Benzo-fused Lactams from a Diversity-oriented Synthesis (DOS) Library as Inhibitors of Scavenger Receptor BI (SR-BI)-mediated Lipid Uptake

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Benzo-fused lactams from a diversity-oriented synthesis (DOS) library as inhibitors of scavenger receptor BI (SR-BI)-mediated lipid uptake


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**Abstract**

We report a new series of 8-membered benzo-fused lactams that inhibit cellular lipid uptake from HDL particles mediated by Scavenger Receptor, Class B, Type I (SR-BI). The series was identified via a high-throughput screen of the National Institutes of Health Molecular Libraries Small Molecule Repository (NIH MLSMR), measuring the transfer of the fluorescent lipid DiI from HDL particles to CHO cells overexpressing SR-BI. The series is part of a previously reported diversity-oriented synthesis (DOS) library prepared via a build-couple-pair approach. Detailed structure-activity relationship (SAR) studies were performed with a selection of the original library, as well as additional analogs prepared via solution phase synthesis. These studies demonstrate that the orientation of the substituents on the aliphatic ring have a critical effect on activity. Additionally, a lipophilic group is required at the western end of the molecule, and a northern hydroxyl group and a southern sulfonamide substituent also proved to be optimal. Compound 2p was found to possess a superior combination of potency (av IC_{50} = 0.10 μM) and solubility (79 μM in PBS), and it was designated as probe ML312.

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Cholesterol has been widely recognized to have a critical impact on cardiovascular health. Our understanding of the connection between plasma cholesterol levels and adverse cardiovascular events has benefited tremendously from the identification of the proteins and macromolecular complexes involved in cholesterol biosynthesis and transport. This basic research has supported the development of numerous pharmacological interventions, including the development of HMG-CoA reductase inhibitors (statins), which lower LDL-C (low-density lipoprotein cholesterol) and decrease substantially the risk of adverse events and death in patients with high levels of LDL-C.

Despite the identification of the key players involved in cholesterol biosynthesis and transport, our mechanistic understanding of how these pathways are regulated is lacking in some important details. For example, it is now apparent that simply elevating HDL-C (high density lipoprotein cholesterol) levels may not necessarily be beneficial, despite a positive clinical correlation between HDL-C levels and cardiovascular outcomes. The receptor for HDL particles, Scavenger Receptor class B, type I (SR-BI),^1,2^ is one of many Pattern Recognition Receptors and is important for the uptake of HDL-C into liver cells (hepatocytes).^3^ Inhibition of SR-BI has been explored in animal models as a means to boost plasma HDL-C levels, but early results suggest that this approach is counterproductive as a means to improve cardiovascular health.^3,4^ Conversely, SR-BI is a co-receptor for the entry of pathogens into hepatocytes, including Hepatitis C virus (HCV)^5–7,^ and malaria (plasmodium) parasites^8–9,^ and its inhibition has recently been explored as a strategy for HCV patients requiring liver transplant.^10,11^

Though it is now recognized as an important target for the study of lipid metabolism and infectious disease, as well as immune response^12–14^ and female fertility,^1^ the structure and mechanism of SR-BI is not understood in full detail. In an effort to identify novel small molecule modulators of this receptor...
that may exert unique effects on lipid transport, as well as more potent and less toxic compounds, we embarked on a high-throughput screen (HTS) supported by the NIH Molecular Libraries Program (MLP). We recently reported two classes of inhibitors from this screen with distinct advantages over prior inhibitors identified in our laboratories. This work culminated in the nomination of ML27820,21 and ML27922,23 as Molecular Libraries probes. Other small molecule inhibitors of SR-BI have been disclosed, including those from the labs of Sankyo24 and iTherX.25,26 We report here a third class of inhibitors identified from a diversity-oriented synthesis (DOS) library, with a SNAr reaction as the key ring-forming step (Scheme 1).27

One of the more promising scaffolds that emerged from this screen was the benzo-fused lactam represented by compound 1a (Table 1). 1a and related compounds had been prepared as part of a diversity-oriented synthesis (DOS) library, with a SNAr reaction as the key ring-forming step (Scheme 1).28 Since only a selection of the original DOS library had been included in our primary assay, we began our follow-up studies by testing DMSO stock solutions of each of the stereoisomers of 1a for activity. The 2R, 5R, 6R stereochemistry of 1a provided the best results (Table 1); compounds

<table>
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<th>Compd</th>
<th>Stereochem.</th>
<th>IC50 (μM)</th>
<th>Compd</th>
<th>Stereochem.</th>
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<td>2R, 5R, 6S</td>
<td>8.9</td>
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</table>

* Average of at least two measurements in Dil uptake assay with DMSO stock solutions.

