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Substituted oxazolidinones as novel NPC1L1 ligands for the inhibition of cholesterol absorption

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Abstract—Cholesterol absorption inhibition (CAI) represents an important treatment option for hypercholesterolemia. Herein, we report the design and evaluation of a series of substituted oxazolidinones as ligands for the Niemann Pick C1 Like 1 (NPC1L1) protein, a key mediator of cholesterol transport. Novel analogs were initially evaluated in a brush border membrane NPC1L1 binding assay; subsequently, promising compounds were evaluated in vivo for acute inhibition of cholesterol absorption. These studies identified analogs with low micromolar NPC1L1 binding affinity and acute in vivo efficacy of >50% absorption inhibition at 3 mg/kg.

Coronary heart disease (CHD) is a leading cause of death in the United States.[1] Given that hypercholesterolemia is a key risk factor for CHD, substantial efforts have been undertaken to mitigate this condition.[2] The standard of care for treating hypercholesterolemia has been the use of HMG-CoA reductase inhibitors, or statins, which block the rate-limiting step of cholesterol biosynthesis.[3] However, since plasma cholesterol results from a combination of biosynthesis and absorption of dietary cholesterol, the approval of the cholesterol absorption inhibitor Zetia® (ezetimibe, **1**) has offered a complementary approach for the treatment of hypercholesterolemia.[4] Clinically, as a monotherapy ezetimibe achieves an average 18% reduction in LDL.[5] When used in combination with a statin, ezetimibe affords an additional 15–18% reduction in LDL beyond that achieved by the statin alone.[5] This combination strategy is particularly valuable for achieving aggressive LDL lowering as well as for treating patients that cannot tolerate high dose statin therapy.

Ezetimibe (**1**) was originally discovered in the absence of a known molecular target.[5c] However, researchers at Schering-Plough/Merck recently demonstrated that it blocks cholesterol absorption via interaction with Niemann Pick C1 Like 1 (NPC1L1).[6] This protein is highly expressed in the proximal intestine and is a key mediator of cholesterol transport.[7] Given that ezetimibe (**1**) is rapidly converted in vivo to its equipotent glucuronide derivative **2,** early NPC1L1 binding experiments were conducted using [^{3}H]ezetimibe-glucuronide as the radioligand.[6] In these studies using brush border membrane (BBM) preparations from rat intestine, Garcia-Calvo and coworkers demonstrated that ezetimibe-glucuronide (**2**) bound selectively to rat NPC1L1 with sub-micromolar affinity (K_i = 0.35 μM).[6] The parent ezetimibe (**1**) was found to have similar binding affinity (K_i = 0.97 μM).[8] Both **1** and **2** were also shown to bind to brush border membrane preparations containing native or recombinant NPC1L1 from various other species, including human, with binding affinities correlating with the observed CAI efficacies in those species.[6,8]

To enable the evaluation of novel NPC1L1 ligands, we established a competition binding assay utilizing [^{3}H]ezetimibe-glucuronide according to the method reported.[8] Using NPC1L1 in brush border membranes isolated from rat, we reproduced the reported binding

Keywords: NPC1L1; Cholesterol absorption inhibitor; Hypercholesterolemia; Dyslipidemia.
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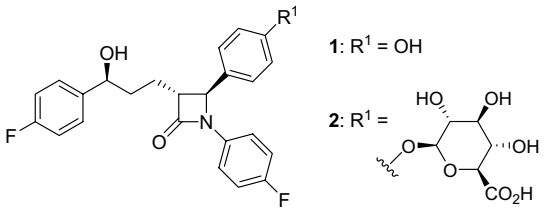


Figure 1. Structure of ezetimibe (**1**) and ezetimibe-glucuronide (**2**).

affinities of both ezetimibe-glucuronide **2** (K_i = 0.18 μM) and ezetimibe **1** (K_i = 0.20 μM) (Fig. 1).

In an effort to identify novel cholesterol absorption inhibitors, we and others have been interested in replacement of the β-lactam core of ezetimibe with alternative heterocycles.[9] A preliminary evaluation of various 4-, 5-, and 6-membered heterocycles suggested that the 3,4,5-trisubstituted oxazolidinone was a promising core replacement. Previously, Dugar and coworkers reported oxazolidinone **3** (Fig. 2) as having modest cholesterol absorption inhibition efficacy in a cholesterol-fed animal model.[9b] Additionally, Carrierea and coworkers described the synthesis of oxazolidinone **4** bearing a substitution pattern analogous to that of ezetimibe.[9c,d] In our hands, resynthesis of these two oxazolidinones and evaluation in the radioligand rat NPC1L1 binding assay revealed that **4** had an affinity (K_i = 9.9 μM) about 50-fold less than ezetimibe (K_i = 0.20 μM), whereas **3** had very weak affinity (K_i > 50 μM).[10] Based on these observations, we anticipated that a diverse survey of the SAR around this oxazolidinone core might afford ligands with increased NPC1L1 binding potency. In addition, these SAR studies would be useful in further understanding the relationship between NPC1L1 binding and CAI efficacy since, to date, correlation data have only been published for a small number of ezetimibe analogs.[6,8]

