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A Novel Class of Apical Sodium Co-dependent Bile Acid Transporter Inhibitors: The 1,2-Benzothiazepines

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Abstract—A series of 5-aryl-3,3-dibutyl-7-(dimethylamino)-1,2-benzothiazepin-4-ol 1,1-dioxides were prepared and were found to inhibit the apical sodium co-dependent bile acid transporter (ASBT) for the potential treatment for hyperlipidemia. Several 1,2-benzothiazepines exhibited low nanomolar in vitro activity. The synthesis and initial in vitro potency data is presented for this novel class of compounds.

Elevated serum low density lipoprotein (LDL) cholesterol levels have been proven to be a major risk factor for the development of arteriosclerosis and coronary heart disease.[1,2] Current therapies to treat hyperlipidemia include the use of niacin, bile acid sequestrants, fibric acid derivatives, HMG-CoA reductase inhibitors and cholesterol absorption inhibitors. The statins represent the HMG-CoA reductase inhibitors, which inhibit the synthesis of cholesterol in vivo while ezetimibe represents the inhibitors of intestinal cholesterol absorption. Statin therapy requires that liver enzymes must be periodically monitored due to potential liver side effects from this therapy.[3]

The normal enterohepatic circulation of bile acids is an important component in lipid homeostasis.[4] The liver produces bile that is excreted into the intestines. The ileum will reabsorb a portion of these bile acids for transport back to the liver. The liver uses cholesterol to synthesize bile acids in order to keep the enterohepatic circulation in balance. Bile acid sequestrants bind to bile acids in the intestine preventing re-absorption. The deficiency of bile acid causes the liver to convert cholesterol to bile acids thereby lowering serum LDL-C levels. Sequestrant therapy suffers from poor patient compliance due to the large doses required and non-palatability. Additional evidence that the prevention of bile acid re-absorption leads to lower LDL cholesterol levels was demonstrated in the POSCH trial. These patients had a partial surgical removal of the ileum that was reported to have lower serum LDL cholesterol levels.[5]

The apical sodium co-dependent bile acid transporter (ASBT) is a potentially appealing target for a novel treatment of hyperlipidemia. ASBT promotes re-absorption of bile acids from the intestinal tract into the enterohepatic circulation. The deficiency of bile acids in the enterohepatic circulation causes a similar physiological effect to the bile acid sequestrants and to ileum removal. The liver compensates for the lower bile acid levels by converting more serum cholesterol to bile acid ultimately lowering serum cholesterol levels. Thus, selective ASBT inhibitors are hypothesized to have a physiological response similar to a bile acid sequestrant without its associated patient compliance problems.

Previously we had reported the use of 2,3-disubstituted-4-phenylquinolines (**1**) as ASBT inhibitors that exhibited in vitro potencies in the low micromolar range.[6] The patent literature has illustrated the use of 1,4- and 1,5-benzothiazepines (**2** (IC_{50} = 48 nM, internal data) and **3**) as alternative templates that are potent inhibitors of ASBT.[7] Herein, we report the use of 1,2-benzothiazepines (**4**) as a new class of nanomolar ASBT inhibitors.