One of the more promising scaffolds that emerged from this screen was the benzo-fused lactam represented by compound 1a (Table 1). 1a and related compounds had been prepared as part of a diversity-oriented synthesis (DOS) library, with a SNAr reaction as the key ring-forming step (Scheme 1).28 Since only a selection of the original DOS library had been included in our primary assay, we began our follow-up studies by testing DMSO stock solutions of each of the stereoisomers of 1a for activity. The 2R, 5R, 6R stereochemistry of 1a provided the best results (Table 1); compounds

<table>
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* Average of at least two measurements in Dil uptake assay with resynthesized or repurified compounds.
with different stereochemistry at the carbons of the 8-membered ring (carbons 5 and 6 according to our convention\textsuperscript{29}) suffered from a sizable drop in potency. Inversion of stereochemistry at the 2-position caused a less substantial drop in activity (compound 1ad).

Full SAR studies continued with reverse-phase HPLC repurification of a large number analogs of 1a with the 2R, 5R, 6R stereochemistry. First, the substituents of the southern amine were examined. A selection of these results is given in Table 2. Our lead compound, 4-chlorophenylsulfonamide 1a, inhibited DiI-HDL uptake with \( IC_{50} = 0.46 \mu M \) when retested as a purified dry powder. The 4-fluorophenyl analog 1b was comparable in potency (\( IC_{50} = 0.56 \mu M \)), but the larger and more electron-rich 4-methoxy analog 1c suffered a significant decrease in potency (\( IC_{50} = 2.0 \mu M \)).

The unsubstituted benzenesulfonamide 1d also showed good potency, but in early assays it was not superior to the 4-chloro analog 1a. Interestingly, thiophene 1e was not a useful isostere (\( IC_{50} = 2.4 \mu M \)). One liability of 1a is its low solubility (0.1 \( \mu M \) PBS with 1\% DMSO). The imidazole analog 1f possessed good solubility (79 \( \mu M \)), but was only weakly potent (\( IC_{50} = 6.4 \mu M \)). The truncated methylsulfonamide 1g also showed good solubility but weak potency. A series of ureas from the original DOS library (including 1h and 1i) were mediocre inhibitors. Many amine compounds with both small (1j, 1k) and larger (1l, 1m, 1n, 1p, 1r) N-alkyl substituents were also mediocre, though the 4-CF\(_3\) benzyl analog 1o showed moderate levels of inhibition (\( IC_{50} = 0.65 \mu M \)).

Keeping the southern 4-chlorophenylsulfonamide of 1a in place, we proceeded to test analogs with variable substitutions at the western nitrogen (Table 3). This region is very sensitive to modification, as moving from the cyclohexyl amide 2e (\( IC_{50} = 0.054 \mu M \)), to the less bulky and lipophilic cyclopropyl amide 2g (\( IC_{50} = 2.1 \mu M \)), gave a nearly 40-fold drop in potency. It is evident that lipophilic, uncharged substituents are highly preferred at this location. The trifluoromethyl substituted amide 2b showed moderate inhibition (\( IC_{50} = 0.26 \mu M \)), but molecules with terminal oxygens or nitrogens that can be protonated or accept hydrogen bonds were poor inhibitors (2c, 2d, 2f, 2h, 2n). The lone

### Table 3

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<tr>
<th>Compd</th>
<th>R(^1)</th>
<th>( IC_{50} ) (( \mu M ))</th>
<th>Compd</th>
<th>R(^1)</th>
<th>( IC_{50} ) (( \mu M ))</th>
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<tr>
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<td>2p</td>
<td>Me,N</td>
<td>0.10 ± 0.02(^b)</td>
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<td>0.19</td>
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\(^a\) Average of at least two measurements in Dil uptake assay with resynthesized or repurified compounds.

\(^b\) Standard error of mean, \( N = 3 \).
exceptions observed were the isoxazole sulfonamide 2t (IC\textsubscript{50} = 0.19 \textmu M), which has a unique steric and electronic environment about its terminal nitrogen, and the 4-methoxyphenyl urea 2m (IC\textsubscript{50} = 0.30 \textmu M). Several other ureas also showed good activity, including 2l (IC\textsubscript{50} = 0.092 \textmu M), 2o (IC\textsubscript{50} = 0.35 \textmu M), and the isopropyl urea 2p (IC\textsubscript{50} = 0.10 \textmu M). Several sulfonamides at this position that were tested showed weak inhibition (2q to 2s). Interestingly, the highly simplified N,N-dimethylaniline 8 (Table 4) gave modest inhibition (IC\textsubscript{50} = 0.63 \textmu M).

To obtain additional insights into the functionality and structural requirements for inhibition of SR-BI, several additional analogs were prepared and tested during the course of this project (Table 4). Changing the position on the benzene ring of the western amine substituent gave an inactive analog (3) of isopropyl urea 2p. Expansion of the lactam scaffold from an 8-membered ring to a 9-membered ring (4) also abrogated the activity. Further investigation of the southern nitrogen substituent indicated that a sulfonamide is optimal, as the southern amide analog 5 of sulfonamide 2e was inactive. The position of the chlorine substituent was also examined with respect to the western cyclohexyl amide 2e. 3-Chloro (6, IC\textsubscript{50} = 0.80 \textmu M) and 2-chlorophenyl (7, IC\textsubscript{50} = 0.18 \textmu M) analogs showed decreased inhibition relative to 2e.