As shown in Figure 3, our SAR strategy focused on the evaluation of analogs with alternative linkers between

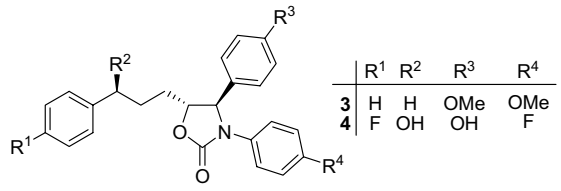


Figure 2. Previous oxazolidinone cholesterol absorption inhibitors.

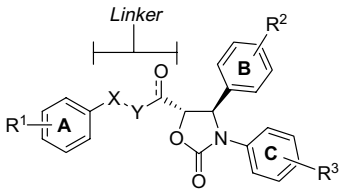


Figure 3. SAR strategy for evaluation of oxazolidinone-based inhibitors.

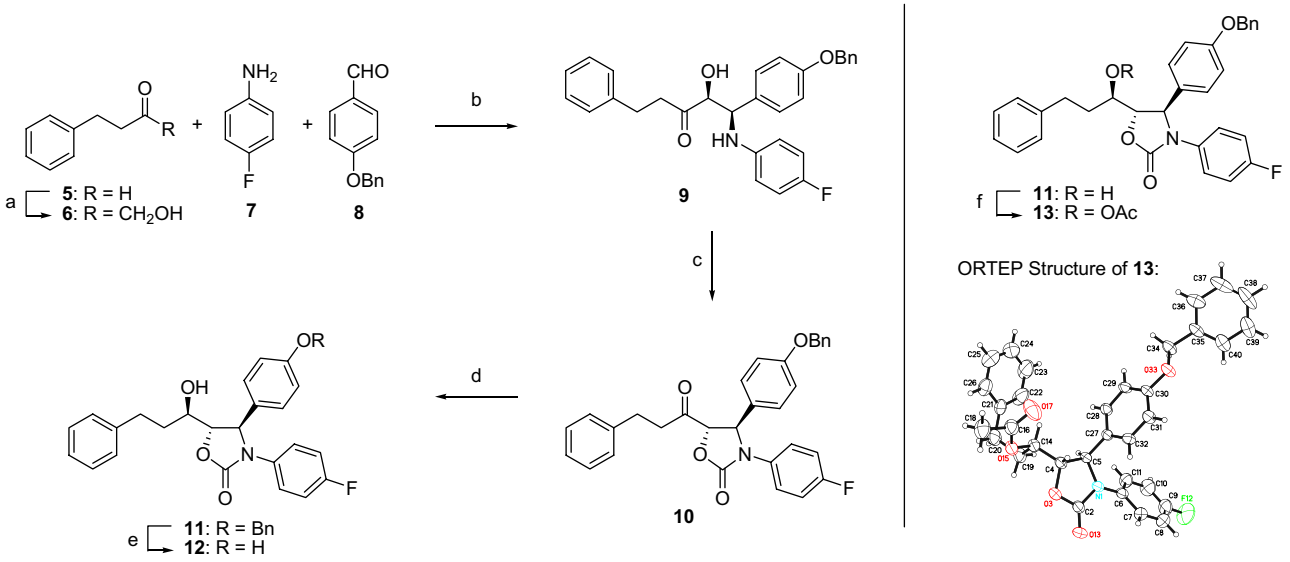
the A-ring and oxazolidinone core as well as alternative substituents on the A-, B-, and C-aryl rings.

Schemes 1–5 outline the preparation of representative oxazolidinone-based NPC1L1 ligands. Scheme 1 illustrates the enantioselective synthesis of analog **12** wherein the secondary hydroxyl of the linker unit has been transposed two carbons closer to the oxazolidinone (relative to compound **4**).[11] Hydrocinnamaldehyde (**5**) was initially converted to α-hydroxyketone **6** via treatment with formaldehyde and *N*-ethylbenzothiazolium bromide.[12] Utilizing an asymmetric three-component Mannich reaction reported by List and coworkers,[13] α-hydroxyketone **6** was subsequently reacted with 4-fluoroaniline (**7**), 4-benzyloxybenzaldehyde (**8**) in the presence of catalytic L-proline to afford 1,2-amino alcohol **9** in moderate yield and excellent stereoselectivity (>15:1). Treatment of **9** with triphosgene and triethylamine resulted in formation of oxazolidinone **10**. Noyori reduction[14] of the side chain ketone provided alcohol **11** as a single diastereomer which was subsequently debenzylated using transfer hydrogenation conditions to afford compound **12**. In order to confirm the relative stereochemistry of **12**, a portion of intermediate **11** was reacted with acetic anhydride and pyridine to give crystalline derivative **13** which was subjected to small molecule X-ray analysis as shown.[15]