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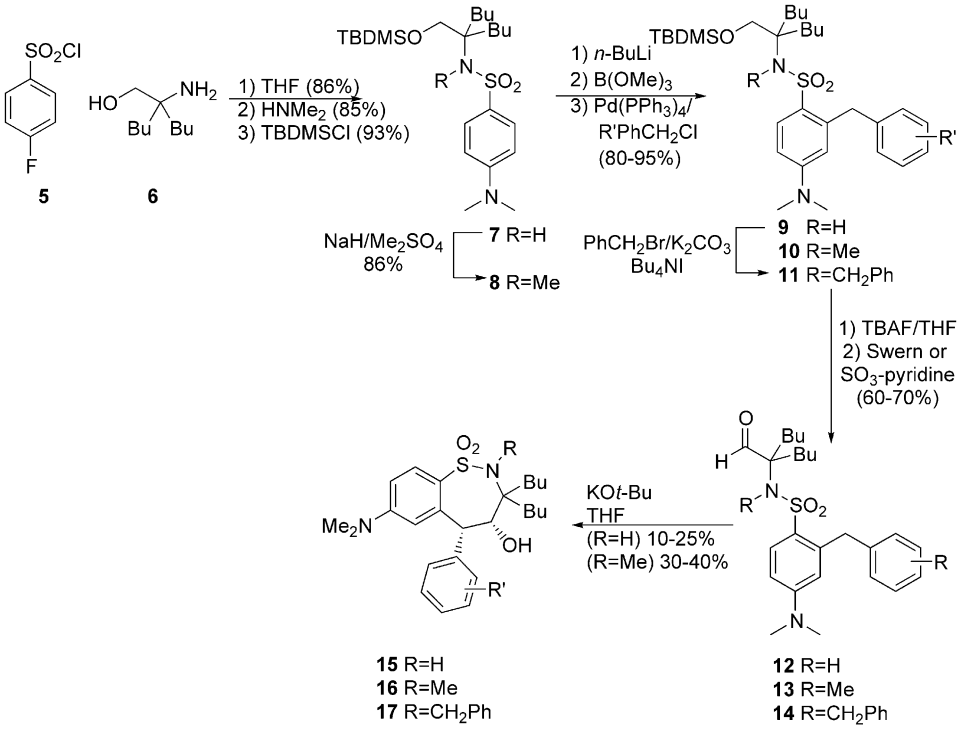
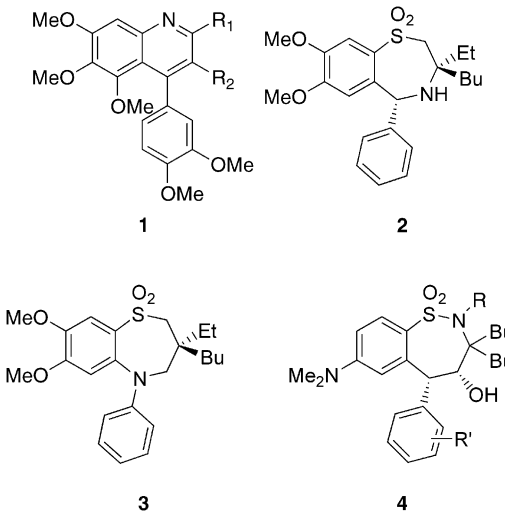


Scheme 1.



The synthesis of the 1, 2-benzothiazepine core (**4**) was accomplished in 8–9 steps (Scheme 1). The amino alcohol **6** was prepared from reduction of the corresponding amino acid prepared analogous to the literature method of Stork.[8] Treatment of *p*-fluorobenzenesulfonyl chloride (**5**) with 2-amino-2-butylhexan-1-ol (**6**) followed by nucleophilic aromatic substitution with dimethylamine and protection of the hydroxyl with TBDMS afforded intermediate **7** in 68% yield over three steps. At this time the sulfonamido nitrogen can be methylated to afford **8** or the synthesis can be continued using the secondary sulfonamide **7**.

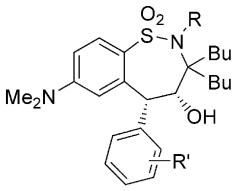
ortho-Lithiation of **7** and **8** was performed by treating the sulfonamide with 3.0 and 2.25 equiv, respectively, of *n*-butyllithium at 0 °C in THF. After 30 min, the reaction was quenched with trimethyl borate, isolated as the boronic acid then the boronic acid was subjected to Suzuki conditions to prepare the diphenylmethylene **9** and **10**. Alternatively, the reaction was quenched with trimethyl borate, the THF was evaporated from the crude borate ester and then the reaction vessel was charged with toluene in order to prepare the diphenylmethane **9** and **10** under Suzuki conditions by reaction with the appropriate benzyl bromide.[9] The TBDMS protected hydroxy sulfonamide **9** can be alkylated at the sulfonamido nitrogen to afford **11** under phase transfer conditions.

