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Discovery of a Potent Nicotinic Acid Receptor Agonist for the Treatment of Dyslipidemia

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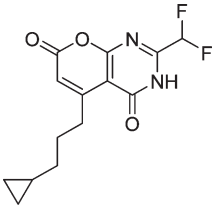
ABSTRACT Nicotinic acid has been used clinically for decades to control serum lipoproteins. Nicotinic acid lowers very low-density lipoprotein (VLDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, and lipoprotein-a (LPa), and it is also effective in raising high-density lipoprotein (HDL)-cholesterol. However, nicotinic acid has several side effects in clinical use. The most notable is intense cutaneous vasodilation "flushing" on the upper body and face. We discovered a pyranopyrimidinedione series to be nicotinic acid receptor agonists. A potent nicotinic acid receptor agonist from this series {5-(3-cyclopropylpropyl)-2-(difluoromethyl)-3*H*-pyrano[2,3-*d*]pyrimidine-4,7-dione}with reduced flushing side effect in dogs was identified.

KEYWORDS Nicotinic acid, agonist, flushing, dyslipidemia, VLDL, HDL



Nicotinic acid (NA) has been used as a drug to lower very low-density lipoprotein (VLDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, and lipoprotein-a (LPa), and it is also effective in raising high-density lipoprotein (HDL)-cholesterol in plasma.[1−7] Studies have demonstrated that nicotinic acid is able to reduce the rate of nonfatal myocardial infarction (MI) and decrease total mortality of patients with a previous history of heart disease.[8,9] In combination with statins, nicotinic acid provided better therapeutic benefit than taking statins alone.[10,11] Despite all of these encouraging clinical benefits, nicotinic acid is known to have side effects that limit its clinical use. The most notable is a severe cutaneous flushing sensation on the upper body and face.[12,13] Other issues include gastrointestinal (GI) side effects[14] and hepatotoxicity.[15−17] Additionally, patients need to take large quantities of the drug (grams per day) due to its short half-life, which also leads to compliance issues. To alleviate this associated flushing side effect, an extended release formulation of nicotinic acid (NIASPAN) is available by prescription.[18] More recently, a combination of extended release nicotinic acid and an antagonist of the prostaglandin D2 receptor (DP) (Tredaptive) has been introduced in Europe. Studies have shown that patients taking this combination of drugs experienced reduced flushing symptoms in comparison with taking nicotinic acid alone.[19]

Nicotinic acid binds with high affinity to a G-protein-coupled receptor, GPR109a, expressed in human adipose tissue. A highly related G-protein-coupled receptor, GPR109b, that shares 95% identity and is only expressed in human and chimpanzee, was also identified.[20−23] GPR109b is a low affinity receptor for nicotinic acid. It has been hypothesized that activation of GPR109a by nicotinic acid decreases intracellular cAMP levels in adipocytes. As a downstream effect, protein kinase A activity is reduced, leading to a decrease in hormone sensitive lipase activity, eventually causing the reduction of intracellular triglyceride (TG) hydrolysis and free fatty acid (FFA) secretion. This decrease of FFA production from adipocytes results in a substrate shortage for liver synthesis and secretion of TG and VLDL, which eventually leads to a decrease of VLDL, LDL, and TG levels in plasma.[24−28] However, the mechanism of the nicotinic acid-induced increase of HDL levels in plasma is not known.[29,30]

Our goal was to identify a compound with better potency and pharmacokinetic profile than nicotinic acid but no flushing side effect. Our structure−activity studies started from the hit compound **1** (Table 1), a thiobarbituric acid derivative which was obtained from a high-throughput screen. It showed moderate in vitro potency as a GPR109a agonist. In order to improve the in vitro activity of **1**, we decided to explore the SAR of the C-2 region first while maintaining the ethyl group as the C-5 substituent. A variety of analogues with different C-2 substituents (R^1) were prepared (Table 1). The type of substitution clearly influenced

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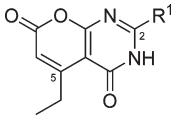
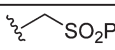
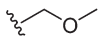
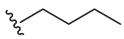
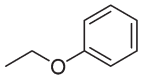
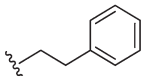
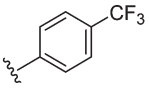
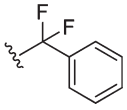
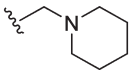
Table 1. Potency of Compounds in the hu-GPR109a cAMP Assay



