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

Chris Dockendorff[a,b,*], **Patrick W. Faloon**[a], **Jun Pu**[a], **Miao Yu**[c], **Stephen Johnston**[a], **Melissa Bennion**[a], **Marsha Penman**[c], **Thomas J. F. Nieland**[c], **Sivaraman Dandapani**[a], **José R. Perez**[a], **Benito Munoz**[a], **Michelle A. Palmer**[a], **Stuart L. Schreiber**[a,d], and **Monty Krieger**[c,*]

[a]Center for the Science of Therapeutics, Broad Institute, 7 Cambridge Center, Cambridge, MA, 02142, USA

[b]Department of Chemistry, Marquette University, P.O. Box 1881, Milwaukee, WI, 53201-1881, USA

[c]Department of Biology, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA, 02139, USA

[d]Howard Hughes Medical Institute, Broad Institute, 7 Cambridge Center, Cambridge, MA, 02142, USA

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.

*Corresponding authors: Tel.: +1-414-288-1617; christopher.dockendorff@marquette.edu (C.D.), Tel.: +1-617-253-6793; krieger@MIT.edu (M.K.).

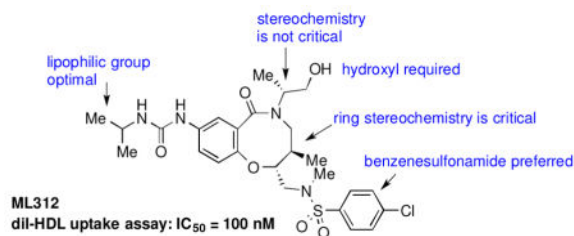Compound **2p** was found to possess a superior combination of potency (avg. IC$_{50}$ = 0.10 μM) and solubility (79 μM in PBS), and it was designated as probe ML312.

Graphical Abstract

Keywords

ML312; SR-BI inhibitor; HDL receptor; cholesterol transport; DOS macrocycle

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,[3] the structure and mechanism of SR-BI is not understood in full detail.[15–18] 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.[19] This work culminated in the nomination of ML278[20–21] and ML279[22–23] as Molecular Libraries probes. Other small molecule inhibitors of SR-BI have been disclosed, including those from the labs of Sankyo[4] and iTherX.[24–28] We report here a third class of inhibitors identified from a diversity-oriented synthesis (DOS) library, some members of which form a subset of the NIH Molecular Libraries Small Molecule Repository. Screening and assay data for this project are also publicly available in the PubChem database (http://pubchem.ncbi.nlm.nih.gov/). The primary assay involved measuring the uptake of the fluorescent lipid 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) from DiI-labeled HDL particles into CHO cells overexpressing mouse SR-BI (ldlA[mSR-BI]).[22] 3,046 compounds (0.96%) were classified as inhibitors of DiI-HDL uptake out of 319,533 compounds tested, with inhibition at 12.5 μM of   70% of the level inhibition with 1 μM BLT-1 (positive control). Hit compounds were omitted that were on plates with $Z' < 0.3$ or that were active in 10% or more of the HTS assays listed in PubChem. A counterscreen was also performed that rejected hit compounds that quenched the fluorescence of DiI-HDL in a dose-dependent manner.

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 S_NAr reaction as the key ring-forming step (Scheme 1).[29] 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 2*R*, 5*R*, 6*R* stereochemistry of **1a** provided the best results (Table 1); compounds with different stereochemistry at the carbons of the 8-membered ring (carbons 5 and 6 according to our convention[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 2*R*, 5*R*, 6*R* 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 $IC_{50} = 0.46$ μM when retested as a purified dry powder. The 4-fluorophenyl analog **1b** was comparable in potency ($IC_{50} = 0.56$ μM), but the larger and more electron-rich 4-methoxy analog **1c** suffered a significant decrease in potency ($IC_{50} = 2.0$ μM). 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 ($IC_{50} = 2.4$ μM). One liability of **1a** is its low solubility (0.1 μM in PBS with 1% DMSO). The imidazole analog **1f** possessed good solubility (79 μM), but was only weakly potent ($IC_{50} = 6.4$ μM). 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 ($IC_{50} = 0.65$ μM).

