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Spiroimidazolidinone NPC1L1 inhibitors. Part 2: Structure–activity studies and in vivo efficacy

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ABSTRACT

Ezetimibe (Zetia®), a cholesterol-absorption inhibitor (CAI) approved by the FDA for the treatment of hypercholesterolemia, is believed to target the intestine protein Niemann-Pick C1-Like 1 (NPC1L1) or its pathway. A spiroimidazolidinone NPC1L1 inhibitor identified by virtual screening showed moderate binding activity but was not efficacious in an in vivo rodent model of cholesterol absorption. Synthesis of analogs established the structure–activity relationships for binding activity, and resulted in compounds with in vivo efficacy, including **24**, which inhibited plasma cholesterol absorption by 67% in the mouse, thereby providing proof-of-concept that non-β-lactams can be effective CAIs.

Hypercholesterolemia resulting from the absorption of both dietary cholesterol and endogenous biliary cholesterol is associated with atherosclerotic diseases.[1] Ezetimibe **1** (shown in Fig. 1), a cholesterol-absorption inhibitor (CAI), has been shown to be effective in decreasing blood low-density lipoprotein cholesterol (LDL-c) levels.[2] Ezetimibe was discovered from a 2-azetidinone lead series initially identified as ACAT inhibitors and subsequent structural modifications of biliary metabolites identified in a bile duct-cannulated rat model.[3] It was later determined that the molecular target of ezetimibe is Niemann-Pick C1-Like 1 protein (NPC1L1), a putative member of the resistance-nodulation-division permease family of transporters.[4] NPC1L1 has been established as a primary regulator for intestinal cholesterol absorption and whole-body cholesterol homeostasis.[5] Ezetimibe **1** is glucuronidated in vivo to the more potent metabolite **2**, which undergoes enterohepatic recirculation to prolong half-life.[6]

These discoveries have improved the ability to identify compounds that function as CAIs, via a correlation between NPC1L1 binding affinity and inhibition of cholesterol absorption in vivo.[7] It has been shown, using ezetimibe and a potent 2-azetidinone lead **3**, that the binding affinity of recombinant human NPC1L1 closely parallels that of native human NPC1L1 from brush border membranes.[7] Furthermore, an in vivo mouse inhibition of cholesterol-absorption assay (MICA) demonstrates correlation between in vivo ED_{50} and in vitro binding affinity for ezetimibe and analog **3**, even though the binding affinity observed for ezetimibe glucuronide **2** in mice is reduced as compared to that of human.[7] This difference in human and animal models is in agreement with previous studies.[4a,8]

These discoveries have improved the ability to identify new compounds that function as CAIs if a correlation between binding affinity and in vivo efficacy is established for non-β-lactam compounds with binding affinity to NPC1L1. This letter describes the synthesis, structure–activity relationships, and in vivo efficacy of a novel class of spiroimidazolidinone NPC1L1 inhibitors which were identified by similarity-based virtual screening and are unrelated to the first-in-class β-lactam compound ezetimibe.[9] Over the course of the preparation of this manuscript, several related reports have disclosed spiro-beta lactam and other non-beta lactam CAIs.[10]

Spiroimidazolidinone analogs were synthesized as shown in Scheme 1. The basic amine of commercially available 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**5**) was protected with a *t*-butoxycarbonyl group, then the amide **6** was deprotonated with sodium hydride, followed by alkylation with ethyl bromoacetate. Repeated deprotections and EDC-mediated couplings afforded the corresponding target analogs **9** in 2–88% yield (three steps).

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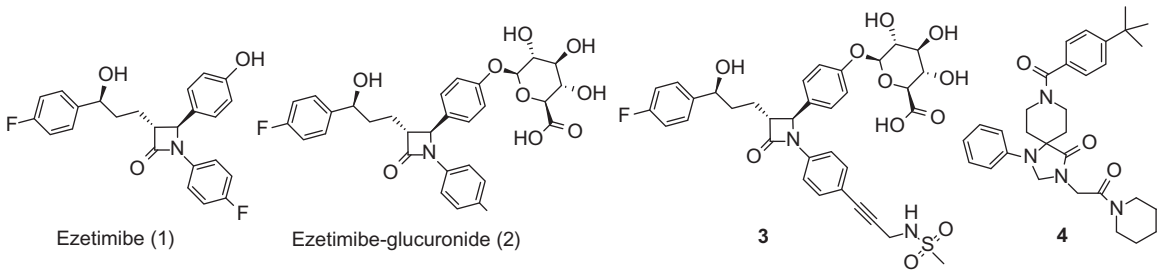
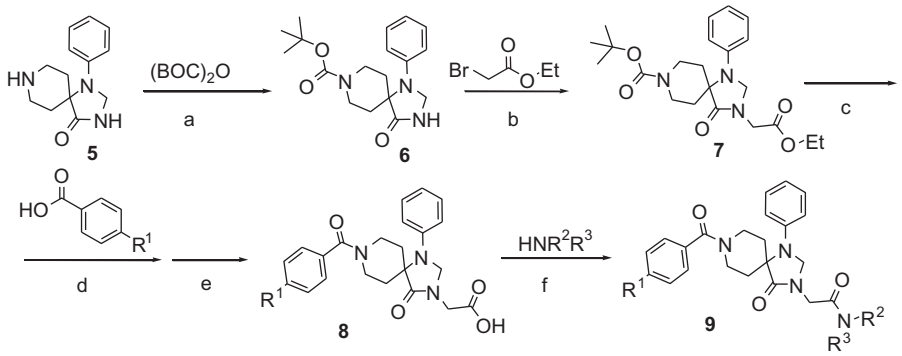


Figure 1. Structures of NPC1L1 inhibitors.



Scheme 1. Reagents and conditions: (a) Et_3N, DMF/CH_2Cl_2, 0 °C, 30 min, 95–99% yld.; (b) NaH, THF/DMSO, 1.5 h, 71–96% yld.; (c) HCl/dioxane, CH_2Cl_2, 16 h, 93–94% yld.; (d) EDC, HOBT, DIEA, CH_2Cl_2, 16 h, 67–97%; (e) LiOH, THF/H_2O, 2 h, 93–94%; (f) EDC, HOBT, DIEA, CH_2Cl_2, 16 h, 4–97% yld.

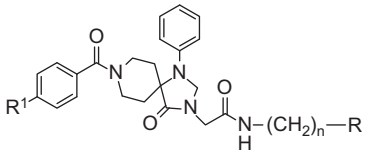
The substituted spirocyclic derivatives shown in Tables 1 and 2 were rapidly synthesized from a library of reagents from commercial sources using similar chemistry. In this manner, approximately 230 analogs of the lead were generated which allowed quick exploration of preliminary structure–activity relationships of this lead series.

Compounds were evaluated in a binding assay of mouse brush border membranes and human embryonic kidney 293 cell line which expressed recombinant human NPC1L1. Binding affinity was determined by displacement of ^{35}S-labeled compound **3** according to procedures previously reported.[3,7,11]

In this binding assay, lead **4**, the original lead which came from the modeling study, exhibited a binding affinity of 2.5 μM in human NPC1L1. Initial SAR studies to improve the binding affinity focused on varying the piperidyl amide and 4-*t*-butyl substituent of the benzamide.

The effect of the right-hand amide side chain was studied in two series of analogs where the left-hand phenyl substituent was either

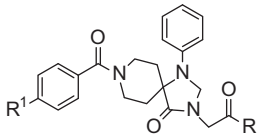
Table 1
Effect of amide side chain on binding to human NPC1L1, IC_{50}, μM

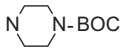
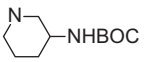
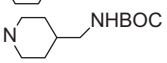
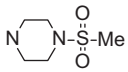
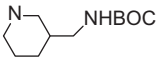
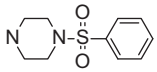
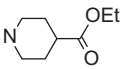
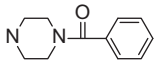
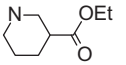
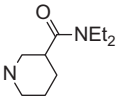
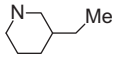
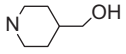
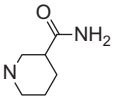
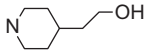
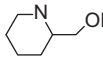
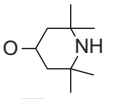
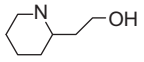
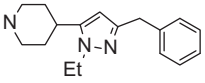
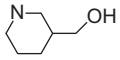
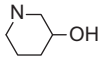
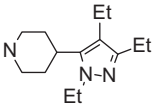
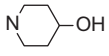
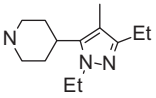
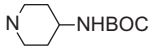
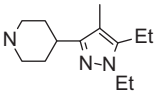


Entry	R^1	R	*n* = 1	*n* = 2	*n* = 3	*n* = 4	*n* = 5
1	*t*-Bu	CO_2Me	16.6	1.0 (44)[a]	4.2 (35)[a]	nd	nd
2	*t*-Bu	CO_2H	nd	23.6	14.5	nd	nd
3	*t*-Bu	OH	nd	19.2	7.7	8.3	3.8
4	*t*-Bu	NH–BOC	nd	9.4	3.2	2.0 (7)[a]	3.1
5	*t*-Bu	NH_2	nd	59.0	49.0	14.0	nd
6	*t*-Bu	$NHSO_2$–Me	nd	10.9	10.4	10.3	nd
7	Cyclohexyl	CO_2Me	11.4	4.5	4.5	nd	nd
8	Cyclohexyl	OH	nd	nd	5.5 (42)[a] (43 ± 27, 70 ± 9)[b]	1.7 (52)[a]	2.1
9	Cyclohexyl	NH–BOC	nd	13.7	3.7	5.5	3.8
10	Cyclohexyl	NH_2	nd	26.5	12.9	6.0	nd
11	Cyclohexyl	$NHSO_2$–Me	nd	7.0	2.5 (42)[a] (<5, <5)[b]	4.6	nd

nd = not determined.
[a] % inhibition in MICA at 10 mg/kg.
[b] % inhibition (mean ± SD) in MICA at 100 mg/kg for plasma and liver, respectively. Data are an av of triplicates.

Table 2
Effect of right-hand-side amide on human IC_{50}, μM



Analog	R	R^1 = *t*-butyl	R^1 = cyclohexyl	Analog	R	R^1 = *t*-butyl	R^1 = cyclohexyl
10a,**b**		1.0 (49)[a]	3.5 (49)[a] (4.5 ± 22, 58 ± 13)[b]	**18c**,**d**		4.2	4.4
11a,**b**		22.0	6.5	**18e**,**f**		4.2	2.5
12a,**b**		19.0	6.3	**18g**,**h**		9.5	3.8
13a,**b**		15.0	14.2	**19a**,**b**		3.1	0.9
14		nd	4.8 (19)[a] (52 ± 23, 46 ± 19)[b]	**19c**,**d**		8.2	4.2
4,**15**		2.5	1.1				
16a,**b**		12	3.6	**20a**,**b**		8.7	3.3
16a,**b**		3.9	2.6				
17a,**b**		4.0	1.1	**20c**,**d**		16.9	3.1
17c		2.6	nd				
17d		11.8	nd	**21a**,**b**		1.3	0.6
17e		6.1	nd	**22**		6.4	nd
17f		8.2	nd				
17g		12.2	nd	**23**		4.6	nd
17h		4.7	nd	**24**		2.5 (40)[a] (35 ± 19, 48 ± 25, 62 ± 11)[c]	nd
18a,**b**		8.3	4.8	**25**		5.4	nd

nd = not determined.
[a] Mouse NPC1L1 binding IC_{50}, μM.
[b] % inhibition (mean ± SE) in MICA at 100 mg/kg in plasma and liver, respectively.
[c] % inhibition (mean ± SE) in MICA at 10, 30, and 100 mg/kg in plasma, respectively.

R^1 = *t*-butyl or cyclohexyl. Binding assay results are shown in Table 1. Of the terminal functional groups studied, non-polar protected heteroatoms (entries 1, 4, 7, and 11) produced the best binding affinities. In general, longer chain lengths afforded higher binding affinities. Compounds with R^1 = cyclohexyl generally gave improved binding affinities as compared to their *t*-butyl analogs.

The binding affinity results for piperidine and piperazine analogs are shown in Table 2. Both series produced examples which had micromolar binding affinities comparable to that of lead **4**. Note that carboxylic acid esters **19a–d** also exhibited similar binding affinities but were not pursued due to their inherent hydrolytic instability. Their carboxylic acid analogs were not tested because

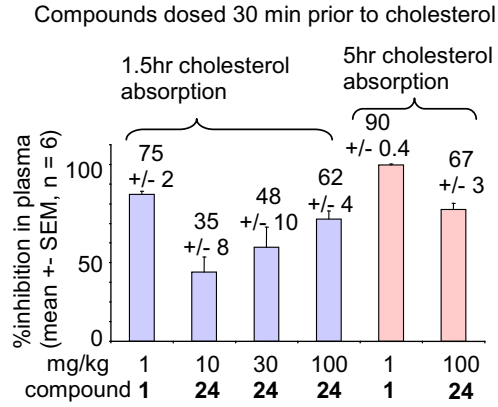


Figure 2. %Inhibition (mean ± SE) of ^{3}H-cholesterol absorption in C57BL/6 mice (values are means of six datapoints).

SAR studies showed carboxylic acids had low binding affinities (Table 1, entry 2; *t*-butyl and phenyl analogs of **8** were 184 and 1523 μM IC_{50} in human, respectively).

The compounds were tested for their ability to inhibit cholesterol absorption in mice. To that end, compounds were pre-dosed orally in C57BL/6 male mice in a 0.2-mL 0.25% methocel suspension. Thirty minutes later, the mice were orally dosed with a 0.2 mL mixture of tritium radiolabeled cholesterol in IntraLipid. After 1.5 h and 5 h, the mice were euthanized and weighed. The blood was collected by cardiac puncture, and the plasma ^{3}H-cholesterol was measured. The liver was collected, weighed, and saponified, and the liver ^{3}H-cholesterol was measured. Six data points were taken per dosage and averaged, and the standard deviation and standard error were determined.

Several of the analogs shown in Tables 1 and 2 were screened in the MICA at 10 or 100 mg/kg, and the results are shown in parentheses.

The most promising efficacy was observed for pyrazole analog **24**. A dose-dependent effect is observed for compound **24**, with the greatest efficacy (62% inhibition) at the 100-mg/kg dose at the 1.5-hour time point (Fig. 2). At the 5-hour time point, the level of inhibition of cholesterol absorption in plasma increased to 67%. Spiroimidazolidinone **24** also reduced cholesterol in the liver (data not shown). These data demonstrate the potential for non-β-lactam compounds to act as cholesterol-absorption inhibitors in mice.

We have identified spiroimidazolidinones as a novel lead class of NPC1L1 inhibitors and explored the SAR of binding affinity of this novel class of CAIs. A series of substituted piperidines were prepared with improvements in binding affinity to NPC1L1 observed for 4-substituted analogs including those containing pyrazole groups. Non-β-lactam compound **24**, which contains both the 4-*t*-butyl benzamide and a 4-pyrazolylpiperidine, demonstrated dose-dependent cholesterol-lowering efficacy in mice. Significant improvements in binding affinity and in vivo efficacy will be required to determine if non-β-lactam CAIs will be viable leads for this class of LDL-reducing therapy.

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