Compound	R^1	EC_{50} (nM)[a]	Compound	R^1	EC_{50} (nM)[a]
1		470±68	**12**		>10000
2		>10000	**13**		1110±25
3		>10000	**14**		3393±386
4		>10000	**15**		3108±715
5		>10000	**16**		1261±547
6		>10000	**17**		353±85
7		>10000	**18**		335±41
8		>10000	**19**		2705±1800
9		>10000	**20**		6065±3935
10		>10000	**21**		>10000
11		>10000			

[a] Data represent an average of multiple determinations ($n \geq 3$) ± standard deviation.

the activity. When the thiol group of **1** was changed to a hydrogen atom (**2**), alkyl (**3**−**5**), cyclopropyl (**6**), alkenyl (**7**), aromatic (**8**), ester (**9**), nitrile (**10**), cycloaminoalkyl (**11**), or phenylsulfonylalkyl group (**12**), the activity was lost. In the case of **13**, when R^1 was a hydroxymethyl group, moderate activity was obtained. However, ether analogues **14** and **15** were 3-fold less potent. On the other hand, the fluoroalkyl group at R^1 displayed an interesting profile. Compound **16** with a trifluoromethyl group showed moderate activity similar to that of **13**. Less fluorinated analogues **17** and **18** were much more potent and exhibited slightly better activity than hit compound **1**. Changing R^1 from difluoromethyl (**17**) to fluorochloromethyl (**19**) was detrimental to the activity. Fluoroalkyl derivatives became less tolerated with increasing steric hindrance (**20** and **21**). Due to the potential to produce toxic α-fluoroacetic acid from **18**, the difluoromethyl group of **17** was chosen to be the optimal R^1 substituent for carrying out additional SAR on the C-5 region.

Having identified the difluoromethyl group as the optimal C-2 substituent, we undertook the SAR of the C-5 region. A series of analogues with different alkyl substituents as R^2 were prepared (Table 2). The activity was very sensitive to subtle changes in the R^2 substituent. Although methyl and propyl analogues, **22** and **23**, were much less potent than the ethyl derivative **17**, compounds **24**−**27** with longer side chains than propyl retained activity similar to that of **17**,

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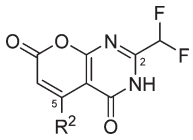
Table 2. Potency of Compounds in the hu-GPR109a cAMP Assay



Compound	R^2	EC_{50} (nM)[a]	Compound	R^2	EC_{50} (nM)[a]
22		6581±3419	**27**		275±141
23		4436±1164	**28**		4152±42
24		245±87	**29**		1402±879
25		128±28	**30**		108±60
26		308±103	**31**		451±161

[a] Data represent an average of multiple determinations ($n \geq 3$) ± standard deviation.

with the pentyl derivative **25** being the best. Introduction of a large cyclohexyl or cyclopentyl group (**28** and **29**) was not tolerated. However, compounds with smaller cyclobutyl or cyclopropyl substituents (**30** and **31**) showed good activity.

Given the promising data of compound **30**, we further explored the SAR on this interesting entity. Several cyclobutyl derivatives with different linker lengths (**32**−**34**) and ring substituents (**35**−**36**) were prepared (Table 3). The cyclobutylethyl group of **30** retained the best in vitro activity. Increasing or decreasing linker length in the case of **32**−**34** had a negative effect on activity. Meanwhile, a methyl substituent on the cyclobutyl ring (**35**) was 3-fold less potent than **30**. However, the spirocyclobutyl analogue **36** maintained similar activity to that of **30**. With the above SAR results we note that potency is very sensitive to subtle changes and difficult to predict.