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 μM), to the less bulky and lipophilic cyclopropyl amide **2g** (IC_{50} = 2.1 μM), 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 μM), 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 exceptions observed were the isoxazole sulfonamide **2t** (IC_{50} = 0.19 μM), which has a unique steric and electronic environment about its terminal nitrogen, and the 4-methoxyphenyl urea **2m** (IC_{50} = 0.30 μM). Several other ureas also showed good activity, including **2l** (IC_{50} = 0.092 μM), **2o** (IC_{50} = 0.35 μM), and the isopropyl urea **2p** (IC_{50} = 0.10 μM). 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_{50} = 0.63 μM).

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_{50} = 0.80 μM) and 2-chlorophenyl (**7**, IC_{50} = 0.18 μM) 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,[30] and also for attachment of library compounds to solid surfaces for small molecule microarray screens.[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 μM in PBS) was reminiscent of the poor water solubility of 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 diisopropyl urea **2p** exhibited the best combination of potency (average IC_{50} = 0.10 μM, Supporting Information Figure 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 2*R*, 5*R*, 6*R* stereochemistry 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 to 4 showed any measurable cytotoxicity after incubation with the cell line used in our assays (ldlA[mSR-BI]) for 24 h, using a CellTiter-Glo assay (Promega) to measure cellular ATP levels. ML312 also exhibited excellent plasma stability, with >99% remaining after 5 h incubation with human or mouse plasma. Plasma protein binding (PPB) studies showed that it was 94.5% bound in 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 DiI from DiI-HDL in the presence of ML312, the uptake of $[^3H]$-labeled cholesteryl oleate ester ($[^3H]CE$) was measured. ML312 inhibited $[^3H]CE$ uptake with a measured IC_{50} of 0.25 μM (Supporting Information Figure S2), a result superior to the clinical compound ITX-5061, but inferior to our alternative probes ML278 and ML279. The binding of Alexa-488-labeled HDL particles to ldlA[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 Figure S3), a phenomenon that we have observed previously.[21] One possibility is that such compounds act to inhibit lipid transport by preventing release of the bound HDL particles from the receptor. Finally, to determine if ML312 might have inhibited SR-BI-mediated lipid uptake by blocking receptor-mediated endocytosis, the endocytosis of Alexa-594-labeled transferrin was monitored. ML312 showed no inhibition of endocytosis by ldlA[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 (19% 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.[32]

In summary, potent inhibitors of SR-BI-mediated lipid (DiI) 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.[33] 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References and notes

1. Acton S, Rigotti A, Landschulz KT, Xu S, Hobbs HH, Krieger M. Science. 1996; 271:518. [PubMed: 8560269]

2. Rigotti A, Miettinen HE, Krieger M. Endocr Rev. 2003; 24:357–387. [PubMed: 12788804]

3. Trigatti B, Rayburn H, Viñals M, Braun A, Miettinen H, Penman M, Hertz M, Schrenzel M, Amigo L, Rigotti A, Krieger M. Proc Nat Acad Sci USA. 1999; 96:9322. [PubMed: 10430941]

4. Kitayama K, Nishizawa T, Abe K, Wakabayashi K, Oda T, Inaba T, Amemiya Y. J Pharm Pharmacol. 2006; 58:1629. [PubMed: 17331327]

5. Voisset C, Callens N, Blanchard E, Op De Beeck A, Dubuisson J, Vu-Dac N. J Biol Chem. 2005; 280:7793. [PubMed: 15632171]

6. Catanese MT, Graziani R, von Hahn T, Moreau M, Huby T, Paonessa G, Santini C, Luzzago A, Rice CM, Cortese R, Vitelli A, Nicosia A. J Virol. 2007; 81:8063. [PubMed: 17507483]

7. Catanese MT, Ansuini H, Graziani R, Huby T, Moreau M, Ball JK, Paonessa G, Rice CM, Cortese R, Vitelli A, Nicosia A. J Virol. 2010; 84:34. [PubMed: 19828610]

8. Rodrigues CD, et al. Cell Host & Microbe. 2008; 4:271–282. [PubMed: 18779053]

9. Yalaoui S, Huby T, Franetich JF, Gego A, Rametti A, Moreau M, Collet X, Siau A, van Gemert GJ, Sauerwein RW, Luty AJ, Vaillant JC, Hannoun L, Chapman J, Mazier D, Froissard P. Cell Host & Microbe. 2008; 4:283. [PubMed: 18779054]