Deprotection of the TBDMS group with TBAF followed by the oxidation of the alcohol to the aldehyde with a sulfur trioxide-pyridine complex (R = Me) or by Swern conditions (R = H, CH_2Ph) afforded aldehydes **12**, **13** and **14**. Cyclization of sulfonamide aldehydes **12**, **13** and **14** was achieved by reaction with potassium *t*-butoxide in THF to afford the corresponding 1,2-benzothiazepine 1,1-dioxides (**15**, **16** and **17**). Typically, the *cis* isomer is the predominant isomer observed.[10] However, in the case where R = H and R′ = *m*-NO_2 essentially no diastereoselectivity was observed.

Standard functional group manipulations of the R′ group in **15**, **16** and **17** afforded the additional analogues listed in Table 1.

The in vitro activity of potential ASBT inhibitors were determined by measuring the uptake of [^{14}C]-taurocholate in baby hamster kidney cells transfected with the cDNA from human ASBT (H14 cells).[11] It was quite evident that the R sulfonamido substituent has a substantial effect on the potency of these compounds (see

Table 1. In vitro assay of 1,2-benzothiazepines 1,1-dioxides that inhibit ASBT-mediated uptake of [^{14}C]-taurocholate in H14 cells



Compd	R	R′	IC_{50} (nM)
2	—	—	48
17a	CH_2Ph	3-NO_2	>1000
17b	CH_2Ph	3-NHEt	2500
16a	Me	3-NO_2	1200
16b	Me	3-NH_2	320
16c	Me	3-OMe	1200
16d	Me	4-OMe	570
16e	Me	4-OH	580
16f	Me	3-($NHCOCH_2Cl$)	970
16g	Me	3-($NHCO(CH_2)_4Br$)	690
16h	Me	3-($NHCOCH_2N^+Et_3$)Cl^-	320
16i	Me	3-($NHCO(CH_2)_4N^+Et_3$)Br^-	83
16j	Me	4-(($(OCH_2CH_2)_3(N^+C_5H_4)$))I^-	310
16k	Me	4-(($(OCH_2CH_2)_3N^+Et_3$)I^-	200
15a	H	H	35
15b	H	3-NO_2	44
15c	H	3-NH_2	6
15d	H	4-OMe	26
15e	H	4-OH	8
15f	H	3-($NHCO(CH_2)_4Br$)	22
15g	H	3-($NHCO(CH_2)_4N^+Et_3$)$CF_3CO_2^-$	1.6
15h	H	4-(($(OCH_2CH_2)_3N^+Et_3$)I^-	3

Table 1). The benzyl substituted compounds (**17a** and **17b**) exhibited weak inhibition in vitro (IC_{50} > 1000 nM). The smaller methyl group (**16a–k**) was a bit more active showing moderate to weak in vitro activities (IC_{50} = 320–1200 nM). The secondary sulfonamides **15a–h** (R = H) were found to be the most potent compounds in this series as their activities were 20–50 times more potent than their respective *N*-methyl analogues (IC_{50} = 3–44 nM). We believe this to be a purely steric effect due to the low activity when R = benzyl or R = methyl. The sulfonamido nitrogen may be in close proximity to the surface of the binding site. Hydrogen bonding at the sulfonamido nitrogen appears not to contribute to binding as illustrated by 1,4- and 1,5-benzothiaepines (**2** and **3**).

A modest electronic effect of the lower 5-aryl ring was observed. The electron withdrawing 3′-NO_2 group (**15b**, IC_{50} = 44 nM) had slightly less or comparable activity than the unsubstituted phenyl ring (**15a**, IC_{50} = 35). The electron donating 3′-NH_2 (**15c** IC_{50} = 6 nM) and 4′-OH (**15e**, IC_{50} = 3 nM) showed a 5-10 fold increase in activity compared to the unsubstituted phenyl ring **15a**. However, the decreased activity of the 4′-OMe analogue (**15d**, IC_{50} = 26 nM) may indicate that the proper placement of the hydrogen as a H-bond donor is required. A similar trend was noted for tertiary sulfonamide (R = Me, see compounds **16a–e**). However, the 4′-OMe analogue (**16d**, IC_{50} = 570 nM) is equipotent with the 4′-OH analogue (**16e**, IC_{50} = 580 nM) unlike in the secondary sulfonamides (R = H). The negative interaction of the N–Me substituent with the transporter changes the orientation of the inhibitor. This altered alignment prevents the beneficial binding of the 4′-OH group as seen in the secondary sulfonamides.

Efforts to prepare a more water soluble 1,2-benzothiazepine resulted in the synthesis of more potent ASBT inhibitors. Several side chains linking a quaternary ammonium salt to the 5-phenyl ring were investigated. An amide linker was constructed from the 3-amino compounds (**15c** and **16b**) and then reacted with triethylamine to afford **15g**, **16h** and **16i**. The quaternary ammonium salts (**15g**, **16h** and **16i**) exhibited potencies 3–14 times greater than their alkyl halide precursors (**15f**, **16f** and **16g**) indicating the importance of the ammonium group for optimal activity. It is also important to note that the potencies of **15g, 16 h** and **16i** are substantially better than the simple 3′-NO_2 and 3′-NH_2 precursors (**15b**, **15c**, **16a**, and **16b**), and that **15g** is one of the most potent compounds to date (IC_{50} = 1.6 nM). We believe that the quaternary ammonium salt may be exposed to solvent and its main function to be increased solubility.

A polyethylene glycol linker was examined at the 4′-position of the 5-phenyl ring with a quaternary ammonium salt attached to its end as well. Once again these compounds (**15h**, **16j** and **16k**) show greater potency than the corresponding 4′-OH compounds (**15d** and **16d**). It appears that one can substitute a fairly large substituent on the 5-phenyl ring as long as water solubility is maintained.

The 1,2-benzothiazepines 1,1-dioxides are a novel class of ASBT inhibitors that show nanomolar activities in vitro. Further evaluation is needed in this series in order to determine the in vivo utility of this novel class of ASBT inhibitors.

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9. **Sample procedure for the conversion of 7 to 9.** To a solution of 2.0 g (4.25 mmol) of **7** in 10 mL of tetrahydrofuran cooled to 0 °C was added 8.0 mL of 1.6 M *n*-butyllithium in hexane. The reaction mixture was stirred at 0 °C for 30 min. To the reaction mixture was added 1.9 mL of trimethyl borate and stirred 10 min at 0 °C then 1 h at room temperature. To the reaction mixture was added 100 mL of water and enough 5% hydrochloric acid to bring the solution to a pH of 6–7 then the volatiles were evaporated. To the aqueous solution was added 100 mL of ethyl acetate then extracted. The ethyl acetate layer was washed with water (100 mL) and brine (100 mL), dried

over magnesium sulfate and concentrated. The residue was dissolved in 7 mL of ethanol and degassed with nitrogen. In a separate flask was placed 150 mg of tetrakis(triphenylphosphine)palladium(0), 10 mL of toluene and 918 mg of 3-nitrobenzaldehyde. The ethanol solution was added to the toluene solution followed by 10 mL of 1 M aqueous sodium carbonate. The reaction mixture was heated to reflux for 1 h then cooled to room temperature and stirred 16 h. The reaction mixture was concentrated and dissolved in 100 mL of ethyl acetate. The ethyl acetate solution was washed with water (100 mL) and brine (100 mL). The ethyl acetate layer was dried over magnesium sulfate and concentrated. The residue was purified by flash chromatography to give 1.72 g **9b** ($R' = 3\text{-}NO_2$).

10. For a discussion of the *cis* selectivity, see: Wang, C.-C.; Li, J. J.; Huang, H.-C.; Lee, L. F.; Reitz, D. B. *J. Org. Chem.* **2000**, *65*, 2711.

11. 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 Hank's 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. 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.