Because of the unpredictable nature of the SAR of the C-5 region, we decided to conduct more detailed SAR on compound **31**. This could potentially provide the opportunity to identify compounds with better activity than the cyclobutyl series. We synthesized a series of cyclopropyl derivatives with different linker lengths and substituents on the cyclopropyl ring. The data in Table 3 again confirms the unpredictable nature of the SAR. When the substituent was changed from the cyclopropylethyl of **31** to cyclopropyl (**37**) or cyclopropylmethyl (**38**), the activity was lost. Fortunately, the cyclopropylpropyl derivative **39** {5-(3-cyclopropylpropyl)-2-(difluoromethyl)-3*H*-pyrano[2,3-*d*]pyrimidine-4,7-dione} showed much improved potency relative to **31** (**39**; EC_{50} = 59 nM), but this was not the case for the cyclopropylbutyl analogue **40**. Introduction of a methyl substituent on the cyclopropyl ring maintained excellent activity (**41**; EC_{50} = 49 nM). However, the substitution that is tolerated on the cyclopropyl ring is very limited, as demonstrated in the cases of **42**−**47**. Steric hindrance played an important role. SAR studies of this cyclopropyl series eventually led to the discovery of compounds **39** and **41** with potent in vitro activity. Compounds tested active, as GPR109a agonists are full agonists and showed much weaker binding affinity for the GPR109b (data not shown). The presence of the NH is important for GPR109a activity, and the acidic NH bond mimics the role of nicotinic acid.

The synthesis of compounds is exemplified by that of **39** in Scheme 1. Condensation of malonamide **48** with ethyl difluoroacetate under basic conditions afforded **49**, which was then cyclized with β-ketoester **51** in acetic acid to provide **39**. β-Ketoester **51** was prepared from acid precursor **50** in one step. The rest of the analogues were synthesized by methods similar to that of **39**.

Although compounds **39** and **41** showed almost identical in vitro potency (Table 4), they behaved very differently in vivo. These two compounds were dosed orally in a rat assay designed to measure reductions in FFA and TG. While **39** demonstrated robust efficacy in vivo (rat FFA −77%, TG −49%, 1 h post 1 mg/kg po dosing), analogue **41** was much less active (rat FFA −14%, TG −23%, 1 h post 1 mg/kg po dosing). A detailed examination of their pharmacokinetics revealed that **39** had much better plasma exposure than **41** (**39** rat AUC_{0-6h} = 34.1 μg/mL·h, **41** AUC_{0-6h} = 3.0 μg/mL·h, 10 mg/kg po dosing).[31] A head-to-head study between **39** and nicotinic acid demonstrated that **39** also had improved potency compared to nicotinic acid in vitro and in vivo (see Supporting Information). Compound **39** dose-dependently inhibited rat plasma FFA with an ED_{50} of 0.2 mg/kg and a maximally effective dose of 1.0 mg/kg. The ED_{50} for nicotinic acid on plasma FFA was difficult to calculate due to the steep dose response effect of nicotinic acid in this model. The maximally effective dose for nicotinic acid on plasma FFA was 10 mg/kg. In addition, **39** dose-dependently lowered plasma triglyceride 1 and 6 h after dosing in rats. In contrast, nicotinic acid lowered plasma triglyceride in rats 1 h after dosing but was not active at 6 h, indicating **39** had a longer duration of action. A time course study of **39** in the rat at 3 mg/kg showed that it significantly reduced plasma triglyceride from 1 to 8 h post dosing. In contrast, the duration of activity with nicotinic acid at 10 mg/kg treatment did not extend beyond 2 h after dosing. Compound **39** was studied for efficacy and flushing profile in fasted male beagle dogs. Although **39** had very similar in vitro potency to nicotinic acid at dog GPR109a, it displayed much greater in vivo efficacy than nicotinic acid. Compound **39** exhibited dose-dependent inhibition of plasma FFA and achieved 70% FFA reduction at 3 mg/kg dose. At this dose, dogs had no overt signs of flushing, as determined by quantification of changes in cutaneous blood flow (Figure 1) and/or changes in skin color and behavior. In contrast, nicotinic acid achieved similar reduction of FFA at 30 mg/kg dose, but this dose produced a pronounced flushing response in 9 of 13 dogs, observed as an average of a 5-fold increase in cutaneous blood flow from baseline

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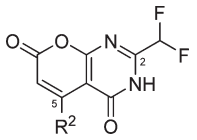
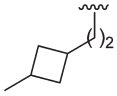
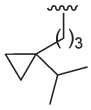
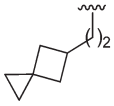
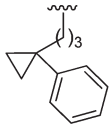
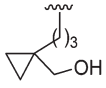
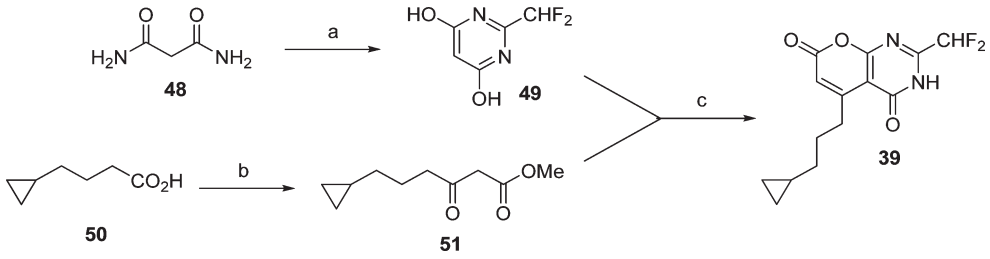
Table 3. Potency of Compounds in the hu-GPR109a cAMP Assay



Compound	R^2	EC_{50} (nM)[a]	Compound	R^2	EC_{50} (nM)[a]
32		>10000	**40**		591±350
33		821±144	**41**		49±11
34		479±113	**42**		327±103
35		379±92	**43**		787±106
36		130±32	**44**		1076±267
37		>10000	**45**		3119±433
38		>10000	**46**		>10000
39		59±56	**47**		281±92

[a] Data represent an average of multiple determinations ($n \geq 3$) ± standard deviation.

Scheme 1. Synthesis of Compound **39**[a]



[a] Reagents and conditions: (a) CHF_2CO_2Et, NaOEt, EtOH, reflux, 70−75%; (b) $MeO_2CCH_2CO_2K$, CDI, $MgCl_2$, THF, 85−95%; (c) HOAc, 120 °C, 20−25%.

(Figure 1) as well as behavioral responses characterized by head shaking and ear scratching. Overall, **39** was approximately 10-fold more potent than nicotinic acid in reducing dog plasma FFA. At the effective dose, both the

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Table 4. In Vitro and in Vivo Potencies of **39**, **41**, and Nicotinic Acid

	39	**41**	nicotinic acid
hu-GPR109a EC_{50} (nM)[a]	59 ± 56	49 ± 11	100 ± 15
rat-GPR109a EC_{50} (nM)[a]	3 ± 1	6 ± 2	39 ± 10
m-GPR109a EC_{50} (nM)[a]	4 ± 3	15 ± 5	29 ± 5
dog-GPR109a EC_{50} (nM)[a]	21 ± 4	126 ± 58	30 ± 14
cyno-GPR109a (nM)[a]	2 ± 1	nd[b]	26 ± 33
hu-GPR109b EC_{50} (nM)[a]	300 ± 105	1120 ± 325	> 10000
rat FFA reduction (1 h post 1 mg/kg dosing)	−77%	−14%	−80%[c]
rat TG reduction (1 h post 1 mg/kg dosing)	−49%	−23%	−60%[c]
dog FFA reduction (1 h post 3 mg/kg dosing)	−70%	nd[b]	−38%

[a] Data represent an average of multiple determinations ($n \geq 3$) ± standard deviation. [b] nd = not determined. [c] 10 mg/kg dosing.

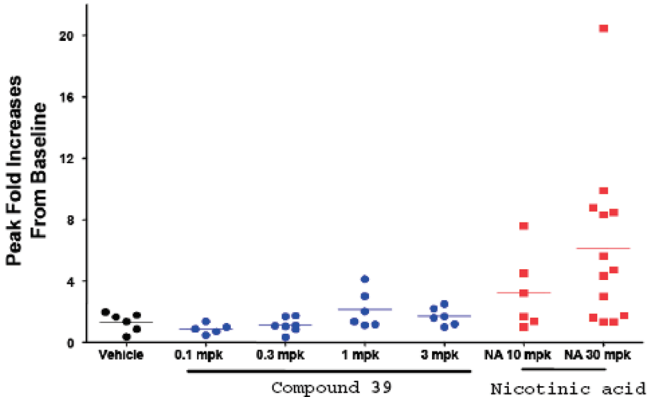

Figure 1. Peak fold increase from baseline in cutaneous blood flow in dogs dosed with **39** (0.1−3 mg/kg, po), nicotinic acid, or vehicle (data for individual dogs).

severity and incidence of flushing were markedly reduced or absent compared to the case of nicotinic acid. This compound also demonstrated an acceptable PK profile in dog (**39** dog AUC_{0-24h} = 70.2 μg/mL·h, 3 mg/kg po dosing). Compound **39** had no effect on hERG at concentrations up to 6 μM, was not an inhibitor of human CYPs 1A2, 2C9, 2D6, or 3A4 (IC_{50} > 50 μM), and did not show induction of CYP3A4 in the human PXR assay up to 10 μM. No significant activity was observed for **39** in the Cerep and GPCR counterscreens. In the radioligand binding experiment, the binding of compound **39** with GPR109a is competitive with nicotinic acid.

In summary, we have identified a potent nicotinic acid receptor agonist **39**, which demonstrated excellent in vivo activity in animal models. In addition, the incidence and magnitude of cutaneous blood flow increases observed with nicotinic acid in the dog models were not observed with compound **39**, suggesting that it has an improved therapeutic window to flushing compared to nicotinic acid. This compound was investigated and developed further for its clinical potential. These results will be the subject of future publications.

SUPPORTING INFORMATION AVAILABLE

Experimental procedures for assay protocols, in vivo studies, and synthesis and characterization of compounds **1**−**47**. This information is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

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ABBREVIATIONS

VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; LPa, lipoprotein-a; HDL, high-density lipoprotein; NA, nicotinic acid; TG, triglyceride; FFA, free fatty acid; DP, prostaglandin D2 receptor; GPR, G-protein-coupled receptor; cAMP, 3′-5′-cyclic adenosine monophosphate.

REFERENCES

(1) Altschul, R.; Hoffer, A.; Stephen, J. D. Influence of nicotinic acid on serum cholesterol in man. *Arch. Biochem.* **1955,** *54*, 558–559.

(2) Carlson, L. A. Nicotinic acid: the broad-spectrum lipid drug. A 50th anniversary review. *J. Int. Med.* **2005,** *258*, 94–114.

(3) Gotto, A. M., Jr. Low high-density lipoprotein cholesterol as a risk factor in coronary heart disease. A working group report. *Circulation* **2001,** *103*, 2213–2218.

(4) Boatman, P. D.; Richman, J. G.; Semple, G. Nicotinic acid receptor agonists. *J. Med. Chem.* **2008,** *51*, 7653–7662.

(5) Imbriglio, J. E.; Chang, S.; Liang, R.; Raghavan, S.; Schmidt, D.; Smenton, A.; Tria, S.; Schrader, T. O.; Jung, J.; Esser, C.; Taggart, A. K. P.; Cheng, K.; Carballo-Jane, E.; Waters, M. G.; Tata, J. R.; Colletti, S. L. GPR109a agonists. Part 1: 5-Alkyl and 5-aryl-pyrazole-tetrazoles as agonists of the human orphan G-protein coupled receptor GPR109a. *Bioorg. Med. Chem. Lett.* **2009,** *19*, 2121–2124.

(6) Boatman, P. D.; Schrader, T. O.; Kasem, M.; Johnson, B. R.; Skinner, P. J.; Jung, J.; Xu, J.; Cherrier, M. C.; Webb, P. J.; Semple, G.; Sage, C. R.; Knudsen, J.; Chen, R.; Taggart, A. K.; Carballo-Jane, E.; Richman, J. G. Potent tricyclic pyrazole tetrazole agonists of the nicotinic acid receptor (GPR109a). *Bioorg. Med. Chem. Lett.* **2010,** *20*, 2797–2800.

(7) Imbriglio, J. E.; Chang, S.; Liang, R.; Raghavan, S.; Schmidt, D.; Smenton, A.; Tria, S.; Schrader, T. O.; Jung, J.; Esser, C.; Holt, T. G.; Wolff, M. S.; Taggart, A. K. P.; Cheng, K.; Carballo-Jane,

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E.; Waters, M. G.; Tata, J. R.; Colletti, S. L. GPR109a agonists. Part 2: Pyrazole-acids as agonists of the human orphan G-protein coupled receptor GPR109a. *Bioorg. Med. Chem. Lett.* **2010,** *20*, 4472–4474.

(8) The Coronary Drug project: design, methods, and baseline results. *Circulation*, **1973**, *47*, 1-179.

(9) Canner, P. L.; Berge, K. G.; Wenger, N. K.; Stamler, J.; Friedman, L.; Prineas, R, J.; Friedewald, W. Fifteen year mortality in Coronary Drug Project patients: long term benefit with niacin. *J. Am. Coll. Cardiol.* **1986,** *8*, 1245–1255.

(10) Brown, B. G.; Zhao, X. Q.; Chait, A.; Fisher, L. D.; Cheung, M. C.; Morse, J. S.; Dowdy, A. A.; Marino, E. K.; Bolson, E. L.; Alaupovic, P.; Frohlich, J.; Albers, J. J. Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. *N. Eng. J. Med.* **2001,** *345*, 1583–1592.

(11) Taylor, A. J.; Sullenberger, L. E.; Lee, H. J.; Lee, J. K.; Grace, K. A. Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol (ARBITER) 2: a double-blind, placebo-controlled study of extended-release niacin on atherosclerosis progression in secondary prevention patients treated with statins. *Circulation* **2004,** *110*, 3512–3517.

(12) Goldsmith, G. A.; Cordill, S. The vasodilating effects of nicotinic acid (relation to metabolic rate and body temperature) *Am, J. med. Sci.* **1943,** *205*, 204–208.

(13) Hiatt, J. G.; Shamsie, S. G.; Schectman, G. Discontinuation rates of cholesterol-lowering medications: implications for primary care. *Am. J. Manag. Care* **1999,** *5*, 437–444.

(14) Knopp, R. H.; Ginsberg, J.; Albers, J. J.; Hoff, C.; Ogilvie, J. T.; Warnick, G. R.; Burrows, E.; Retzlaff, B.; Poole, M. Contrasting effects of unmodified and time-release forms of niacin on lipoproteins in hyperlipidemic subjects: clues to mechanism of action of niacin. *Metabolism* **1985,** *34*, 642–650.

(15) Dalton, T. A.; Berry, R. S. Hepatotoxicity associated with sustained-release niacin. *Am. J. Med.* **1992,** *93*, 102–104.

(16) Lawrence, S. P. Transient focal hepatic defects related to sustained-release niacin. *J. Clin. Gastroenterol* **1993,** *16*, 234–236.

(17) Etchason, J. A.; Miller, T. D.; Squires, R. W.; Allison, T. G.; Gau, G. T. Niacin-induced hepatitis: a potential side effect with low-dose time-release niacin. *Mayo Clin. Proc.* **1991,** *66*, 23–28.

(18) Morgan, J. M.; Capuzzi, D. M.; Guyton, J. R.; Centor, R. M.; Goldberg, R.; Robbins, D. C.; Dipette, D.; Jenkins, S.; Marcovina, S. Treatment effects of Niaspan, a controlled-release niacin, in patients with hypercholesterolemia: a placebo-controlled trial. *J. Cardiovasc. Pharmacol. Ther.* **1996,** *1*, 195–202.

(19) Paolini, J. F.; Mitchel, Y. B.; Reyes, R.; Kher, U.; Lai, E.; Watson, D. J.; Norquist, J. M.; Meehan, A. G.; Bays, H. E.; Davidson, M.; Ballantyne, C. M. Effects of laropiprant on nicotinic acid-induced flushing in patients with dyslipidemia. *Am. J. Cardiol.* **2008,** *101*, 625–630.

(20) Lorenzen, A.; Stannek, C.; Lang, H.; Andrianov, V.; Kalvinsh, I.; Schwabe, U. Characterization of a G protein-coupled receptor for nicotinic acid. *Mol. Pharmacol.* **2001,** *59*, 349–357.

(21) Wise, A.; Foord, S. M.; Fraser, N. J.; Barnes, A. A.; Elshourbagy, N.; Eilert, M.; Ignar, D. M.; Murdock, P. R.; Steplewski, K.; Green, A.; Brown, A, J.; Dowell, S. J.; Szekeres, P. G.; Hassall, D. G.; Marshall, F. H.; Wilson, S.; