difluorophenyl) amide was then installed through a conventional saponification/amidation/ethylation sequence, followed by deprotection of benzyl ether under a hydrogenation condition to give **10**. Etherification of **10** with α,α′-dichloro-*p*-xylene in the presence of K_2CO_3 gave the corresponding ether, which was treated with DABCO in acetonitrile to afford the desired ammonium compound **11**.

Next, based on the structures of **3** and **6**, we designed **16a**, and started the synthesis from methyl 3,5-dihydroxybenzoate **12** (Scheme 2).

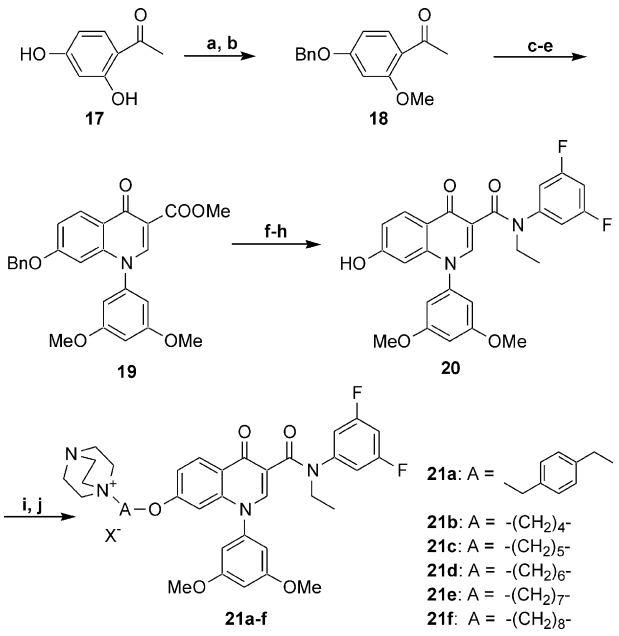


Scheme 1. (a) NaH, $CO(OMe)_2$ (79%); (b) $Me_2NCH(OMe)_2$ (68%); (c) 4-benzyloxyaniline, K_2CO_3, DMF (27%); (d) aq NaOH, EtOH (94%); (e) ClCOO*i*-Bu, Et_3N, 3,5-difluoroaniline (quant); (f) NaH, EtI, DMF (83%); (g) H_2/Pd–C, EtOH (95%); (h) α,α′-dichloro-*p*-xylene, K_2CO_3, DMF (54%); (i) DABCO, MeCN (75%).



Scheme 2. (a) NaH, BnBr, DMF (39%); (b) K_2CO_3, MeI, DMF (96%); (c) aq NaOH, EtOH (95%); (d) DPPA, Et_3N, toluene, *t*-BuOH (90%); (e) HCl–AcOEt (78%); (f) **8**, K_2CO_3, DMF (64%); (g) aq NaOH, MeOH (97%); (h) $(COCl)_2$, *N*-ethyl 3,5-difluoroaniline (62%); (i) H_2/Pd–C, EtOH (92%); (j) alkylene dihalide, NaH, DMF (31–82%); (k) DABCO, MeCN (77–85%).

Monobenzylation and subsequent methylation of **12** provided the resulting ether, which was subjected to saponification and Curtius rearrangement using DPPA to give **13**. Treatment of **13** with **8** in DMF followed by cyclization led to **14** in moderate yield. Saponification of **14** followed by amidation with *N*-ethyl 3,5-difluoroaniline via acid chloride gave an amide, and removal of the



Scheme 3. (a) K_2CO_3, BnBr, acetone (90%); (b) K_2CO_3, MeI, DMF (98%); (c) NaH, $CO(OMe)_2$ (86%); (d) $Me_2NCH(OMe)_2$; (e) 3,5-dimethoxyaniline, K_2CO_3, DMF (73% for two steps); (f) aq NaOH, EtOH (quant); (g) $(COCl)_2$, *N*-ethyl 3,5-difluoroaniline (97%); (h) H_2/Pd–C, EtOH (quant); (i) alkylene dihalide, K_2CO_3, DMF (67–97%); (j) DABCO, MeCN (53–81%).

Table 1. In vitro assay of compounds that inhibit IBAT-mediated uptake of [^{3}H]-taurocholate

Compd	X^-	IC_{50} (μM)	% inhibition @ 30 μg/mL	mp
3		0.89		
5			64	194
6			64	193
11	Cl^-		41	182
16a	Cl^-		71	167
16b	I^-		83	147
16c	Br^-		86	Foam
16d	Br^-		83	Foam
21a	Cl^-		71	180–182
21b	I^-		24	146–149
21c	I^-		67	137–140
21d	I^-		88	130–133
21e	Br^-	0.77	100	130–132
21f	Br^-	1.07	94	128–130

benzyl protecting group provided **15**. Etherification of **15** with α,α′-dichloro-*p*-xylene and subsequent treatment with DABCO afforded the desired **16a**. In order to investigate SAR, carbon chain analogues **16b–d** were also prepared by employing 1,6-diiodohexane, 1,7-dibromoheptane and 1,8-dibromooctane as linkers.

Finally, compounds **21a–f** were designed and synthesized as outlined in Scheme 3. Selective monobenzylation successfully proceeded to give a monobenzyl ether, and subsequent methylation provided **18** in good yield. Compound **18** was converted to **21a–f** under the same conditions as those described above.

