Bioorganic & Medicinal Chemistry Letters xxx (xxxx) xxxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of novel pyrrole derivatives as potent agonists for the niacin receptor GPR109A

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ARTICLE INFO

Keywords:
GPR109A
Niacin
Pyrrole
Lipolysis
Non-esterified fatty acid

ABSTRACT

Novel pyrrole derivatives were discovered as potent agonists of the niacin receptor, GPR109A. During the derivatization, compound **16** was found to be effective both in vitro and in vivo. The compound **16** exhibited a significant reduction of the non-esterified fatty acid in human GPR109A transgenic rats, and the duration of its in vivo efficacy was much longer than niacin.

Niacin (nicotinic acid) is a group B vitamin, which has been used in humans to treat dyslipidemia since the 1950s.[1,2] Niacin reduces total plasma cholesterol, very low-density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), and lipoprotein a (Lp(a)).[2,3] In addition, it favorably elevates high-density lipoprotein cholesterol (HDL-C) whose levels are inversely correlated with the risk of coronary heart disease.[4] However, continuous flushing is the most common side effect of niacin therapy, which leads to patient non-adherence and discontinuation.[5] Furthermore, niacin is rapidly metabolized and requires frequent dosing or improvement of formulation (e.g., extended release product) to sustain pharmacologically active drug concentrations.

In 2003, a G-protein-coupled receptor, GPR109A (PUMA-G in mice) was identified as a receptor for niacin.[6] It is expressed in adipose tissue and various immune cells including monocytes, macrophages, dendritic cells and neutrophils. In adipose tissue, activation of GPR109A reduces intracellular cAMP levels, and this reduction in cAMP levels leads to lower plasma non-esterified fatty acids (NEFA) levels by suppressing lipolysis in adipocytes. Although the reduction of plasma NEFA has been hypothesized as a cause of improvement dyslipidemia, some clinical studies implies the less contribution of GPR109A to lipid disorders.[7] The niacin-induced flushing also occurs via GPR109A.[8]

Numerous structures of GPR109A agonists have been reported in patents and scientific literature by GlaxoSmithKline,[9] Merck,[10–14] Arena,[15] Hoffmann-La Roche,[16] Schering-Plough,[17] Incyte,[18] and A. P. Ijzerman's group at Leiden University.[19] In these reports, some agonists of GPR109A, like pyrazole analogs[20] and anthranilic acid derivatives[10,11,21] from Merck and Arena, for example **1** and **2** in Fig. 1, were indicated to suppress the flushing. We presumed the carboxylic acid group on the pyrazole scaffold of **1** was in a similar position to the one on the benzene scaffold of **2** from their SAR studies and the benzene scaffold of **2** could be replaced with the monocyclic pyrazole. Therefore

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https://doi.org/10.1016/j.bmcl.2020.127105
Received 29 December 2019; Received in revised form 23 February 2020; Accepted 8 March 2020

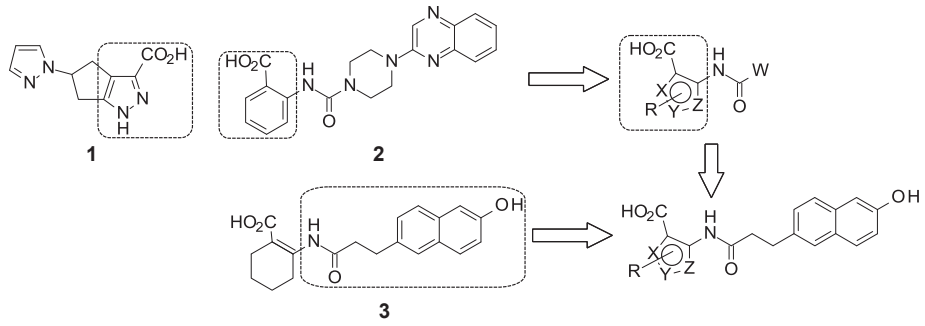


Fig. 1. Structures of small molecule-based GPR109A agonists.

we initiated the research on a 5-membered aryls based pyrazole scaffold, whose motif is shown in Fig. 1, to identify long acting GPR109A agonists without flushing.

Herein, we would like to disclose our efforts into SAR studies of 5-membered aryls, derived pyrazole, as GPR109A agonists.

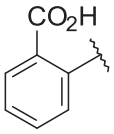
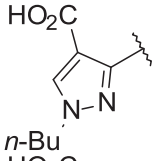
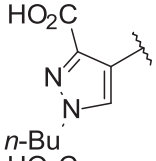
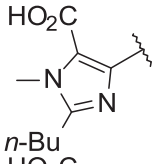
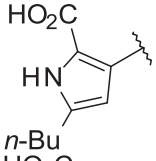
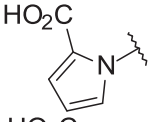
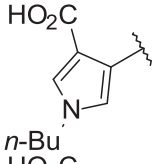
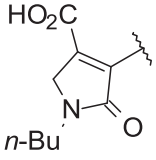
First, we synthesized the compounds in Table 1, in which we provisionally replaced the right hand moiety (W in Fig. 1) with the naphthol side chain, for example **3**, which was likely to increase the in vitro potency.[12,14,22] The side chains on the left 5-membered aryls (R in Fig. 1) were tentatively held on the *n*-butyl group except compound **9**. A comparison of the in vitro activity of the compounds between synthesized compounds and anthranilic acid derivative **4**[12] is shown in Table 1. The in vitro activity of the compounds against GPR109A was measured by the ^{3}H-niacin competition binding assay[23] and the guanine nucleotide exchange (^{35}S-GTPγS) functional assay.[24] Pyrazole derivatives **5** and **6** were inferior to anthranilic acid derivative **4**, but they had better activity than niacin in both the binding and functional assays. Whereas, imidazole derivative **7**, a regioisomer of the pyrazole scaffold, showed a 6-fold decrease in binding activity compared to niacin. Then pyrrole analogs **8**, **9** and **10**, in which we replaced one nitrogen atom in the pyrazole with carbon, were synthesized. Though compound **8** and **9** were less active than niacin, compound **10** resulted in favorable activity being maintained compared to **4** in the binding assay. On the other hand, pyrrole derivative **11**, substituted the pyrrole ring of compound **10** into the α, β-unsaturated lactam ring, showed modest activity. These results suggested the need for the nitrogen atom in the Y position of a 5-membered ring (Fig. 1) and the aromaticity of the pyrrole scaffold to improve the in vitro activities. In addition, the compound-induced inhibition of lipolysis was tested using the human adipocyte lipolysis assay.[25] Compound **10** showed excellent activity in the *h*-lipolysis assay (IC_{50} = 0.065 μM) compared to niacin (IC_{50} = 0.16 μM), which resulted in 4-fold more potency than the benzene scaffold **4** (IC_{50} = 0.29 μM). Therefore, the benzene moiety of **4** could be replaced with a pyrrole scaffold, and we selected the pyrrole derivative **10** as a lead compound to investigate the novel GPR109A agonists.

Next, we explored further SAR study of pyrrole derivatives disclosed the *N*-substituent on the pyrrole ring, as shown in Table 2. We replaced the right hand moiety from naphthol to aryl oxadiazole moiety as a representative biaryl side chain, which was reported to improve in vitro activities,[11,26] to examine the compatibility of the pyrrole scaffold. Methyl substituents **12a** and **12b** decreased 3 to 5-fold in binding potency compared to *N*-*n*-butyl analog **16**. Ethyl compound **13** had nearly comparable binding activity to **16** and slightly low functional activity. Branched alkyl substituent **14** and cyclopropyl derivative **15** showed further low activities in both the binding and functional assays. Compound **23** with the benzyl group as a bulkier substituent than the *n*-butyl group resulted in modest activities. Derivatives possessing polar groups on *N*-substituent, **17**, **18**, **21** and **22**, resulted in a loss of activity in both the binding and functional assays. On the other hand, the in vitro potency was recovered by protecting the polar hydroxyl group with methyl, compound **19**, and inducing a fluorine to the *n*-alkyl side chain, compound **20**. In the *h*-lipolysis assay, compound **16** showed 3-fold more favorable activity (IC_{50} = 0.020 μM) compared to the lead compound **10** (IC_{50} = 0.065 μM). These results suggested the biaryl series could be appropriate side chains of the pyrrole scaffold.