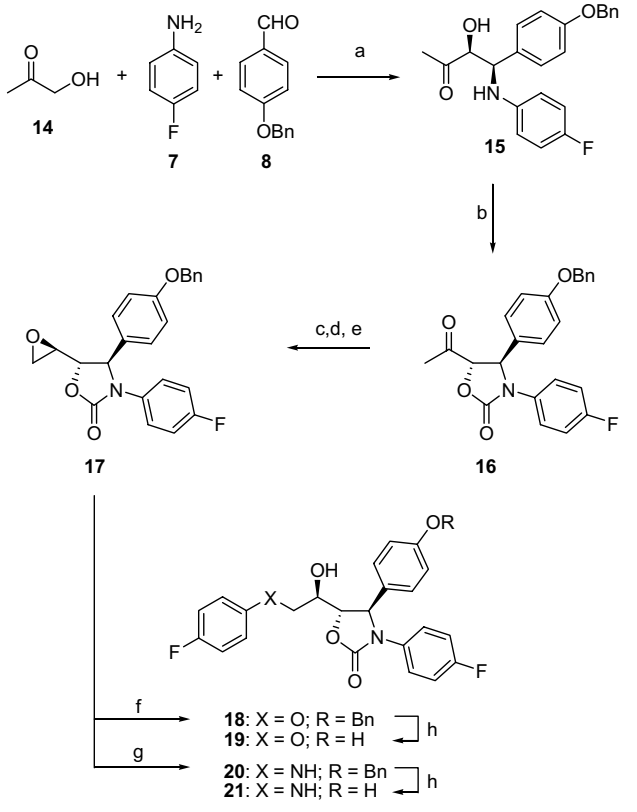
Scheme 2 illustrates the preparation of ligands that contain ether or amine functionalities in the linker backbone.[16] Utilizing the same asymmetric Mannich reaction described above, hydroxyacetone (**14**), 4-fluoroaniline (**7**), and 4-benzyloxybenzaldehyde (**8**) were condensed in the presence of catalytic L-proline to stereoselectively provide 1,2-amino alcohol **15** which cyclized to oxazolidinone **16** upon treatment with triphosgene and triethylamine.

Epoxide **17** (Scheme 2) was prepared from ketone **16** via a three-step sequence commencing with α-bromination followed by carbonyl reduction ($NaBH_4$) and finally base-mediated closure of the intermediate halohydrin to give a mixture (ca. 3:1) of diastereomeric epoxides. Chromatographic separation provided epoxide **17** as the major diastereomer (43% yield over three steps). With **17** in hand, analogs bearing an ether linkage were accessible via base-mediated addition of phenols as illustrated for compound **18**. In parallel, analogs bearing an amine linkage could be accessed via treatment of epoxide **17** with anilines in the presence of $LiClO_4$ as illustrated for compound **20**. Finally, **18** and **20** were debenzylated utilizing standard transfer hydrogenation conditions to provide **19** and **21**, respectively.

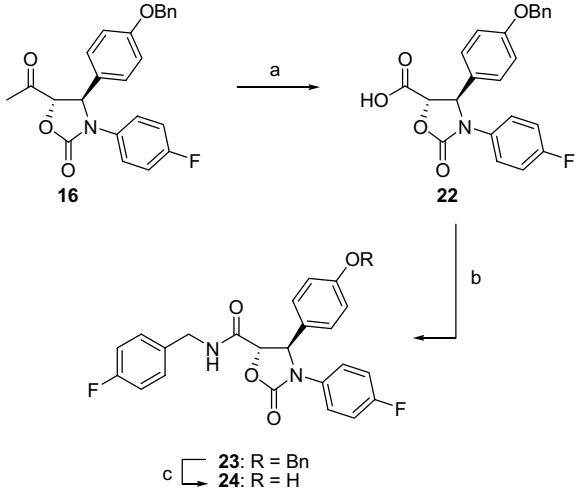
Analogs containing amide functionalities in the linker unit were prepared as outlined in Schemes 3 and 4. As shown in Scheme 3, ketone **16** (from Scheme 2) was initially subjected to a haloform reaction (Br_2, NaOH) to generate carboxylic acid **22**. EDAC-mediated coupling of acid **22** with 4-fluorobenzyl amine afforded amide **23** which was subsequently debenzylated to provide compound **24** as shown. The reverse amide was subsequently prepared as outlined in Scheme 4 by initial



