Bioorganic & Medicinal Chemistry Letters xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Identification of substituted benzothiazole sulfones as potent and selective inhibitors of endothelial lipase

Soong-Hoon Kim*, James A. Johnson, Ji Jiang, Brandon Parkhurst, Monique Phillips, Zulan Pi, Jennifer X. Qiao, George Tora, Alice Ye Chen, Eddie Liu, Xiaohong Yin, Richard Yang, Lei Zhao, David S. Taylor, Michael Basso, Kamelia Behnia, Joelle Onorato, Xue-Qing Chen, Lynn M. Abell, Hao Lu, Gregory Locke, Christian Caporuscio, Leonard P. Adam, David Gordon, Ruth R. Wexler, Heather J. Finlay

Research and Development, Bristol-Myers Squibb, Princeton, NJ 08543, United States

ARTICLE INFO

Keywords:
Endothelial lipase
HDL cholesterol
Cardiovascular diseases
Phospholipids
Hepatic lipase

ABSTRACT

A low level of high density lipoprotein (HDL) is an independent risk factor for cardiovascular disease. HDL reduces inflammation and plays a central role in reverse cholesterol transport, where cholesterol is removed from peripheral tissues and atherosclerotic plaque. One approach to increase plasma HDL is through inhibition of endothelial lipase (EL). EL hydrolyzes phospholipids in HDL resulting in reduction of plasma HDL. A series of benzothiazole sulfone amides was optimized for EL inhibition potency, lipase selectivity and improved pharmacokinetic profile leading to the identification of Compound **32**. Compound **32** was evaluated in a mouse pharmacodynamic model and found to show no effect on HDL cholesterol level despite achieving targeted plasma exposure (C_{trough} > 15 fold over mouse plasma EL IC_{50} over 4 days).

HDL-C is an independent risk factor for cardiovascular disease (CVD) and routine screening for HDL-C is recommended for all adults.[1] The plasma level of HDL-C inversely correlates with cardiac events, and low levels are present in patients who have CVD.[2] The cardio-protective function of HDL and apo A-I (the major protein constituent of HDL) is believed to be related to anti-oxidant, anti-thrombotic and anti-inflammatory activity.[3] In addition, HDL-C has a central role in reverse cholesterol transport where cholesterol is removed from atherosclerotic plaque and peripheral tissues for removal from the body via the liver.[4] The potential benefit of increasing HDL-C has encouraged multiple therapeutic approaches, including inhibition of endothelial lipase (EL).[5]

EL is a 69-k_D phospholipase that is structurally related to lipoprotein lipase (LPL), pancreatic lipase (PL) and hepatic lipase (HL). EL is a serine hydrolase with a catalytic triad of serine, aspartic acid and histidine, and the active form is a homodimer with head to tail conformation.[6]The primary substrates for EL are the phospho-lipids in HDL, a complex composed of apo A-I/A-II proteins, triglycerides, phospholipids and cholesterol. This selectivity for HDL places EL as a key modulator of HDL metabolism and associated HDL-C plasma level.[7]

Homozygote EL KO (-/-) mice show an increase in HDL-C plasma level,[8] while transgenic expression of EL in mice result in a reduction of HDL-C levels.[9] These findings from genetic models are further supported by direct inhibition of EL by neutralizing polyclonal antibodies.[10] In addition, a small molecule EL inhibitor, XEN455 (Fig. 1), has been disclosed.[11] This approach has been reported to elevate HDL-C plasma concentration *in vivo*, further supporting EL as a potential target for HDL modulation.

As previously described, compound **2** (EL IC_{50} 0.15 μM) did not elevate HDL-C level in mice when dosed for 6 days.[12] This observation was attributed to a disconnect between the *in vitro* assays, which were run in the absence of plasma or serum, and the potency in physiological conditions for EL inhibition. When evaluated in an assay where lipid hydrolysis was measured in HDL, and in the presence of human serum, **2** had an IC_{50} value of > 476 μM. Evaluating multiple series for target potency in the plasma and serum assays led to the optimization of sulfonylated benzothiazole-oxadiazole **3**, a potent inhibitor in the presence of mouse plasma (mPlasma IC_{50} 0.02 μM) with selectivity versus HL (45 fold).[13] Selectivity versus HL was important since HL inhibition has been reported to elevate low density lipoprotein (LDL), which is a known risk factor for CVD.[14] Despite having achieved C_{min} ~ 7 fold above mPlasma IC_{50} over a period of 24 h, an increase in HDL-C was not observed with **3** in C57BL/6 wild-type (WT) mouse model. The