Synthetic routes of the compounds in Tables 1 and 2 are outlined in Schemes 1 and 2. Pyrazole intermediates **25** and **28** were accomplished by the alkylation of the commercially available **24** and **26**, which were confirmed by NOE. Imidazole intermediate **30** was constructed via amination of methyl *N*-cyanopentanedioate **29**[27] followed by cyclization. Intermediate **33** was provided by the cyanomethylation of ethyl pentanoate, *N*-alkylation with diethyl 2-aminopropanedioate, then cyclization with sodium ethoxide. 3-Amino-4-carboxylpyrrole derivative **35**, the regioisomer of **33**, was constructed from the primary amine. *N*-alkylation, followed by cyclization with the cyanoacetate and decarboxylation afforded intermediate **35**. The synthesis of 4-Amino-1-butyl-5-oxo-2H-pyrrole-3-carboxylate **37** began with *N*-alkylation of *n*-butylamine followed by cyclization with diethyl oxalate to elaborate the pyrrole core. Amination of the hydroxyl group led to the desired intermediate **37**. These intermediates (**25**, **28**, **30**, **35** and **37**), ethyl 1-aminopyrrole-2-carboxylate **38**[28] were then subjected to amidation with another intermediate of the carboxylic acid **39**, which was prepared as described in the patents.[29] For intermediate **33**, acetyl-protected **39** was used to avoid decarboxylation under the BBr_3 deprotection condition. Compounds **12a**-**23** were provided by the condensation of corresponding amines prepared similarly to **35** with intermediate **40**.[11,26] An ensuing deprotection afforded the desired GPR109A agonists for biological evaluation.

The reduction of plasma NEFA levels induced by oral administration of **16** as a typical compound in several highly potent pyrrole derivatives was measured in our pharmacodynamics model rats,[30] which were transgenic rats expressing human GPR109A (Table 3). Significant reductions of plasma NEFA levels were observed in a dose dependent manner, and the maximum efficacy of plasma NEFA reduction in 30 mg/kg of **16** was comparable to that in 10 mg/kg of niacin. The duration of **16** was quite different from niacin, that is, the reduction of plasma NEFA of **16** was still observed 3 h after dosing, whereas niacin

Table 1
In vitro SAR of 5-menbered aryl analogs.

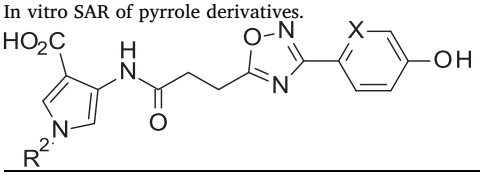
Compd		^{3}H-Niacin Binding assay IC_{50} (μM)	^{35}S-GTPγS Functional assay EC_{50} (μM)
Niacin		0.50	5.0
4		0.024	0.015
5		0.23	1.5
6		0.46	1.0
7		3.1	7.2
8		1.8	2.9
9		3.9	low active[a]
10		0.051	0.10
11		0.41	1.4

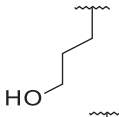
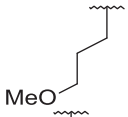
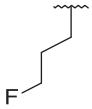
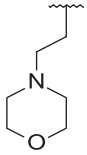
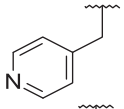
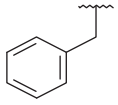
[a] Low active means the EC_{50} was > 1000 μM before adjusted Niacin data to 5.0 μM.

did not. Despite its remarkable duration of in the vivo efficacy, compound **16** showed poor oral exposure ($AUC_{0\text{-}7h}$ = 0.63 μg hr/mL, 10 mg/kg p.o.) in rat pharmacokinetic studies.

In summary, replacement of the anthranilic acid to the pyrrole scaffold and optimization of the alkyl chain on the pyrrole nitrogen led to the discovery of novel and potent GPR109A agonists. The location of

Table 2
In vitro SAR of pyrrole derivatives.



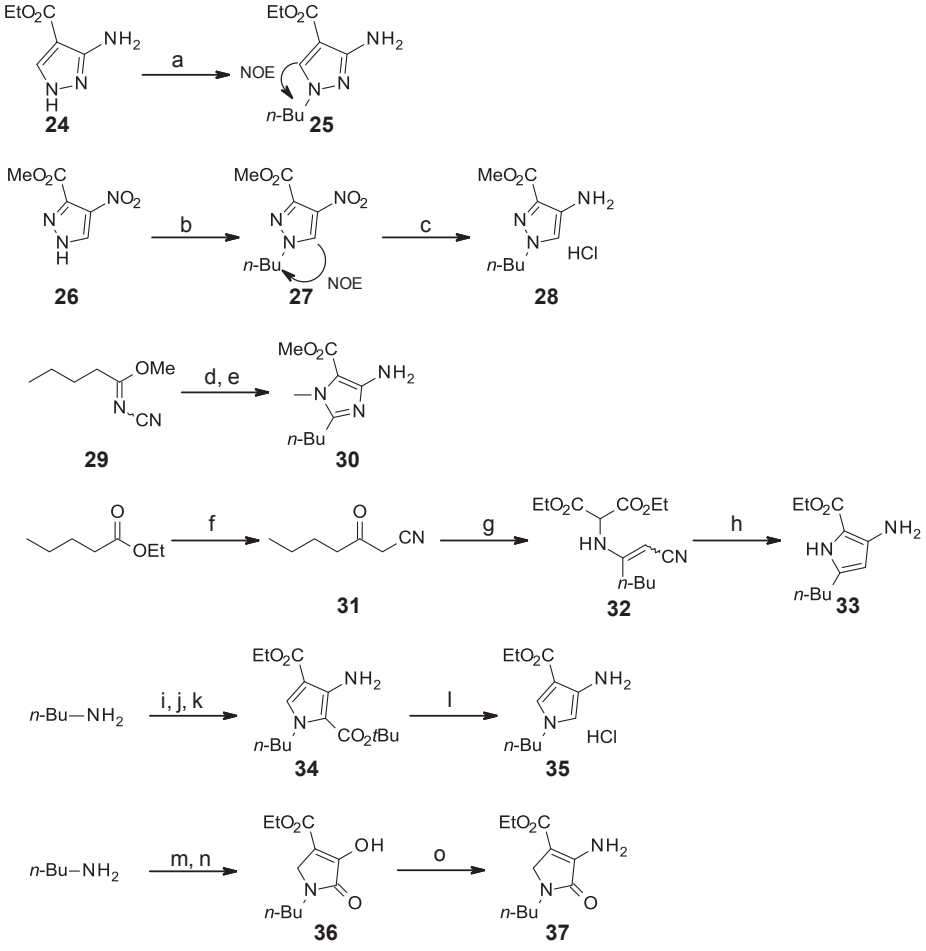
Compd	R^2	X	^{3}H-Niacin Binding assay IC_{50}(μM)	^{35}S-GTPγS Functional assay EC_{50}(μM)
Niacin			0.50	5.0
12a	-Me	CH	0.55	4.8
12b	-Me	N	0.97	2.0
13	-Et	CH	0.22	1.6
14	-*i*-Pr	N	1.9	9.5
15	-cycloPr	N	1.6	8.1
16	-*n*-Bu	N	0.18	0.64
17		CH	4.0	14
18		N	2.6	7.6
19		N	1.1	2.8
20		N	0.74	1.5
21		CH	100	low active[a]
22		CH	11	26
23		CH	0.62	3.2

[a] Low active means the EC_{50} was > 1000 μM before adjusted Niacin data to 5 μM.

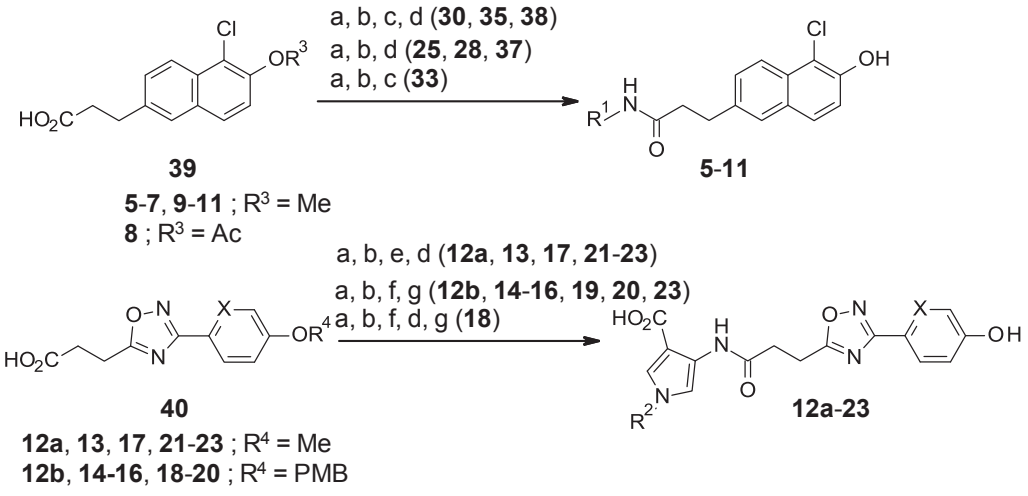
the nitrogen atom on the 5-membered hetero rings and the polarity of the substitution from the nitrogen atom on pyrrole influenced in vitro activity greatly. Further structural optimizations and biological evaluations of the novel pyrrole-based GPR109A agonists to improve PK profiles will be reported in due course.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.



Scheme 1. Reagents and conditions: (a) NaH, *n*-BuI, MeCN, rt to 60 °C, 38%; (b) NaH, *n*-BuI, MeCN, rt, 62%; (c) $Pd(OH)_2/C$, H_2, MeOH, then 4 N HCl, rt, 59%; (d) sarcosine methyl ester hydrochloride, Et_3N, rt to 60 °C; (e) NaOMe, MeOH, 0 °C to rt, 90% (two steps); (f) MeCN, NaH, THF, 70 °C, 81%; (g) diethyl 2-aminopropanedioate, NaOEt, NaOAc, EtOH, rt, 33%; (h) NaOEt, EtOH, 50 °C, 30%; (i) *tert*-butyl 2-bromoacetate, *t*-BuOMe, rt, quant.; (j) ethyl 2-(ethoxymethylene)-2-cyanoacetate, Et_3N, toluene, rt, 86%; (k) NaH, THF, 0 °C to rt, 78%; (l) conc.HCl, 1,4-dioxane, rt, 99%; (m) Ethyl acrylate, EtOH, rt; (n) diethyl oxalate, NaOEt, reflux, 69% (two steps); (o) NH_4OAc, AcOH, 110 °C, 88%.



Scheme 2. Reagents and conditions: (a) oxalyl chloride, DMF, CH_2Cl_2, rt; (b) amine (**25**, **28**, **30**, **33**, **35**, **37**, **38**, corresponding amine to **12a-23**), Et_3N or pyridine/CH_2Cl_2, or DMA, 0 °C to rt, 21%-quant. (two steps); (c) 1 N LiOH, THF, EtOH, rt to 50–90 °C, 53%-quant.; (d) BBr_3, CH_2Cl_2, −78 °C to rt, 15%-quant.; (e) 1 N NaOH, EtOH, 100 °C, 39–96%.; (f) triisopropylsilane, TFA, CH_2Cl_2, 0 °C to rt, 47%-quant.; (g) 1 N LiOH, EtOH or THF, 70–100 °C, 50–78% or 14–27% (two steps for **12b**, **16**).

Table 3
Reductions of plasma NEFA levels of **16** and niacin compared with vehicle by p.o. dosing[a] in fasted rats (n = 5).

Compd	0.5 h after dosing[b] (%)	3 h after dosing[b] (%)
vehicle	0	0
16 (10 mg/kg)	−66	−21
16 (30 mg/kg)	−76	−56
Niacin (3 mg/kg)	−86	+6
Niacin (10 mg/kg)	−86	+10

[a] PG/Tween = 4/1.
[b] Percent changes from average plasma NEFA levels in the vehicle group 0.5 h and 3 h after dosing, respectively.

Acknowledgments

We are grateful to Dr. Yoshikazu Uto at Daiichi Sankyo Co., Ltd. for his review and comments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127105.

References

1. Altschul R, Hoffer A, Stephen JD. *Arch Biochem Biophys.* 1995;54:558–559.
2. Carlson LA. *J Intern Med.* 2005;258:94–114.
3. (a) Knopp RH. *N Engl J Med.* 1999;341:498–511
   (b) Offermanns S. *Trends Pharmacol Sci.* 2006;27:384–390.
4. (a) Gotto AM. *Circulation.* 2001;103:2213–2218l
   (b) Gille A, Bodor ET, Ahmed K, Offermanns S. *Annu Rev Pharmacol Toxicol.* 2008;48:79–106.
5. Paolini JF, Mitchel YB, Reyes R, et al. *Am J Cardiol.* 2008;101:625–630.
6. (a) Schaoub A, Fütterer A, Pfeffer K. *Eur J Immunol.* 2001;31:3714–3725
   (b) Wise A, Foord SM, Fraser NJ, et al. *J Biol Chem.* 2003;278:9869–9874
   (c) Tunaru S, Kero J, Schaub A, et al. *Nat Med.* 2003;9:352–355.
7. (a) Lauring B, Taggart AK, Tara JR, et al. *Sci Transl Med.* 2012;4:148ra115
   (b) Olson EJ, Mahar KM, Haws TF, et al. *Clin Pharmacol Drug Dev.* 2019;8:871–883.
8. Benyó Z, Gille A, Kero J, et al. *J Clin Invest.* 2005;115:3634–3640.
9. (a) Campbell M, et al. Patent WO 2005/016867; (b) Campbell M, et al. Patent WO 2005/016870; (c) Pinto IL, et al. Patent WO 2005/077950; (d) Campbell M, et al. Patent WO 2006/085108; (e) Mason AM, et al. Patent WO 2006/085111; (f) Hatley RJ, et al. Patent WO 2006/085112; (g) Pinto IL, et al. Patent WO 2006/085113; (h) Hatley RJD, et al. Patent WO 2006/045564 A1; (i) Hatley RJD, et al. Patent WO 2006/045565; (j) Heer J, et al. Patent WO 2007/017265.
10. Shen HC, Szymonifka MJ, Kharbanda D, et al. *Bioorg Med Chem Lett.* 2007;17:6723–6728.
11. Shen HC, Ding FX, Luell S, et al. *J Med Chem.* 2007;50:6303–6306.
12. Raghavan S, Tria GS, Shen HC, et al. *Bioorg Med Chem Lett.* 2008;18:3163–3167.
13. (a) Shen HC, Ding FX, Deng Q, et al. J Med Chem. 2009;52;2587–2602; (b) Semple G, et al. Patent WO 2005/044816; (c) Colletti SL, et al. Patent WO 2006/057922; (d) Raghavan S, et al. Patent WO 2006/002557; (e) Colletti SL, et al. Patent WO 2007/027532; (f) Colletti SL, et al. Patent WO 2007/035478.
14. Colletti SL, et al. Patent WO 2006/052555.
15. (a) Gharbaoui T, Skinner PJ, Shin Y-J, et al. Bioorg Med Chem Lett. 2007;17;4914–4919; (b) Skinner PJ, Cherrier MC, Webb PJ, et al. Bioorg Med Chem Lett. 2007;17;5620–5623; (c) Boatman DP, et al. Patent WO 2006/069242.
16. (a) Peters JU, Kühne H, Dehmiow H, et al. Bioorg Med Chem Lett. 2010;20;5426–5430; (b) Dehmlow H, et al. Patent US 2006/0281810; (c) Dehmlow H, et al. Patent US 2007/0072873.
17. Palani A, et al. Patent WO 2006/124490.
18. Cao G, et al. Patent WO 2007/015744.
19. Blad CC, van Veldhoven JP, Klopman C, et al. *J Med Chem.* 2012;55:3563–3567.
20. (a) Semple G, Skinner PJ, Gharbaoui T, et al. *J Med Chem.* 2008;51:5101–5108
   (b) Imbriglio JE, Chang S, Liang R, et al. *Bioorg Med Chem Lett.* 2009;19:2121–2124
   (c) Imbriglio JE, Chang S, Liang R, et al. *Bioorg Med Chem Lett.* 2010;20:4472–4474
   (d) Boatman PD, Schrader TO, Kasem M, et al. *Bioorg Med Chem Lett.* 2010;20:2797–2800
   (e) Boatman PD, Lauring B, Schrader TO, et al. *J Med Chem.* 2012;55:3644–3666.
21. Shen HC, Ding FX, Deng Q, et al. *J Med Chem.* 2009;52:2587–2602.
22. Shen HC, Ding FX, Raghavan S, et al. *J Med Chem.* 2010;53:2666–2670.
23. Soga T, Kamohara M, Takasaki J, et al. *Biochem Biophys Res Commun.* 2003;303:364–369.
24. Wise A, Foord SM, Fraser NJ, et al. *J Biol Chem.* 2003;278:9869–9874.
25. Taggart AK, Kero J, Gan X, et al. *J Biol Chem.* 2005;280:26649–26652.
26. Schmidt D, Smenton A, Raghavan S, et al. *Bioorg Med Chem Lett.* 2010;20:3426–3430.
27. Che J, Raghavendra MS, Lam Y. *J Comb Chem.* 2009;11:378–384.
28. Jacobi PA, Buddhu SC. *Tetrahedron Lett.* 1998;29:4823–4826.
29. (a) Colletti SL, et al. Patent WO 2007/120575; (b) Suzuki K, et al. Patent WO 2010/004972.
30. Masuda Y, Kurikawa N, Nishizawa T. *Atherosclerosis.* 2018;272:182–192.