Scheme 1. Synthesis of oxazolidinone **12**. Reagents and conditions: (a) $(CHO)_n$, Et_3N, *N*-ethylbenzothiazolium bromide, EtOH, 80 °C, 36 h, 25%; (b) L-proline (10 mol%), DMSO, 25 °C, 72 h, 75%; (c) triphosgene, Et_3N, CH_2Cl_2, −20 → 25 °C, 12 h, 63%; (d) $RuCl_2$-(*S*)-(DM-BINAP)-(*S*)-DAIPEN, KO*t*-Bu, H_2 (50 psi), *i*-PrOH:THF, 25 °C, 6 h, 92%; (e) Pd/C, cyclohexene, EtOH:EtOAc, 80 °C, 5 h, 91%; (f) Ac_2O, pyridine, MeCN, 25 °C, 16 h, 85%.



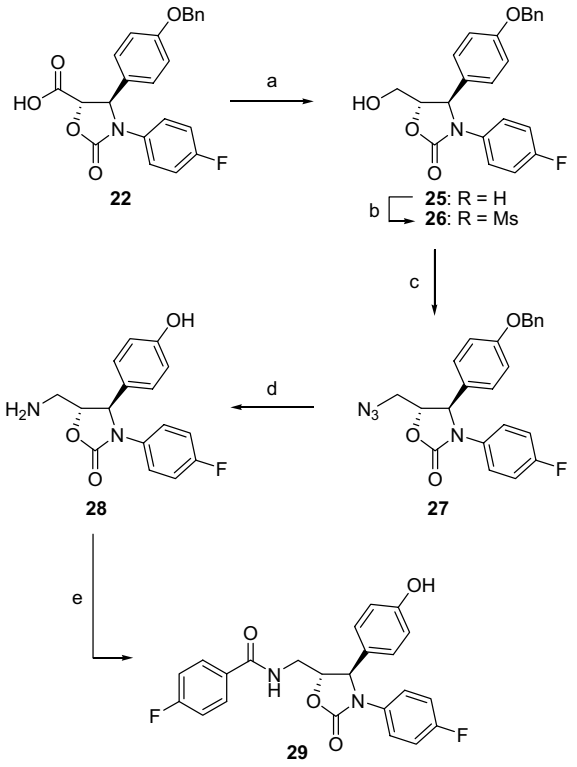
Scheme 2. Synthesis of oxazolidinones **19** and **21**. Reagents and conditions: (a) L-proline (20 mol%), DMSO, 25 °C, 12 h, 95%; (b) triphosgene, Et_3N, CH_2Cl_2, −20 → 25 °C, 12 h, 41%; (c) trimethylphenylammonium tribromide, THF, 0 °C, 22 h; (d) $NaBH_4$, EtOH, 0 → 25 °C, 3 h; (e) NaO*t*-Bu, THF, 0 → 25 °C, 2 h, 43% over three steps; (f) 4-fluorophenol, Cs_2CO_3, DMSO, 70 °C, 3 h, 78%; (g) 4-fluoroaniline, $LiClO_4$, Et_2O, 25 °C, 16 h, 50%; (h) Pd/C, cyclohexene, EtOH:EtOAc, 80 °C, 5 h, 75–90%.



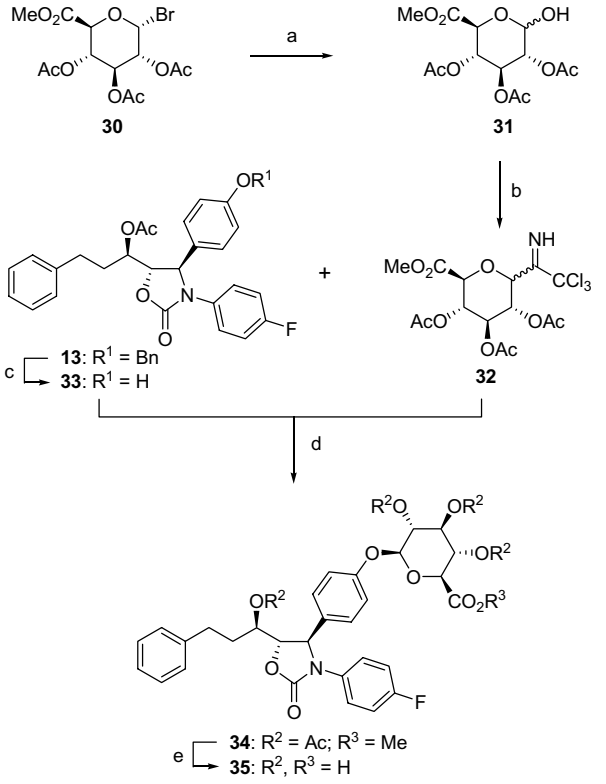
Scheme 3. Synthesis of oxazolidinone amide **24**. Reagents and conditions: (a) Br_2, NaOH, H_2O:dioxane, 0 °C, 2 h, 80%; (b) 4-fluorobenzyl amine, EDAC–HCl, HOBt, CH_2Cl_2, 25 °C, 4 h, 50%; (c) Pd/C, cyclohexene, EtOH:EtOAc, 80 °C, 3 h, 79%.

reduction of carboxylic acid **22** to the corresponding alcohol **25**. Conversion of alcohol **25** to the corresponding mesylate **26** followed by reaction with NaN_3 resulted in formation of azide **27**. Treatment of **27** with Pd/C under transfer hydrogenation conditions resulted in reduction of the azide functionality to a primary amine with concomitant removal of the *O*-benzyl protecting group to afford amino-phenol **28**. Finally, **28** was reacted with 4-fluorobenzoyl chloride in the presence of triethylamine to generate amide **29**.

Finally, to enable comparison with the known ezetimibe-glucuronide **2**, the glucuronide conjugate of an oxa-



Scheme 4. Synthesis of oxazolidinone amide **29**. Reagents and conditions: (a) BH_3·THF, THF, 0 → 25 °C, 16 h, 68%; (b) methanesulfonic anhydride, Et_3N, CH_2Cl_2, 25 C, 5 h, 75%; (c) NaN_3, DMF, 60 °C, 16 h, 72%; (d) Pd/C, cyclohexene, EtOH:EtOAc, 80 °C, 1.5 h; (e) 4-fluorobenzoyl chloride, Et_3N, CH_2Cl_2, 25 C, 1.5 h, 31% over two steps.



Scheme 5. Synthesis of oxazolidinone–glucuronide **35**. (a) $CdCO_3$, H_2O, MeCN, 70 °C, 4 h, 78%; (b) Cl_3CCN, DBU, CH_2Cl_2, 0 °C, 1 h, 50%; (c) Pd/C, cyclohexene, EtOH:EtOAc, 80 °C, 3 h, 73%; (d) BF_3·OEt_2, CH_2Cl_2:THF, −20 → 25 °C, 2.5 h, 23%; (e) Et_3N, H_2O, MeOH, 25 °C, 16 h, 31%.

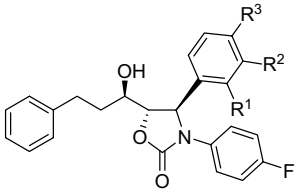
zolidinone analog, **35**, was prepared as outlined in Scheme 5. Commercially available 1-bromo-2,3,4-tri-*O*-acetyl-α-D-glucuronic acid methyl ester (**30**) was hydrolyzed with aqueous $CdCO_3$ and then treated with trichloroacetonitrile in the presence of DBU to generate trichloroacetimidate **32**.[17] Reaction of **32** with phenol **33** in the presence of catalytic BF_3·OEt_2 selectively generated β-glucuronide **34** which underwent subsequent global deprotection upon treatment with triethylamine/MeOH/H_2O to provide oxazolidinone glucuronide **35**.[18]

By appropriate substitution of starting materials, the routes outlined in Schemes 1–5 were subsequently utilized to prepare the compounds utilized in the SAR studies described below.

All analogs were initially screened in the NPC1L1 BBM binding assay using native rat enterocytes and [^{3}H]ezetimibe-glucuronide (**2**) as the radioligand.[19] Compounds with observed binding activity (K_i < 50 μM) were then evaluated in an acute in vivo cholesterol absorption inhibition assay. In this efficacy model, chow-fed hamsters were dosed orally with analogs at 3 mg/kg. Two hours post-dose, the animals were administered a bolus of [^{14}C]cholesterol, and after an additional two hours, serum cholesterol samples were taken and analyzed. Percent reduction of total cholesterol was determined relative to the vehicle-treated control group.[20]

Our structure–activity studies focused largely on modification of the linker region along with evaluation of alternative A-, B-, and C-ring substitution patterns. As shown in Table 1, initial transposition[11] of the secondary hydroxyl group from the aryl side (reference com-

Table 1. NPC1L1 binding and acute CAI efficacy of oxazolidinones **12** and **35**–**40**[19,20]



Compound	R^1	R^2	R^3	BBM NPC1L1 K_i (μM)	Acute CAI at 3 mg/kg
1	—	—	—	0.20	−89%
4	—	—	—	9.9	−64%
12	H	H	OH	>50	NT
35	H	H	O-Gluc	>50	NT
36	H	OH	H	33	−4%
37	OH	H	H	12	0%
38	OH	H	OH	9.2	−55%
39	OH	H	OMe	4.8	−33%
40	OMe	H	OMe	>50	NT

NT, not tested; Gluc, glucuronide.