* Corresponding author.
E-mail address: soonghoon.kim@bms.com (S.-H. Kim).

https://doi.org/10.1016/j.bmcl.2019.05.048
Received 29 March 2019; Received in revised form 22 May 2019; Accepted 24 May 2019

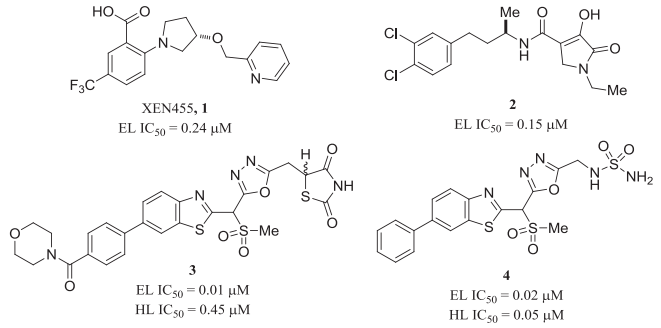
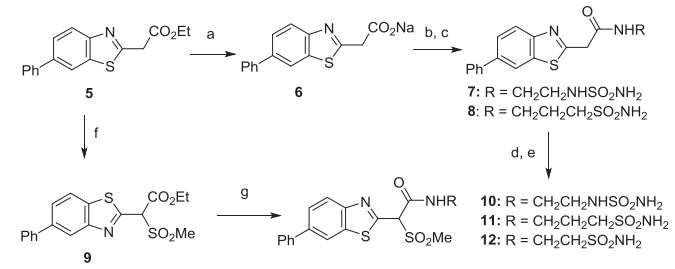


Figure 1. Endothelial Lipase inhibitors.

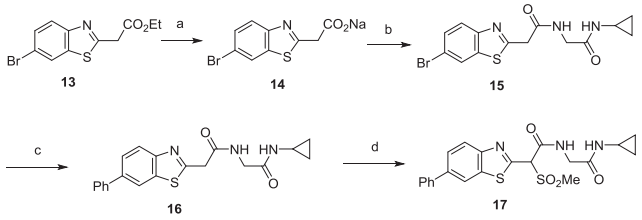
possibility that a pharmacodynamic increase in HDL-C required sustained concentration at even higher multiples above mPlasma IC_{50} over a longer period (> 24 h) could not be explored with **3** because of its dose limiting solubility. We sought to maintain EL potency and selectivity versus HL, concomitant with a *p.o.* PK profile,that would enable evaluation of PD at higher plasma concentrations of the compound *in vivo*.

Our strategy focused on improving ADME properties to increase oral PK exposure. Heterocycle bio-isosteres of the central oxadiazole were significantly less potent. Previously disclosed compounds in this series suggested that replacing the oxadiazole with an amide would retain potency.[15] In addition, SAR optimization of benzothiazole-linked oxadiazole series[13] demonstrated that polar groups, such as the thiazolidinedione in **3** and sulfonyl urea **4** (EL IC_{50} 0.02 μM), in the terminal position were required for potency. Based on these observations, benzothiazole sulfones with terminal amide groups were targeted to maintain potency and improve ADME properties. Synthesis of sulfones **10–12** (Table 1) is shown in Scheme 1. Compound **17** was prepared as shown in Scheme 2. The route closely followed Scheme 1, with the added flexibility of late-stage Suzuki coupling (step c) to introduce the aryl ring.

The direct amide sulfone analog of oxadiazole **4**, compound **10**, showed only modest EL inhibition (~20 fold loss compared to **4**). Further modification of the terminal sulfamide to a sulfonamide, compound **11**, further reduced potency. However, we were gratified to see that the one carbon homologue of **11**, sulfonamide **12**, showed a significant improvement in potency (EL IC_{50} 10 nM). Importantly, **12** also demonstrated potency in the mPlasma assay (IC_{50} 210 nM). To target compounds which maintained or enhanced potency and improved ADME properties, additional non-sulfonamide groups were



