Bioorganic & Medicinal Chemistry Letters 26 (2016) 1901–1904

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Identification of nonabsorbable inhibitors of the scavenger receptor-BI (SR-BI) for tissue-specific administration

Steven M. Sparks *, Huiqiang Zhou, Claudia Generaux, Lindsey Harston, David Moncol, Channa Jayawickreme, Janet Parham, Patrick Condreay, Thomas Rimele

GlaxoSmithKline, Enteroendocrine Discovery Performance Unit and Platform Technology and Science, 5 Moore Drive, Research Triangle Park, NC 27709, United States

ARTICLE INFO

Article history:
Received 14 December 2015
Revised 7 March 2016
Accepted 8 March 2016
Available online 9 March 2016

Keywords:
Scavenger receptor class B type I
SR-BI inhibitor
Nonabsorbed SR-BI inhibitor
Intestinal SR-BI

ABSTRACT

The identification of a low-permeability scavenger receptor BI (SR-BI) inhibitor starting from the ITX-5061 template is described. Structure–activity and structure–permeability relationships were assessed for analogs leading to the identification of compound **8** as a potent and nonabsorbable SR-BI inhibitor.

Introduction

The scavenger receptor class B type I (SR-BI) is a multifunctional transmembrane receptor present in a variety of tissues and cell types where its main functions govern the selective uptake of sterols and lipids.[1–4] SR-BI has the highest expression in tissues regulating sterol metabolism including the liver, intestine, and the steroidogenic tissues, the adrenal glands, ovaries and testes. The receptor has been widely studied for its role in cholesterol and cholesterol ester exchange between cells and lipoprotein particles including high-density lipoprotein (HDL). With these functions SR-BI plays a key role in the process of reverse cholesterol transport in the liver, where it provides a mechanism for the reuptake of cholesterol from HDL.[5] SR-BI has also been implicated in inflammatory processes governed by oxidized lipids via the receptor's recognition of oxidized lipoprotein particles including oxidized low density lipoprotein (OxLDL).[6]

In addition to lipid particle binding and the subsequent role played in lipid metabolism, SR-BI has been implicated in the entry of viral particles and parasites into host cells.[7,8] The receptor's role as a key co-factor required for Hepatitis C Virus (HCV) entry has been extensively studied, with inhibitors of SR-BI now advancing in clinical trials as HCV entry inhibitors.[9,10] Entry of *Plasmodium* sporozoites, the causative agents of malaria, into hepatocytes has also been postulated to occur through interaction with SR-BI.[11–13]

Our interest in SR-BI results from the numerous papers detailing the intestinal physiology mediated by the receptor where it also acts as a mediator of sterol and hydrophobic small molecule (vitamins and carotenoids) uptake.[14–17] Postprandial micelles (PPM) composed of dietary lipids resulting from the breakdown of triglycerides to fatty acids and monacylgycerol mix with bile salts in the lumen to form micelles which are recognized by SR-BI at the enterocyte brush border membrane. Upon PPM binding, SR-BI acts as a lipid sensor which initiates apolipoprotein B trafficking from the apical membrane within the enterocytes and is thus involved in intestinal lipoprotein production.[18] Indeed, upregulation of intestinal SR-BI under conditions of insulin resistance in rodents is associated with the overproduction of apolipoprotein B48-containing lipoproteins from the intestine.[19] In addition, overexpression of SR-BI has been shown to accelerate lipid absorption in rodents.[20]

While intestinal modulation of SR-BI may provide beneficial effects for ailments caused by dysregulated intestinal lipid metabolism including dyslipidemia, diabetes and obesity, to date only tissue selective transgenic modulation has allowed for the study of the receptor at the intestinal level. To further explore the biology of SR-BI in the intestine, we sought to develop low-permeability inhibitors of the receptor. These compounds are designed to be orally administered and nonabsorbable, eliciting their potential therapeutic effects via inhibition of SR-BI locally in the GI-tract.[21,22]

* Corresponding author at present address: Viamet Pharmaceuticals, 4505 Emperor Blvd., Suite 300, Durham, NC 27703, United States. Tel.: +1 919 467 8539x317.

E-mail address: ssparks@viamet.com (S.M. Sparks).

http://dx.doi.org/10.1016/j.bmcl.2016.03.025
0960-894X/

After a survey of known SR-BI inhibitors,[23–28] we choose ITX-5061 (**1**) as our starting point (Fig. 1). ITX-5061 (**1**) was developed as a p38 mitogen-activated protein kinase (MAPK) inhibitor which showed increased HDL-cholesterol levels in humans that was subsequently attributed to the compound's activity as a SR-BI inhibitor.[29] Owing to the compound's high polar surface area (tPSA = 123 Å^2), we surmised that addition of polar or charged functionalities off of either the sulfonamide or morpholino termini of ITX-5061 would limit cellular permeation and allow for the identification of a nonabsorbed SR-BI inhibitor.

Chemistry for the preparation of modified sulfonamide analogs of ITX-5061 is shown in Scheme 1. Alkylation of 1-napthol followed by Friedel–Crafts acylation and saponification provided the desired ketoacids **A**. Coupling with 5-*t*-butyl-2-methoxybenzene-1,2-diamine followed by treatment with the appropriate sulfonyl chloride afforded the substituted sulfonamides **B**. For compounds **5**–**8**, SEM protection of the sulfonamide nitrogen was conducted to prevent intramolecular cyclization of the 3-chloropropylsulfonamide intermediate. Subsequent treatment with the desired amines followed by deprotection provided the final compounds **C**. In select cases ester saponification was required to afford the final carboxylic acid-containing analogs.

Chemistry for the preparation of preparation of morpholino replacements of ITX-5061 is shown in Scheme 2. Bromide derivative **D** was treated directly with substituted amines to provide either tertiary amine **15**, quaternary amines **9** and **13**, or intermediate esters which required saponification to yield the desired carboxylic acids **14** and **16**. Amide derivatives were formed starting from bromide **E**. Azide displacement followed by saponification, amide bond formation, and sulfonamide formation provided intermediate **F**. Reduction followed by coupling and deprotection afforded amides **11** and **12**.

The analogs were evaluated for their ability to block the cellular uptake of the fluorescent lipid 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) from HDL particles into the human osteosarcoma U2OS cell line overexpressing human SR-BI.[24] The potency of the compounds was determined using a ten-point dose response curve and reported as a pIC$_{50}$. Compounds with acceptable inhibition of DiI uptake were progressed into a cellular permeability assay utilizing Madin-Darby canine kidney (MDCK) cells. Passive permeation was evaluated in this assay by using a P-glycoprotein (PGP) inhibitor added to the apical media to provide an apparent permeability (P_{app}) value.[30] Compounds with low to medium permeability (P_{app} < 20 nM/s) were assessed for lower GI stability by incubation in rat cecal contents under an anaerobic atmosphere. This luminal stability assay was devised to measure the potential for gut bacterial metabolism of test

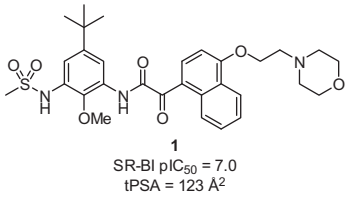
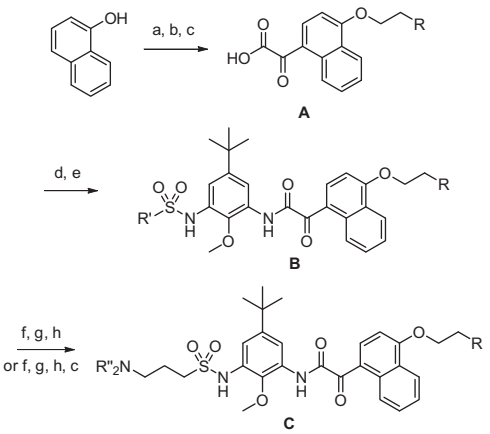
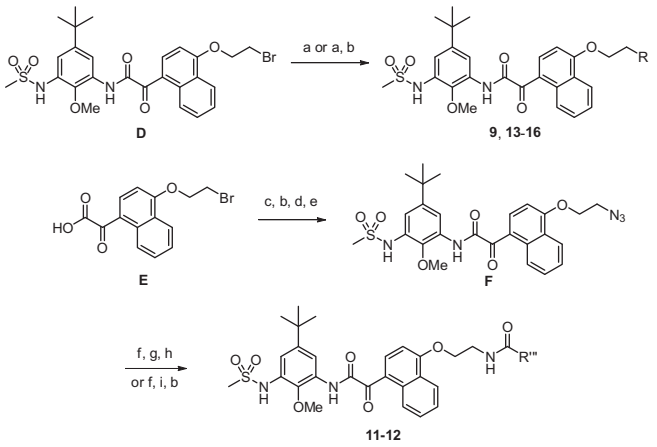


Figure 1. SR-BI inhibitor ITX-5061 (**1**).

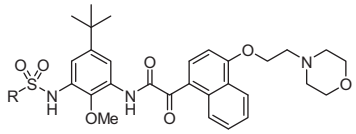


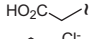
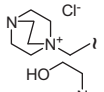
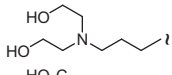
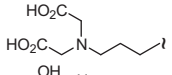
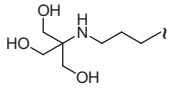
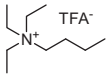
Scheme 1. Reagents and conditions: (a) RCH_2CH_2X, K_2CO_3, DMF; (b) $ClCOCO_2Et$, $AlCl_3$, DCM; (c) LiOH, H_2O, THF; (d) 5-*t*-butyl-2-methoxybenzene-1,3-diamine, HATU, DIEA, DMF; (e) $R'SO_2Cl$, pyr; (f) For R′ = $(CH_2)_3Cl$, SEM-Cl, Et_3N, 0 °C; (g) KI, R''_2NH, CH_3CN, 80 °C; (h) HCl, MeOH or HF-pyr.



Scheme 2. Reagents and conditions: (a) amine, CH_3CN, 90 °C; (b) LiOH, H_2O, THF; (c) NaN_3, DMF, 90 °C; (d) 5-*t*-butyl-2-methoxybenzene-1,3-diamine, HATU, DIEA, DMF; (e) CH_3SO_2Cl, pyr; (f) PPh_3, H_2O, THF; (g) RCO_2H, HATU, DIEA; (h) TFA; (i) RCOCl, DIEA.

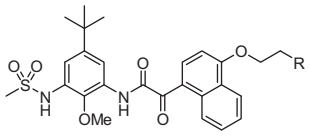
Table 1
SR-BI inhibition: sulfonamide SAR, SPR, and luminal stability

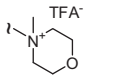
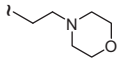
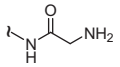
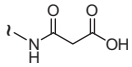
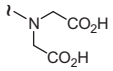
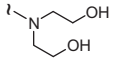
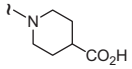


Compound	R	pIC$_{50}$[a]	MDCK P_{app}[b] (nM/s)	Luminal stability[b] $T_{1/2}$ (h)
1	Methyl	7.0 ± 0.2	126	—
2	Propyl	7.3 ± 0.4		
3		6.7 ± 0.1	2	>12
4		6.8 ± 0.3	1	>12
5		7.4 ± 0.5	8	11
6		5.3 ± 0.1	—	—
7		7.3 ± 0.3	<1	11
8		6.8 ± 0.2	<1	>12

[a] pIC$_{50}$ values are expressed as the mean of ⩾2 replicates.
[b] —Indicates not tested.

Table 2
SR-BI inhibition: sulfonamide SAR, SPR, and luminal stability



Compound	R	pIC$_{50}$[a]	MDCK P_{app}[b] (nM/s)	Luminal stability[b] $T½$ (h)
9		6.0 ± 0.1	<1	>12
10		7.3 ± 0.3	—	—
11		7.3 ± 0.2	3	3
12		6.0 ± 0.4	3	11
13		6.3 ± 0.2	<1	>12
14		<4.5	—	—
15		7.1 ± 0.1	45	>12
16		6.0 ± 0.4	5	>12

[a] pIC$_{50}$ values are expressed as the mean of ⩾2 replicates.
[b] —Indicates not tested.

Table 3
DMPK properties of select SR-BI inhibitors[a,b,c]

Compound	Portal C_{max} (ng/mL)	Systemic C_{max} (ng/mL)	Fecal drug recovery[b] (%)
8	77	13	83 ± 3
9	28	10	84 ± 34
12	25	3	46 ± 17
13	51	13	61 ± 14
16	2003	590	—

[a] Dosed via oral gavage at 30 mg/kg to male CD rats in 0.5% HPMC/0.1% Tween 80/water.
[b] Fecal recovery values are expressed as the mean ± standard error of ⩾2 replicates.
[c] —Means not determined.

compounds. Compounds with a half-life ($T½$) of 11 h or greater were considered for progression.

Initial studies explored modifications to the sulfonamide portion of ITX-5061 (Table 1). ITX-5061 (**1**) was evaluated to establish a baseline for potency and permeability and profiled with a pIC$_{50}$ of 7 and high permeability, with a P_{app} value of 126 nM/s. Propyl analog **2** was profiled to assess the possibility of extending the sulfonamide group and was equipotent, suggesting further modifications off the sulfonamide region would be tolerated. Adjusting the oxidation state of the terminal carbon atom to a carboxylic acid (**3**) provided acceptable potency with low permeability and good GI-stability. Quaternary amines (**4** & **8**) were well tolerated and provided low permeability and excellent in vitro GI-stability. Amines extended by a propyl linker terminated with diol- (**5**) or triol-functionality (**7**) provided excellent potency, however, adjustment of the oxidation state to the dicarboxylic acid (**6**) greatly eroded activity.

Encouraged by the results obtained with the sulfonamide analogs, we next explored modifications to the morpholine moiety (Table 2). While direct quaternization of the morpholino-group (**9**) provided a 1-log reduction in potency, extending the morpholine group out by two additional methylene groups (**10**) maintained potency equivalent to **1**. Amide containing compounds **11** and **12** highlighted that a terminal amine was well tolerated while a terminal carboxylic acid was not, however glycine amide **11** suffered from rapid degradation in the luminal stability assay. Quaternary amine **13** showed moderate potency with diacid **14** reinforcing that acidic functionality did not provide potent inhibition of SR-BI. Diol **15** provided the desired potency (pIC$_{50}$ = 7.1) but suffered from moderate permeability (P_{app} = 45 nM/s). Piperidine derivative **16** provided further evidence that acidic functionality was not well tolerated.

With a stable of molecules meeting our desired in vitro profile for further evaluation as nonabsorbed SR-BI inhibitors, we progressed select compounds into DMPK studies (Table 3). Utilizing male CD rats, portal and jugular drug blood concentrations were measured following oral dosing (30 mg/kg) out to a 24 h timepoint. Fecal drug recovery was evaluated for compounds with portal vein drug concentrations of less than 100 ng/mL with the goal of achieving greater than 80% recovery for progression. In general, portal C_{max} values were below the 100 ng/mL cutoff with the exception of compound **16** (Portal C_{max} = 2003 ng/mL), which we hypothesize may achieve higher drug levels due to active transport. The fecal drug recovery of the remaining compounds identified quaternary amines **8** and **9** as promising candidates for evaluation as nonabsorbed SR-BI inhibitors, with compound **8** affording the best overall profile with higher potency (pIC$_{50}$ = 6.8) and lower variability in the fecal recovery assay (fecal drug recovery = 83 ± 3%) compared with amine **9**. Finally, the aqueous solubility of solid compound **8** in simulated fasted intestinal fluid was measured and showed excellent solubility (FaSSIF solubility = 808 μg/mL).

In summary, optimization of the structure–activity and structure–permeability relationships starting from the ITX-5061 template afforded compound **8** as a nonabsorbed inhibitor of SR-BI. Following assessment of SR-BI inhibition, in vitro permeability and GI-stability were assessed to identify low permeability-high stability compounds which were progressed into in vivo DMPK studies keying on identification of compounds with low portal vein drug concentrations. Finally, fecal drug recovery allowed for the identification of compound **8** as an optimal agent to study the biological effects of small molecule SR-BI inhibition in the GI-tract. Further studies evaluating the gastrointestinal effects of SR-BI inhibition utilizing nonabsorbed inhibitor **8** will be reported in due course.

Supplementary data

Supplementary data (experimental procedures for compound **8** and assays protocols) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.03.025.

References and notes

1. Brown, J. M.; Yu, L. *Subcell. Biochem.* **2010**, *51*, 337.
2. Platt, N.; Gordon, S. *Chem. Biol.* **1998**, *5*, R193.
3. Rigotti, A.; Miettinen, H. E.; Krieger, M. *Endocr. Rev.* **2003**, *24*, 357.
4. Hoekstra, M.; Van Berkel, T. J. C.; Van Eck, M. *World J. Gastroenterol.* **2010**, *16*, 5916.
5. Rader, D. J.; Alexander, E. T.; Weibel, G. L.; Billheimer, J.; Rothblat, G. H. *J. Lipid Res.* **2009**, S189.
6. Sun, B.; Boyanovsky, B. B.; Connelly, M. A.; Shridas, P.; van der Westhuyzen, D. R.; Webb, N. R. *J. Lipid Res.* **2007**, *48*, 2560.
7. Bartosch, B.; Vitelli, A.; Granier, C.; Goujon, C.; Dubuisson, J.; Pascale, S.; Scarselli, E.; Cortese, R.; Nicosia, A.; Cosset, F. L. *J. Biol. Chem.* **2003**, *278*, 41624.