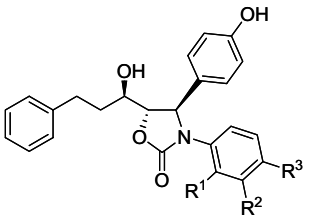
pound **4**) of the linker to the oxazolidinone side (compound **12**) resulted in a loss of binding activity. The corresponding glucuronide of phenol **12**, compound **35**, was also inactive. While maintaining the transposed linker, various phenol isomers were then evaluated. Relocating the B-ring phenol from the 4-position (**12**, K_i = 50 μM) to the 3-position (**36**, K_i = 33 μM) and then to the 2-position (**37**, K_i = 12 μM) resulted in increased binding activity albeit without in vivo efficacy. Encouragingly, combination of the 2-OH substituent with either a 4-OH (**38**, K_i = 9.2 μM) or a 4-OMe (**39**, K_i = 4.8 μM) afforded increased NPC1L1 binding and also acute in vivo efficacy. This increase in efficacy with the 2-OH substituent was consistent with previous observations in the ezetimibe series.[21] The importance of the 2-OH substituent to binding affinity was further demonstrated by comparison of compounds **39** (2-OH, 4-OMe) and **40** (2,4-OMe) with NPC1L1 binding affinities of 4.8 and >50 μM, respectively.

Limited examination of C-ring substitution was also undertaken as shown in Table 2. Comparison of the 4-F (**12**, K_i >50 μM), 3-F (**41**, K_i = 11 μM), and 2-F (**42**, K_i >50 μM) substitution patterns revealed that 3-F substitution was preferred on this ring. Interestingly, **41** also had very good acute in vivo efficacy (−69% at 3 mg/kg).

Table 3 illustrates the combination of the A- and B-ring improvements described above along with further refinement of the B-ring SAR. Analog **43**, with the optimized B-ring (2-OH, 4-OMe) and C-ring (3-F) combination, had improved binding activity (K_i = 2.3 μM) relative to either compound **39** or **41**; however, despite increased affinity for NPC1L1, **44** had only modest acute efficacy (−33% at 3 mg/kg). Maintaining the B-ring 2-OH and examining additional substitution including methoxy (**44**–**45**), fluorine (**46**–**48**) or methyl (**49**) to the B-ring did not offer improvements in binding affinity relative to **43**.

Next, the introduction of heteroatoms into the linker unit was examined as outlined in Table 4.[22] Ether analog **18** (K_i = 28 μM) had somewhat improved binding affinity relative to the all carbon comparator **12** (K_i > 50 μM)

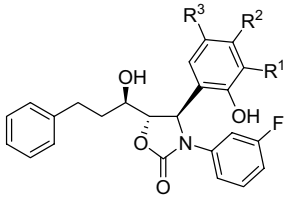
Table 2. NPC1L1 binding and acute CAI efficacy of oxazolidinones **12** and **41**–**42**[19,20]



Compound	R^1	R^2	R^3	BBM NPC1L1 K_i (μM)	Acute CAI at 3 mg/kg
12	H	H	F	>50	NT
41	H	F	H	11	−69%
42	F	H	H	>50	NT

NT, not tested.

Table 3. NPC1L1 binding and acute CAI efficacy of oxazolidinones **43**–**49**[19,20]



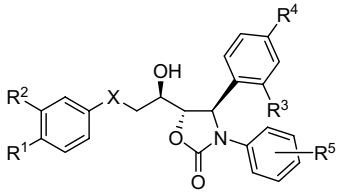
Compound	R^1	R^2	R^3	BBM NPC1L1 K_i (μM)	Acute CAI at 3 mg/kg
43	H	OMe	H	2.3	−33%
44	OMe	H	H	5.7	NT
45	H	H	OMe	>50	NT
46	H	F	H	19	0%
47	H	H	F	18	0%
48	F	H	H	>50	0%
49	H	Me	H	16	−19%

NT, not tested.

and had unexpectedly good acute efficacy (−55% at 3 mg/kg). Unfortunately, installation of the previously optimized B- and C-ring substitution pattern to give **50** did not afford improved binding affinity. Additionally, increasing the size of the R^1 substituent on the A-Ring (i.e., **51** and **52**) resulted in loss of binding activity. A parallel chemistry array scanning alternative A-ring substitution (complete data not shown) identified the 3-OMe group as a preferred substituent on this ring as illustrated by **53** (K_i = 15 μM) and **54** (K_i = 8.7 μM). Despite increased binding affinity however, both **53** and **54** had inferior acute efficacy relative to **18**. Lastly, incorporation of a secondary amine into the linker (i.e., **21**) did not offer improved efficacy.

A final set of modifications, as highlighted in Table 5, involved installation of secondary and tertiary amides into the linker unit. While 4-fluorobenzyl amide **24** had modest affinity (K_i = 30 μM), evaluation of isomeric **29** revealed that introduction of an amide functionality on opposite side of the linker unit was not tolerated. Following up on amide **24**, N-methylation to afford the corresponding tertiary amide **55** resulted in a 2-fold increase in binding affinity (K_i = 14 μM) with modest acute efficacy. Encouragingly, combination of this 4-fluorobenzyl-*N*-methyl tertiary amide with the previously optimized B- and C-ring substitution patterns gave compound **56** which had a 10-fold increase in binding affinity (K_i = 1.4 μM). Disappointingly, despite this improved binding affinity, **56** did not have in vivo efficacy. As shown for **57**, incorporation of the tertiary amide into a ring system retained binding affinity (K_i = 3.6 μM) and modest efficacy. Lastly, **58**, bearing (3-OMe)-benzyl-*N*-methyl amide, was found to have activity similar to its 4-F counterpart **55**. The fact that these oxaolidinones with amide linkers such as **56** and **57** have low micromolar NPC1L1 binding affinity and modest efficacy is encouraging given that to our knowledge, such modifications have not been reported on the extensively studied ezetimibe template. This suggests

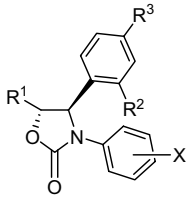
Table 4. NPC1L1 binding and acute CAI efficacy of oxazolidinones **18**, **21**, and **50–54**[19,20]

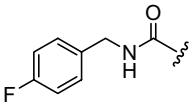
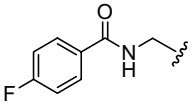
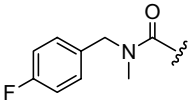
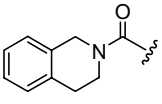
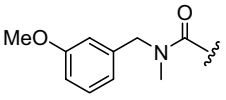


Compound	R^1	R^2	X	R^3	R^4	R^5	BBM NPC1L1 K_i (μM)	Acute CAI at 3 mg/kg
18	F	H	O	H	OH	4-F	28	−55%
50	F	H	O	OH	OMe	3-F	28	0%
51	Cl	H	O	H	OH	4-F	49	NT
52	OMe	H	O	H	OH	4-F	>50	NT
53	H	OMe	O	H	OH	4-F	15	−13%
54	H	OMe	O	OH	OMe	3-F	8.7	−26%
21	F	H	NH	H	OH	4-F	19	−5%

NT, not tested.

Table 5. NPC1L1 binding and acute CAI efficacy of oxazolidinones **24**, **29**, and **55–57**[19,20]



Compound	R^1	R^2	R^3	X	BBM NPC1L1 K_i (μM)	Acute CAI at 3 mg/kg
24		H	OH	4-F	30	NT
29		H	OH	4-F	>50	NT
55		H	OH	4-F	14	−26%
56		OH	OMe	3-F	1.4	0%
57		OH	OMe	3-F	3.6	−21%
58		H	OH	4-F	11	−24%

NT, not tested.

that the novel substituent vectors provided by the current oxazolidinone core may offer further additional opportunity in this region of the template.

In addition to analog optimization, the current studies also offered an opportunity to evaluate the still developing relationship between NPC1L1 binding and CAI efficacy.[6,8] Figure 4 highlights a retrospective analysis wherein acute in vivo efficacy is plotted against NPC1L1 binding for analogs evaluated in both assays. While the impact of pharmacokinetic differences amongst the analogs cannot be discounted, binning analogs by color

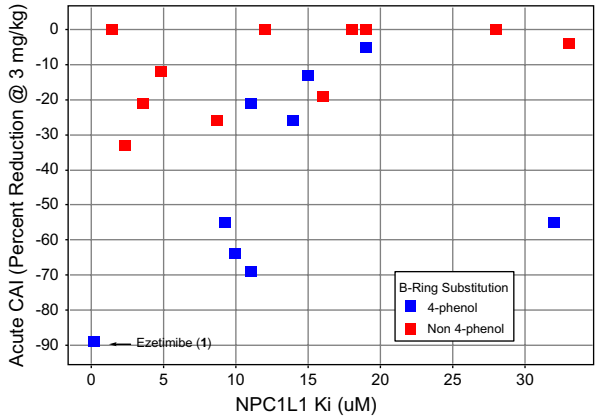


Figure 4. Relationship between acute efficacy and NPC1L1 affinity.

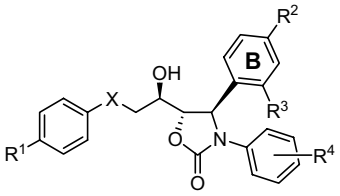
with regard to whether they contained (blue) or did not contain (red) a 4-OH substituent on the B-ring revealed an interesting trend. Specifically, among the analogs tested, NPC1L1 binding affinity and the presence of a 4-OH substituted B-ring were necessary for significant efficacy (>50% reduction at 3 mg/kg).

It is well established that the 4-phenol of ezetimibe is rapidly glucuronidated in vivo and the resulting glucuronide adduct has activity comparable to the parent molecule (vida supra).[6] To understand how such glucuronidation might relate to efficacy in the current oxazolidinone series, we compared the metabolism of ezetimibe and a representative set of oxazolidinone analogs (Table 6: **18**, **38**, **39**, and **41**) in a rat hepatocyte assay and performed metabolite identification studies on each compound. For ezetimibe (**1**) and oxazolidinones (**18**, **38**, **41**) all bearing a B-ring 4-OH (Table 6, R^2 = OH), the corresponding 4-*O*-β-glucuronide was the predominant if not the exclusive metabolite. By contrast, **39**, lacking a B-ring 4-OH (Table 6, R^2 = OMe), underwent oxidative metabolism. For compounds where glucuronidation was the predominant metabolic pathway, the half-life ($T_{1/2}$) of the parent molecule in the hepatocyte assay may be taken as an estimate of the efficiency with which that molecule is glucuronidated. As shown in Table 6, ezetimibe (**1**) had the shortest half-life ($T_{1/2}$ = 43 min) suggesting that it is very efficiently converted to its glucuronide adduct. By this same measure, oxazolidinones **18** and **41** were converted to their corresponding glucuronides more efficiently than either **38** or **39**. Interestingly, despite the fact that **39** (K_i = 4.8 μM) is 5-fold more potent than **18** (K_i = 28 μM), the latter is more efficacious in vivo, perhaps due to its ability to be efficiently converted to its glucuronide. Similarly, while **38** (K_i = 9.2 μM) and **41** (K_i = 11 μM) have similar binding potencies, **41** is glucuronidated more efficiently as suggested by its lower hepatocyte $T_{1/2}$ (71 vs 136 min) perhaps accounting for its somewhat greater in vivo efficacy.

The suggested importance of glucuronidation to efficacy in this oxazolidinone series is interesting and may parallel the situation for ezetimibe (**1**). It is known that rapid glucuronidation of ezetimibe in vivo facilitates enterohepatic recirculation of the drug thereby increasing its half-life and facilitating localization (via bile delivery) of the equipotent glucuronidated drug to its intestinal site of action. Additionally, one might propose that the formation of equipotent glucuronide conjugates might protect the ligands from oxidative metabolism that would otherwise render the compounds inactive.[23] While understanding the role of glucuronidation in the efficacy of this oxazolidinone series will require additional experimentation, the preliminary data suggest that further structure–activity optimization may require attention to both NPC1L1 binding and glucuronidation efficiency.

We have described the optimization of a series of oxazolidinone-based NPC1L1 ligands. These efforts resulted in the identification of analogs with low micromolar NPC1L1 affinity and acute in vivo efficacy. The unique substituent vectors offered by the oxazolidinone enabled the identification of novel modifications such as amide linkers. Finally, we highlighted the impact that glucuronidation may have on analog efficacy.

Table 6. Comparison of relative rates of glucuronidation for **1**, **18**, and **38**–**39**



Compound	R^1	X	R^2	R^3	R^4	BBM NPC1L1 K_i (μM)	Acute CAI at 3 mg/kg	R^2 = O-Gluc major metabolite (%)	Rat hepatocyte $T_{1/2}$ (min)
1	—	—	—	—	—	0.20	−89	Yes	43
18	F	O	OH	H	4-F	28	−55	Yes	67
41	H	CH_2	OH	H	3-F	11	−69	Yes	71
38	H	CH_2	OH	OH	4-F	9.2	−55	Yes	136
39	H	CH_2	OMe	OH	4-F	4.8	−33	No	249

Acknowledgments

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23. For example, evaluation of both ezetimibe (**1**) and its glucuronide (**2**) in a rat liver microsomal stability assay (lacking the cofactors required for glucuronidation) revealed that the glucuronide conjugate was substantially more resistant to oxidative metabolism **1**, $T_{1/2} = 9.5$ min; **2**, $T_{1/2} > 60$ min.