Scheme 1. (a) 1N NaOH, THF, RT, 100%; (b) For Compound **7**: $NH_2CH_2CH_2NHBoc$, PyBOP, TEA, DMF, RT then TFA, DCM, 47% for two steps; $NH_2SO_2NH_2$, dioxane, 100 °C, 53%; (c) For Compound **8**: $NH_2CH_2CH_2CH_2SO_2NH_2$ hydrochloride, PyBOP, TEA, DMF, RT, 34%; (d) For Compound **10**: NaHMDS, MeSO2Cl, −20 °C, 22%; (e) For Compound **11**: NaHMDS, $MeSO_2Cl$, −20 °C, 18%; (f) DBU, $MeSO_2Cl$, DMF, RT, 32%; (g) Compound **12**: $NH_2CH_2CH_2SO_2NH_2$ hydrochloride, TEA, dioxane, 145 °C, 15%.

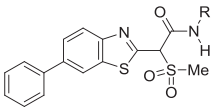


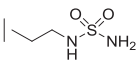
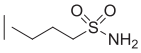
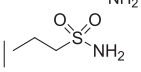
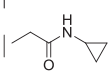
Scheme 2. (a) 1N NaOH, THF, RT, 99%; (b) 2-amino-*N*-cyclopropylacetamide hydrochloride, PyBOP, TEA, DMF, RT, 66%; (c) $PhB(OH)_2$, $Pd(PPh_3)_4$, dioxane, 2 M Na_2CO_3, 90 °C, 30%; (d) NaHMDS, $MeSO_2Cl$, −20 °C, 7.8%.

prepared. A series of terminal amides were explored and cyclopropyl amide **17** was identified some potency for EL inhibition.

However, compounds **10–12** and **17** in Table 1 all showed poor selectivity versus HL. This was consistent with the earlier SAR work[13] on benzothiazole-linked oxadiazole sulfones where selectivity was improved by incorporating polar substituents on the terminal ring (for example, compound **3** compared to compound **4**). The SAR for selectivity versus HL proved to be consistent, and synthesis of polar-substituted aryl and heterocyclic analogs led to compounds with improved selectivity (compounds **21** and **22** shown in Table 2). Additional heterocycles were explored at the C6 position and compound **23** was identified with single digit nanomolar potency for EL and excellent selectivity versus HL. In addition to improved selectivity, **21–23** showed good potency in the mPlasma assay (IC_{50} 190–320 nM) and

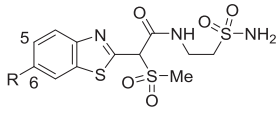
Table 1
Amide SAR.

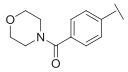
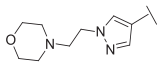


Cmpd	R	EL IC_{50} (nM)[a]	HL IC_{50} (nM)[a]	mPlas (nM)[a]
10		450	81	
11		7700	52	
12		10	15	210
17		150	47	

[a] Inhibition was measured in duplicate, and the mean values were used to calculate IC_{50} values

Table 2
Selectivity Versus HL.



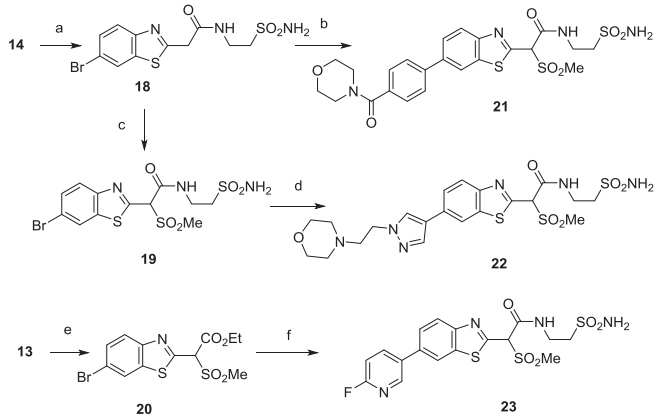
Cmpd	R	EL IC_{50} (nM)[a]	HL IC_{50} (nM)[a]	mPlas (nM)[a]
12		10	15	100
21		10	280	320
22		10	310	190
23		5	4400	200

[a] Inhibition was measured in duplicate, and the mean values were used to calculate IC_{50} values

Table 3
Mouse *p.o.* PK (10 mg/kg)[a]

Cmpd	C_{max} (nM)	C_{7hr} (nM)	C_{24hr} (nM)	T_{max} (hr)	AUC_{total} (nM*hr)[b]	Stability (Mouse, %)[c]	PAMPA (pH 5.5/7.4, nm/s)	cLog P
12	3,500	430	130	0.5	14,000	91	365/368	0.52
21	36	8	5	2.6	160	100	12/13	−0.42
22	15	4	1	3	100	74	0/0	−2.10
23	30	1			50	96	17/78	−0.21

[a] PK studies were carried out in fasting balb/c male mice. Compounds were formulated in ethanol / PEG 400 / water (10:60:30). [b]AUC_{total} was calculated based on compound concentration to 24 h. [c]Percentage of compound (0.5 μM) remaining after 10 min incubation in mouse liver microsomes.

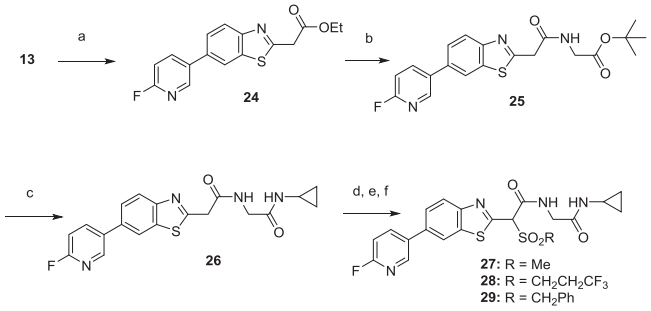


Scheme 3. (a) $NH_2CH_2CH_2SO_2NH_2$ hydrochloride, PyBOP, TEA, DMF, RT, 66%; (b) $PdCl_2$(dppf), morpholino(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)methanone, K_3PO_4, dioxane, 105 °C, 67%; NaHMDS, $MeSO_2Cl$, THF, −20 °C, 24%; (c) NaHMDS, $MeSO_2Cl$, THF, −20 °C, 21%; (d) 4-(2-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazol-1-yl)ethyl) morpholine, $Pd(PPh_3)_4$, dioxane/2M Na_2CO_3, 100 °C, 47%; (e) DBU, $MeSO_2Cl$, DMF, RT, 49%; (f) $PdCl_2$(dppf), 2-fluoro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine, K_3PO_4, dioxane, 105 °C, 56%; $NH_2CH_2CH_2SO_2NH_2$ hydrochloride, TEA, HOBT, $ZrCl_4$, dioxane, 145 °C, 9%.

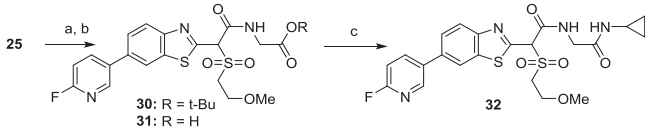
were selected for mouse oral PK evaluation (Table 3). The synthesis of compounds **21–23** is shown in Scheme 3.

As shown in Table 3, compound **12** exhibited good exposure (C_{max} 3,500 nM, AUC_{total} 14,000 nM*hr). Unfortunately, EL selective compounds **21–23** showed poor oral exposure in mice. The low absorption (C_{max} < 36 nM for **21–23**) was likely related to poor permeability. Compounds **21–23** were stable in mouse liver microsomes, but PAMPA (parallel artificial membrane permeability assay) permeability values were low at pH 5.5 and 7.4 (< 78 nm/s). The striking difference in oral exposure for **21–23** and **12** was likely due to the difference in permeability. Since polarity in the benzothiazole aryl portion was critical for selectivity (**21–23** in Table 2), structural changes to improve permeability were sought elsewhere. Cyclopropyl amide **17** (Table 1) had a clogP value of 1.24 compared to 0.52 for sulfonamide **12** and demonstrated higher permeability (PAMPA 824 nm/s and 1,054 nm/s at pH 5.5 and 7.4 respectively). We therefore focused on synthesis of cyclopropyl amide sulfones **27–29** with C5 and C6 polar groups to increase selectivity while maintaining permeability (Schemes 4 and 5).

The PAMPA permeability values (pH 5.5/7.4) for cyclopropyl amide **27** showed significant improvement over corresponding sulfonamide **23** (252/389 nm/s vs 17/78 nm/s, respectively). However, as shown in Table 4, improved permeability was accompanied by loss of selectivity versus HL. Cyclopropyl amide **27** was only ~3 fold selective versus HL compared to 880 fold selectivity observed for **23**. In an effort to further improve selectivity versus HL, additional sulfone substitutions were explored while keeping the cyclopropyl amide and C6 substituent constant (representative compounds include **28–29** and **32**). Trifluoropropyl analogue **28** and benzyl analogue **29** were less potent and less selective versus HL compared to **23**. Polar methoxy ethyl sulfone, **32** showed improved selectivity (50 fold) with good potency in the

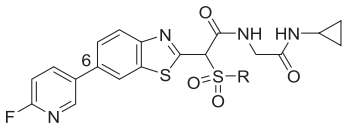


Scheme 4. (a) $PdCl_2$(dppf), K_3PO_4, (6-fluoropyridin-3-yl)boronic acid, dioxane, 110 °C, 83%; (b) 1 N NaOH, THF, RT; *tert*-butyl 2-aminoacetate hydrochloride, HATU, DIEA, DMF, 34% for two steps; (c) TFA/DCM, RT then cyclopropyl amine, HATU, DIEA, DMF, 88% yield for two steps; (d) for **27** (R = Me), NaHMDS, $MeSO_2Cl$, DMF, −20 °C, 56%; (e) for **28** (R = $CH_2CH_2CF_3$), NaHMDS, $CF_3CH_2CH_2SO_2Cl$, DMF, −20 °C, 8%; (f) for **29** (R = CH_2Ph), NaHMDS, $PhCH_2SO_2Cl$, THF, −20 °C, 41%



Scheme 5. (a) NaHMDS, $MeOCH_2CH_2SO_2Cl$, THF, −20 °C, 35% (b) TFA/DCM, RT, 98%; (b) cyclopropyl amine, HATU, DIEA, DMF, 91%

Table 4
Sulfone SAR.



Cmpd	R	EL IC_{50} (nM)	HL IC_{50} (nM)	Selectivity	mPlasma IC_{50}(nM)	hSerum IC_{50} (nM)
27	Me	47	130	2.8	290	7
28	F, F, F	29	79	2.7	600	660
29	Ph	25	7	0.3	94	65
32	MeO	4.6	230	84	120	83

Inhibition was measured in duplicate, and the mean values were used to calculate IC_{50} values.

mPlasma assay. The selectivity was confirmed using EL (HL KO IC_{50} 310 nM) and HL (EL KO IC_{50} 26,000 nM) mPlasma assays derived from KO mice, an approach more representative of physiological inhibition. Inhibition of EL in the presence of added human serum (hSerum IC_{50}) was comparable to mPlasma IC_{50}. Cyclopropyl amide **32** had increased

Table 5
Compound **32** mouse *p.o.* PK.[a]

Dose (mg/kg)	C_{max} (nM)	C_{7hr} (nM)	C_{24hr} (nM)	T_{max} / $T_{1/2}$(hr)	AUC_{total} (nM)[b]
10	39,000	1,700	14	1.0 / 2.2	111,000
20	143,000	7,400	45	0.5 / 2.3	400,000
30	142,000	2,420	25	0.25 / 2.3	240,000

[a] PK studies were carried out in fasting balb/c male mice. Compound 32 was formulated in ethanol / PEG 400 / water (10:60:30). [b]AUC_{total} was calculated based on compound concentration to 24 h

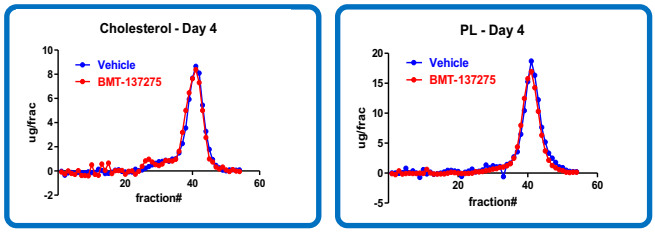


Figure 2. Effect of EL inhibitor **32** (red) and vehicle (blue) on HDL-cholesterol and phospholipids (PL) plasma concen-tration on day 4.

permeability (PAMPA at pH 5.5 = 94 nm/s) compared to sulfonamide **23** (PAMPA at pH 5.5 = 17 nm/s), while retaining good mouse liver microsome stability (81% remaining after 10 min incubation).

Compound **32** was evaluated in mouse *p.o.* PK and results are shown in Table 5. Compound **32** showed higher exposure (C_{7h} 1,700 nM) than compound **3** when dosed at 10 mg/kg, as well as significant improvement over sulfonamides **21–23**. As described previously, compound **3** had dose limiting solubility[13] and could not be dosed higher than 10 mg/kg *in vivo*. When the dose was increased for compound **32**, and increase in exposure was observed at 20 mg/kg (~3.6 fold increase in AUC); however, no significant increase was observed from 20 to 30 mg/kg. To target the highest feasible exposures at C_{24h}, we elected to dose *b.i.d.* with projected exposure based on the observed PK exposure at 20 mg/kg *q.d.*, a C_{min} concentration of 2,700 nM, which was 23 fold over mPlasma IC_{50} of 120 nM in mouse.

Wild-type C57BL/6 mice were dosed with compound **32** at 20 mg/kg *p.o. b.i.d.* over 4 days. A satellite PK co-hort showed exposure consistent with projected values (C_{8h} = 5,200 nM, C_{24h} = 1,800, C_{102h} = 6,500 nM), with C_{min} > 15 fold over the mPlasma IC_{50} throughout the 4 day period. The lipid profile was measured using fast protein liquid chromatography (FPLC) on day 1 and day 4 from plasma pooled from 8 mice. There were no differences observed between mice treated with vehicle control (blue) and Compound **32** (red) on day 1 or on day 4 for HDL-C or LDL-C (Fig. 2). This result was consistent with our earlier observation[13] for compound **3**, however, in contrast with studies where neutralizing EL antibody[9] elevated HDL-C and (phospholipids) PL in mice, and with HDL-C elevation observed with small molecule EL inhibitor XEN455 **1**.[11]

In summary, further optimization of the benzothiazole sulfone series led to the discovery of a potent, highly selective, and orally bioavailable inhibitor, Compound **32**. Compound **32** had an improved PK exposure profile at 10 mg/kg compared to earlier compound **3** and could be dose escalated to 20 mg/kg *b.i.d.* dosing to enable a C_{min} concentration of 1,800 nM in the mouse PD study achieving > 15 fold over mouse IC_{50} at Cmin. Surprisingly, sustained EL inhibition over a 4 day period in a mouse pharmacodynamic model failed to demonstrate an increase in plasma HDL-C levels. This led us to evaluate in further detail the kinetics of EL inhibition and incorporation of an assay with pre-incubation which will be disclosed in due course.

Acknowledgements

The authors would like to thank Dr. Arvind Mathur, Rick Rampulla and colleagues at Department of Discovery Synthesis at Biocon Bristol-Myers Squibb RD Center for the preparation of intermediates.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2019.05.048.

References

1. Toth PP. *Circulation.* 2005;111:e89.
2. Castelli WP. *Can J Cardiol.* 1988;4(supple A):5A–10A.
3. Rader DJ. *Am J Cardio.* 2002;90(suppl):62i–70i.
4. Annema W, Tietge UJ. *Curr Atheroscler Rep.* 2011;13:257–265.
5. deGoma EM, Rader DJ. *Nat Rev Cardiol.* 2011;8:266–277.
6. Griffon N, Jin W, Petty TJ, et al. *J Biol Chem.* 2009;284:23322–23330.
7. McCoy MG, Sun G, Marchadier D, Maugeais C, Glick JM, Rader DJ. *J Lipid Res.* 2002;43:921–929.
8. Brown RJ, Lagor WR, Sankaranaravanan S, et al. *Circ Res.* 2010;107:357–364.
9. Ishida T, Choi S, Kundu RK, Hirata K, Rubin EM, Cooper AD, Quertermous T. *J Clin Invest.* 2003;111:347–355.
10. Jin W, Millar JS, Broedl U, Click JM, Rader DJ. *J Clin Invest.* 2003;111:357–362.
11. Greco MN, Connelly MA, Leo GC, et al. *Bioorg Med Chem Lett.* 2013;23:2595–2597.
12. Hengeland J, Abell LM, Adam LP, et al. *ACS Med Chem Lett.* 2018;9(7):673–678.
13. Johnson JA, Tora G, Pi Z, et al. Sulfonylated benzothiazole based inhibitors of endothelial lipase. *ACS Med Chem Lett.* 2018;9(12):1263–1268.
14. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel B. *Circulation.* 1998;97:1837–1847.
15. Masuda, Kouji; Kida, Shiro; Hayashi, Kyohei; Katou, Manabu; Yoshikawa, Naoki; Kugimiya, Akira; Nakajima, Mado; Tanaka, Nobuyuki. WO2010044441, 2010.