10. Zhu H, Wong-Staal F, Lee H, Syder A, McKelvy J, Schooley RT, Wyles DL. J Infect Dis. 2012; 205:656. [PubMed: 22279172]

11. Sulkowski MS, Kang M, Matining R, Wyles D, Johnson VA, Morse GD, Amorosa V, Bhattacharya D, Coughlin K, Wong-Staal F, Glesby MJ. J Infect Dis. 2014; 209:658. [PubMed: 24041792]

12. Fioravanti J, Medina-Echeverz J, Berraondo P. Immunotherapy. 2011; 3:395. [PubMed: 21395381]

13. Guo L, Song Z, Li M, Wu Q, Wang D, Feng H, Bernard P, Daugherty A, Huang B, Li XA. J Biol Chem. 2009; 284:19826. [PubMed: 19491399]

14. Zhu P, Liu X, Treml LS, Cancro MP, Freedman BD. J Biol Chem. 2009; 284:22878. [PubMed: 19542230]

15. Yu M, Romer KA, Nieland TJ, Xu S, Saenz-Vash V, Penman M, Yesilaltay A, Carr SA, Krieger M. Proc Nat Acad Sci USA. 2011; 108:12243. [PubMed: 21746906]

16. Papale GA, Hanson PJ, Sahoo D. Biochemistry. 2011; 50:6245. [PubMed: 21675794]

17. Gaidukov L, Nager AR, Xu S, Penman M, Krieger M. J Biol Chem. 2011; 286:18452. [PubMed: 21454587]

18. Neculai D, Schwake M, Ravichandran M, Zunke F, Collins RF, Peters J, Neculai M, Plumb J, Loppnau P, Pizarro JC, Seitova A, Trimble WS, Saftig P, Grinstein S, Dhe-Paganon S. Nature. 2013; 504:172. [PubMed: 24162852]

19. Nieland TJ, Penman M, Dori L, Krieger M, Kirchhausen T. Proc Nat Acad Sci USA. 2002; 99:15422. [PubMed: 12438696]

20. [accessed February 4, 2015] See http://www.ncbi.nlm.nih.gov/books/NBK133420/

21. Dockendorff C, Faloon PW, Yu M, Youngsaye W, Penman M, Nieland TJF, Nag PP, Lewis TA, Pu J, Bennion M, Negri J, Paterson C, Lam G, Dandapani S, Perez JR, Munoz B, Palmer MA, Schreiber SL, Krieger M. ACS Med Chem Lett. 2015 In press. 10.1021/ml500154q

22. [accessed February 4, 2015] http://www.ncbi.nlm.nih.gov/books/NBK143554/

23. Dockendorff C, Faloon PW, Germain A, Yu M, Youngsaye W, Nag PP, Bennion M, Nieland TJF, Dandapani S, Perez JR, Munoz B, Palmer MA, Schreiber SL, Krieger M. Bioorg. Med. Chem. Lett. 2015 submitted (insert details here).

24. Masson D, Koseki M, Ishibashi M, Larson CJ, Miller SG, King BD, Tall AR. Arterioscler Thromb Vasc Biol. 2009; 29:2054. [PubMed: 19815817]

25. Wong-Staal F, Syder AJ, McKelvy JF. Viruses. 2010; 2:1718. [PubMed: 21994703]

26. Syder AJ, Lee H, Zeisel MB, Grove J, Soulier E, Macdonald J, Chow S, Chang J, Baumert TF, McKeating JA, McKelvy J, Wong-Staal F. J Hepatol. 2011; 54:48. [PubMed: 20932595]

27. Mittapalli GK, Jackson A, Zhao F, Lee H, Chow S, McKelvy J, Wong-Staal F, Macdonald JE. Bioorg Med Chem Lett. 2011; 21:6852. [PubMed: 21978675]

28. Mittapalli GK, Zhao F, Jackson A, Gao H, Lee H, Chow S, Pal Kaur M, Nguyen N, Zamboni R, McKelvy J, Wong-Staal F, Macdonald JE. Bioorg Med Chem Lett. 2012; 22:4955. [PubMed: 22784640]