8. Fioravanti, J.; Medina-Echeverz, J.; Berraondo, P. *Immunotherapy* **2011**, *3*, 395.
9. Sulkowski, M. S.; Kang, M.; Matining, R.; Wyles, D.; Johnson, V. A.; Morse, G. D.; Amorosa, V.; Bhattacharya, D.; Coughlin, K.; Wong-Staal, F.; Glesby, M. J. *J. Infect. Dis.* **2014**, *209*, 658.
10. Zhu, H.; Wong-Staal, F.; Lee, H.; Syder, A.; McKelvy, J.; Schooley, R. T.; Wyles, D. L. *J. Infect. Dis.* **2012**, *205*, 656.
11. Rodrigues, C. D.; Hannus, M.; Prudencio, M.; Martin, C.; Goncalves, L. A.; Portugal, S.; Epiphanio, S.; Akinc, A.; Hadwiger, P.; Jahn-Hofmann, K.; Rohl, I.; van Gemert, G. J.; Franetich, J. F.; Luty, A. J. F.; Sauerwein, R.; Mazier, D.; Koteliansky, V.; Vornlocher, H. P.; Echeverri, C. J.; Mota, M. M. *Cell Host Microbe* **2008**, *4*, 271.
12. Foquet, L.; Verhoye, L.; Leroux-Roels, G.; Meuleman, P.; Hermsen, C. C.; van, G. G.-J.; Sauerwein, R. W.; Cortese, R.; Nicosia, A. *J. Antimicrob. Chemother.* **2015**.
13. Yalaoui, S.; Huby, T.; Franetich, J. F.; Gego, A.; Rametti, A.; Moreau, M.; Collet, X.; Siau, A.; van Gemert, G. J.; Sauerwein, R. W.; Luty, A. J. F.; Vaillant, J. C.; Hannoun, L.; Chapman, J.; Mazier, D.; Froissard, P. *Cell Host Microbe* **2008**, *4*, 283.
14. Levy, E.; Spahis, S.; Sinnett, D.; Peretti, N.; Maupas-Schwalm, F.; Delvin, E.; Lambert, M.; Lavoie, M. A. *Curr. Opin. Lipidol.* **2007**, *18*, 310.
15. Xiao, C.; Hsieh, J.; Adeli, K.; Lewis, G. F. *Am. J. Physiol.* **2011**, *301*, E429.
16. Hauser, H.; Dyer, J. H.; Nandy, A.; Vega, M. A.; Werder, M.; Bieliauskaite, E.; Weber, F. E.; Compassi, S.; Gemperli, A.; Boffelli, D.; Wehrli, E.; Schulthess, G.; Phillips, M. C. *Biochemistry* **1998**, *37*, 17843.
17. Lobo, M. V. T.; Huerta, L.; Ruiz-Velasco, N.; Teixeiro, E.; De la Cueva, P.; Celdran, A.; Martin-Hidalgo, A.; Vega, M. A.; Bragado, R. *J. Histochem. Cytochem.* **2001**, *49*, 1253.
18. Beaslas, O.; Cueille, C.; Delers, F.; Chateau, D.; Chambaz, J.; Rousset, M.; Carriere, V. *PLoS One* **2009**, *4*, e4278.
19. (a) Hayashi, A. A.; Webb, J.; Choi, J.; Baker, C.; Lino, M.; Trigatti, B.; Trajcevski, K. E.; Hawke, T. J.; Adeli, K. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2011**, *301*, G326; (b) Lino, M.; Farr, S.; Baker, C.; Fuller, M.; Trigatti, B.; Adeli, K. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2015**, *309*, G350.
20. Bietrix, F.; Yan, D.; Nauze, M.; Rolland, C.; Bertrand-Michel, J.; Comera, C.; Schaak, S.; Barbaras, R.; Groen, A. K.; Perret, B.; Terce, F.; Collet, X. *J. Biol. Chem.* **2006**, *281*, 7214.
21. Charmot, D. *Curr. Pharm. Des.* **2012**, *18*, 1434.
22. Kramer, W.; Glombik, H. *Curr. Med. Chem.* **2006**, *13*, 997.
23. Dockendorff, C.; Faloon, P. W.; Yu, M.; Youngsaye, W.; Penman, M.; Nieland, T. J. F.; Nag, P. P.; Lewis, T. A.; Pu, J.; Bennion, M.; Negri, J.; Paterson, C.; Lam, G.; Dandapani, S.; Perez, J. R.; Munoz, B.; Palmer, M. A.; Schreiber, S. L.; Krieger, M. *ACS Med. Chem. Lett.* **2015**. Ahead.
24. Nieland, T. J. F.; Penman, M.; Dori, L.; Krieger, M.; Kirchhausen, T. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15422.
25. Mittapalli, G. K.; Jackson, A.; Zhao, F.; Lee, H.; Chow, S.; McKelvy, J.; Wong-Staal, F.; MacDonald, J. E. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6852.
26. Nieland, T. J. F.; Shaw, J. T.; Jaipuri, F. A.; Duffner, J. L.; Koehler, A. N.; Banakos, S.; Zannis, V. I.; Kirchhausen, O. T.; Krieger, M. *Biochemistry* **2008**, *47*, 460.
27. Mittapalli, G. K.; Zhao, F.; Jackson, A.; Gao, H.; Lee, H.; Chow, S.; Kaur, M. P.; Nguyen, N.; Zamboni, R.; McKelvy, J.; Wong-Staal, F.; MacDonald, J. E. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4955.
28. Nishizawa, T.; Kitayama, K.; Wakabayashi, K.; Yamada, M.; Uchiyama, M.; Abe, K.; Ubukata, N.; Inaba, T.; Oda, T.; Amemiya, Y. *Atherosclerosis (Amsterdam, Neth.)* **2007**, *194*, 300.
29. Masson, D.; Koseki, M.; Ishibashi, M.; Larson, C. J.; Miller, S. G.; King, B. D.; Tall, A. R. *Arterioscler. Thromb. Vasc. Biol.* **2009**, *29*, 2054.
30. Wu, Y.; Aquino, C. J.; Cowan, D. J.; Anderson, D. L.; Ambroso, J. L.; Bishop, M. J.; Boros, E. E.; Chen, L.; Cunningham, A.; Dobbins, R. L.; Feldman, P. L.; Harston, L. T.; Kaldor, I. W.; Klein, R.; Liang, X.; McIntyre, M. S.; Merrill, C. L.; Patterson, K. M.; Prescott, J. S.; Ray, J. S.; Roller, S. G.; Yao, X.; Young, A.; Yuen, J.; Collins, J. L. *J. Med. Chem.* **2013**, *56*, 5094.