All the compounds synthesized here have no chiral centers, and their structures are so simple that they can be easily prepared even on a large scale.

3. Results and discussion

ASBT inhibitory activities of the synthesized compounds are summarized in Table 1. The in vitro activity was determined by measuring the uptake of [^{3}H]-taurocholate in hamster ileal rings.[10]

Although compound **11** was initially thought to be structurally close to **3** and expected to inhibit ASBT potently, it turned out to be a relatively weak inhibitor of ASBT. On the other hand, **16a** was actually more potent than the non-amphiphilic **6**. Substitution of the linker of **16a** with C6–C8 chain linkers led to an additional improvement in ASBT inhibition, although the results did not suggest clear relationships between ASBT inhibition and the length of the tether. Compound **21a** prepared by modification at the 7 position of the quinoline ring showed slightly improved potency compared to **6**. In the carbon chain linker analogues (**21b–f**), the potency was apparently dependent on the length of the linker. The heptamethylene derivative **21e** exhibited an optimal improvement and was found to have $IC_{50} = 0.77$ μM.

We next assessed the cholesterol-lowering effect of **21e** in hamsters.

Table 2 shows the effects of Compound **21e** on serum total cholesterol, non HDL-cholesterol, HDL-cholesterol,

Table 2. Effect of compound **21e** on serum lipids in hamsters

Group	Dose (mg/kg)	TC (mg/dL)	Non HDL-C (mg/dL)	HDL-C (mg/dL)	Ratio: non HDL-C/HDL-C	TG (mg/dL)
Vehicle	0	183.4±1.0	103.5±3.4	79.9±3.6	1.3±0.1	301.7±28.7
Compd **21e**	0.3	157.4±7.5*	75.7±6.5**	81.8±4.2	0.9±0.1	142.7±37.0**
	1.0	166.9±3.8	85.2±1.5	81.7±3.9	1.1±0.1	249.3±26.4
	3.0	174.3±4.7	94.1±4.4	80.2±3.2	1.2±0.1	270.8±48.4
	10.0	144.1±6.6***	74.3±6.1**	69.8±3.2	1.1±0.1	217.6±30.6
	30.0	142.8±7.1***	78.9±5.4*	63.9±4.6*	1.3±0.1	235.0±24.4
	100.0	118.9±7.8***	57.0±7.1***	61.9±2.0**	0.9±0.1*	217.7±32.2

Each value represents the mean±SEM ($n = 3$–5/group). *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$, as compared with the vehicle-treated group using Dunnett's Test.

the cholesterol ratio, and triglycerides in chow-fed male Syrian golden hamsters following 14 days of treatment.[11] Compound **21e** treatment reduced serum total cholesterol by 35%, non HDL-cholesterol by 45%, HDL-cholesterol by 23%, the cholesterol ratio by 30% maximum. Compound **21e** significantly reduced triglycerides without apparent dose-dependency. Compound **21e** did not affect the body weights of any of the groups throughout the treatment (data not shown). Accordingly, compound **21e** was concluded to be useful for lipid lowering and improving the cholesterol ratio in the blood. The biological activities of the other derivatives will be reported in a full account in due course.

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9. All new compounds are fully characterized by their spectroscopic and analytical data.
10. The freshly obtained ileum segment from a hamster (Syrian, male, 5–7 weeks old; Japan SLC, Inc.) was everted by a stainless steel rod and washed in ice-cold oxygenated Krebs Ringer solution (115 mM NaCl, 5.9 mM KCl, 1.2 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 2.5 mM $CaCl_2$, 1.2 mM Na_2SO_4 and 25 mM $NaHCO_3$, pH = 7.4). The everted ileum was divided into about 5-mm pieces, which were randomized into several groups based on the gradient of ASBT activities. Next, tissue pieces were incubated for 5 min at 37 °C in oxygenated Krebs Ringer solution supplemented with 37 μM [^{3}H]-taurocholate (TCA, 74 GBq/mmol; NEN/Perkin–Elmer), 2 mg/mL bovine serum albumin (BSA, essential fatty acid free; Sigma) and 30 μg/mL of each test compound. Nonspecific uptake was measured by the addition of 37 mM of non-radiolabeled TCA. After incubation, the pieces were washed 3 times in ice-cold Krebs Ringer solution containing 2 mg/mL BSA, weighted, and solubilized in NCS-II tissue solubilizer (Amersham Pharmacia). Thereafter, each radioactivity (dpm) was determined in a liquid scintillation counter. TCA-uptake was corrected for nonspecific counts and expressed as dpm/mg of wet weight. Values obtained for the test compounds were the mean of triplicate incubations.
11. Compound **21e** was administered daily at doses of 0.3, 1, 3, 10, 30, and 100 mg/kg/day to chow-fed male Syrian golden hamsters on 14 consecutive evenings by oral gavage in 0.5% carboxymethylcellulose (dosing vehicle). The animals were allowed free access to rodent chow and water throughout the study. The animals were weighed daily and sacrificed on the morning after the 14th dose. Blood serum was assayed for serum triglycerides (TG), total cholesterol (TC), and lipoprotein cholesterol profiles; very low density lipoprotein (VLDL) plus low density lipoprotein (LDL), high density lipoprotein (HDL) cholesterol, and the ratio of non HDL-cholesterol (VLDL plus LDL cholesterol) to HDL-cholesterol.