The hydroxyl group present in all compounds reported thus far served as an anchor for attachment of the DOS scaffolds to a solid support for library preparation,\textsuperscript{30} and also for attachment of library compounds to solid surfaces for small molecule microarray screens.\textsuperscript{31} Therefore, the SAR around this region remained unexplored. Treatment of 1a with DPPA and DBU, followed by Staudinger reduction with triphenylphosphine in THF/water, provided primary amine 9, which proved to be inactive. Displacement of the intermediate azide with dimethylamine gave the tertiary amine 10, which was also inactive. The methyl ether analog of 2p was also prepared and found to be only weakly active (11). Limited attempts at reductive deoxygenation of 2p were unsuccessful, so the deoxy analog 12 was prepared via a de novo route (see Supporting information) and also found to have weak activity, suggesting that the hydroxyl group is required for substantial inhibition of SR-BI with this scaffold.

The water solubility of the lead compound in this series (1a, 0.1 \textmu M in PBS) was reminiscent of the poor water solubility of

<table>
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<th>Structure</th>
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</table>

\textsuperscript{a} Average of at least two measurements in Dil uptake assay.
one of our previous probes (ML278, 0.57 μM in PBS). Fortunately, several of the potent analogs screened in these studies showed much higher solubility. In particular, the disopropyl urea 2p exhibited the best combination of potency (average IC_{50} = 0.10 μM, Supporting Information Fig. S1) and solubility (79 μM in PBS). As this compound was present in our original DOS library along with all its stereoisomers, we were able to confirm that the 2R, 5R, 6R stereochirality of 2p is optimal, in line with the results with 1a. 2p was nominated as Molecular Libraries Probe ML312. Its re-synthesis is outlined in Scheme 2.

Our most promising compounds in this project were profiled in a series of secondary assays to gain insights into their mode of action and potential for further development. None of the compounds in Tables 1–4 showed any measurable cytotoxicity after incubation with the cell line used in our assays (lilA[mSR-BI]) for 24 h, using a CellTiter-Glo assay (Promega) to measure cellular ATP levels. After incubation with mouse and human liver microsomes for 1 h, the uptake of [3H]CE labeled transferrin was monitored. ML312 showed no inhibition of receptor-mediated endocytosis, the endocytosis of Alexa-594-labeled HDL in the presence of ML312, the uptake of [3H]CE after incubation with mouse and human plasma, and 94.7% bound in mouse plasma. ML312 has limited metabolic stability, with 25% and 10% remaining after incubation with mouse and human liver microsomes for 1 h.

Additional studies with ML312 were performed to study its mode of action. In addition to measuring the uptake of Dil from Dil-HDL in the presence of ML312, the uptake of [3H]CE was measured. ML312 inhibited [3H]CE uptake with a measured IC_{50} of 0.25 μM (Supporting information Fig. S2), a result superior to the clinical compound ITX-2104, but inferior to our alternative probes ML278 and ML279. The binding of Alexa-488-labeled HDL particles to lilA[mSR-BI] cells via SR-BI was also measured. As with our prior probes, ML312 enhanced the level of HDL binding to SR-BI, with an EC_{50} of 0.4 μM (Supporting information Fig. S3), a phenomenon that we have observed previously. One possibility is that such compounds act to inhibit lipid transport by preventing release of the endocytosed lipid by blocking receptor-mediated endocytosis, the endocytosis of Alexa-594-labeled transferrin was monitored. ML312 showed no inhibition of endocytosis by lilA[mSR-BI] cells at concentrations up to 35 μM. Additionally, binding studies were performed with a panel of 67 different receptors and secondary targets (see Supporting information for details). At a concentration of 10 μM, no targets were inhibited by 20% or more, with the highest level of inhibition observed with the L-type Ca^{2+} channel (18% inhibition). It thus appears to have better selectivity than our previously reported probe ML278, perhaps due to its multiple stereocenters and more complex 3D structure.

In summary, potent inhibitors of SR-BI-mediated lipid (Dil) uptake were discovered as part of the NIH Molecular Libraries Probe Production Centers Network (MLPCN) initiative. Profiling and SAR analyses of several top compounds led to the nomination of the benzo-fused lactam 2p (ML312) as a probe compound for SR-BI. ML312 has superior solubility to the other probes identified in this project, is not cytotoxic, has no significant chemical liabilities, and appears to act selectively at SR-BI, though it is less potent than ML278 (IC_{50} = 6 nM) or ML279 (IC_{50} = 17 nM) in the primary assay.

Acknowledgments

We thank Eamon Comer for providing compound 13 and Carrie Mosher, Travis Anthoine, and Mike Lewandowski for analytical chemistry support.

Supplementary data

Supplementary data (preparation and characterization of 2p (ML312), 11, and 12; compound profiling protocols; representative dose–response curves of ML312 in Dil-HDL uptake assay, [3H]CE uptake assay, and HDL binding assay; assay protocols; and secondary target screening data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.03.073.

References and notes