29. Marcaurelle LA, et al. J Am Chem Soc. 2010; 132:16962. [PubMed: 21067169]

30. Ryba TD, Depew KM, Marcaurelle LA. J Comb Chem. 2009; 11:110. [PubMed: 19049425]

31. Miao H, Tallarico JA, Hayakawa H, Munger K, Duffner JL, Koehler AN, Schreiber SL, Lewis TA. J Comb Chem. 2007; 9:245. [PubMed: 17348730]

32. Lovering F, Bikker J, Humblet CJ. Med Chem. 2009; 52:6752.

33. [accessed February 4, 2015] http://www.ncbi.nlm.nih.gov/books/NBK143554/
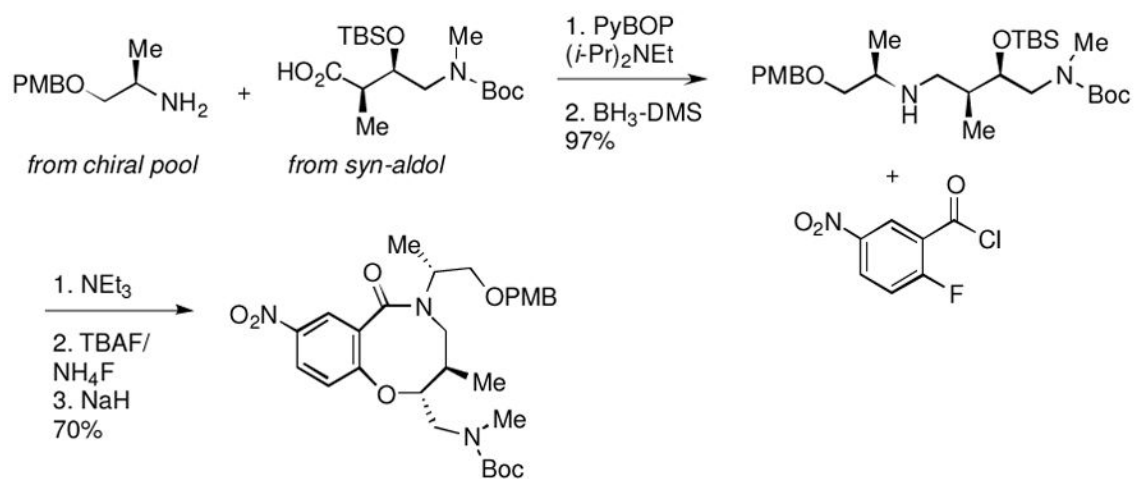
Scheme 1.
Previous synthesis of core scaffold for library synthesis via S$_N$Ar reaction
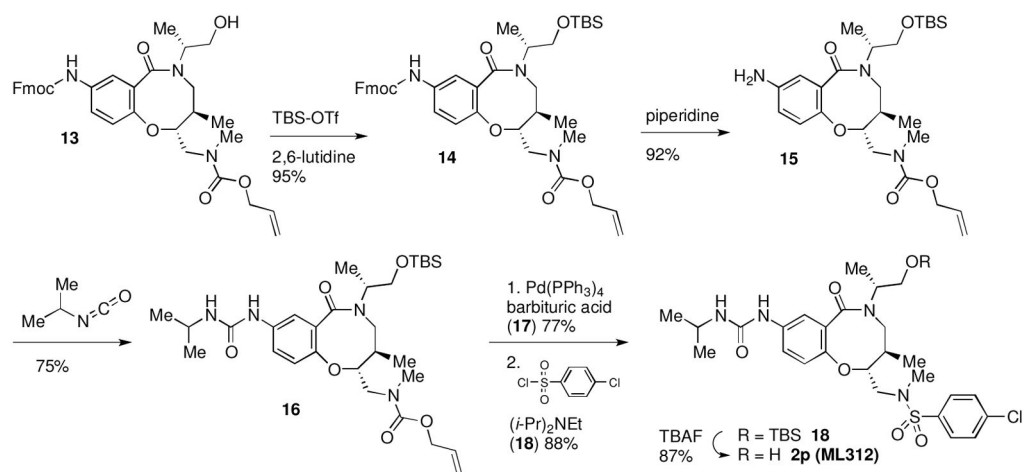
Scheme 2.
Synthesis of **2p** (ML312)

Table 1

SSAR of lead compound **1a**
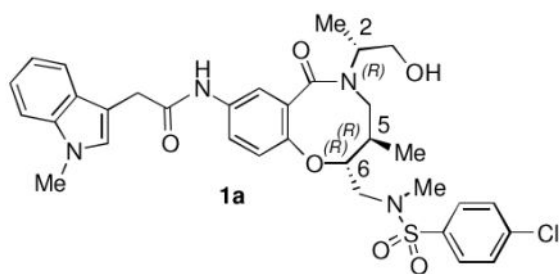
Cmp	Stereochem.	$IC_{50}(\mu M)^{a}$
1a	*2R, 5R, 6R*	0.18
1ab	*2R, 5R, 6S*	11.5
1ac	*2R, 5S, 6R*	>25
1ad	*2S, 5R, 6R*	0.91
1ae	*2S, 5S, 6S*	17.4
1af	*2S, 5S, 6R*	12.3
1ag	*2S, 5R, 6S*	10.0
1ah	*2R, 5R, 6S*	8.9

[a]Average of at least two measurements in DiI uptake assay with DMSO stock solutions.

Table 2

SAR of southern amine substituent
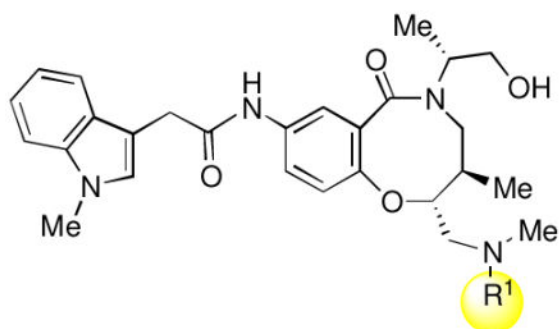
Cmp	R¹	IC$_{50}$(μM)^a
1a		0.46
1b		0.56
1c		2.0
1d		0.22
1e		2.4
1f		6.4
1g		9.9
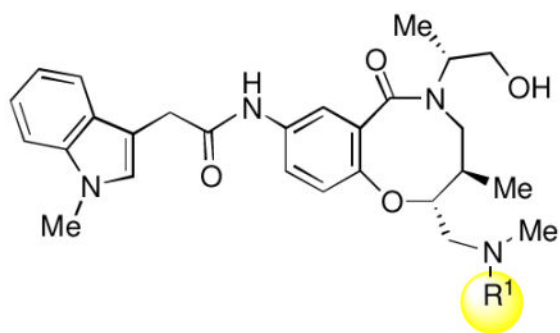
Cmp	R^1	$IC_{50}(\mu M)^a$
1h		4.9
1i		9.2
1j		>25
1k		18
1l		9.2
1m		1.6
1n		2.6
1o		0.65
1p		>25
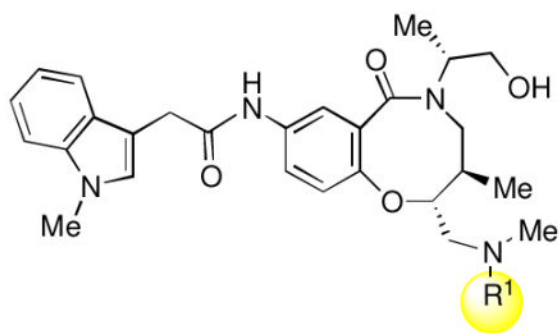
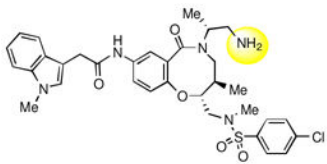| Cmp	R^1	IC$_{50}$(μM)^a
1q		0.89
1r		1.3

a Average of at least two measurements in DiI uptake assay with resynthesized or repurified compounds.

Table 3

SAR of western amine substituent
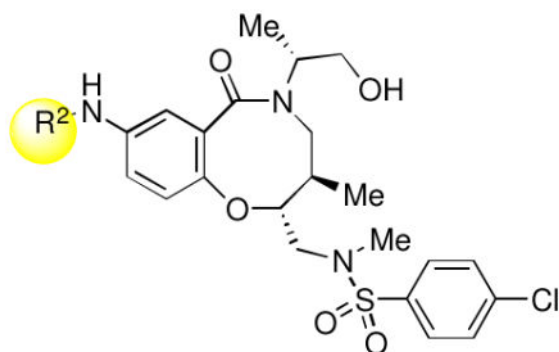
Cmp	R¹	IC$_{50}$(µM)^{a}
1a		0.46
2b		0.26
2c		>25
2d		5.0
2e		0.054
2f		>25
2g		2.1
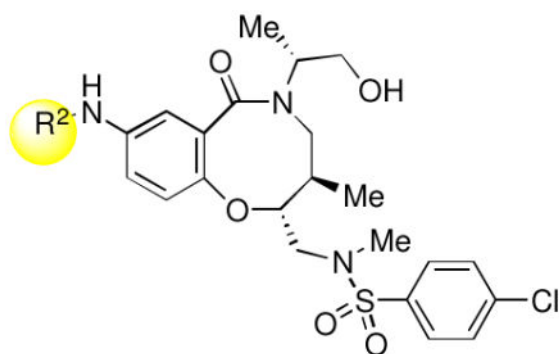
Cmp	R^1	IC$_{50}$(µM)^a
2h		10
2i		>25
2l		0.092
2m		0.30
2n		7.1
2o		0.35
2p		0.10 ± 0.02^b
2q		1.6
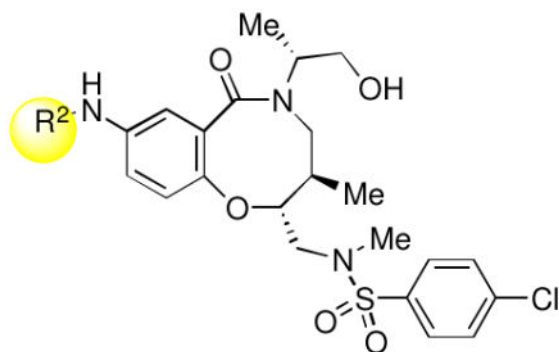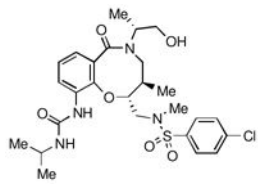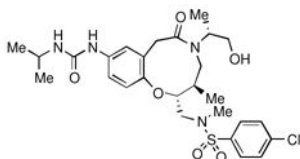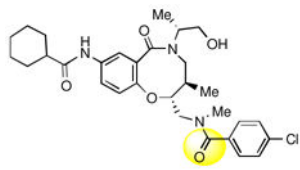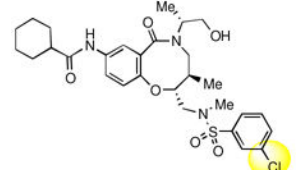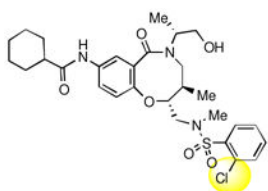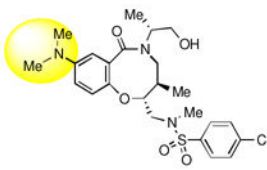
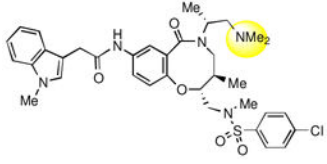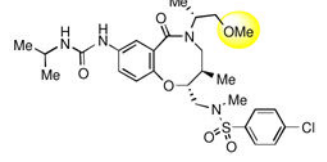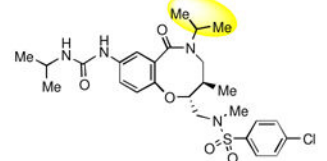| Cmp	R^1	IC$_{50}$(µM)^a
2r		3.2
2s		14
2t		0.19

^{a}Average of at least two measurements in DiI uptake assay with resynthesized or repurified compounds.

^{b}Standard error of mean, N = 3.

Table 4

Various analogs

Cmp	Structure	IC$_{50}$(μM)^a
3		>25
4		>25
5		>25
6		0.80
7		0.18
8		0.63

Cmp	Structure	IC$_{50}$(μM)^a
9		>25
10		>25
11		8.0
12		9.9

^{a}Average of at least two measurements in DiI uptake assay.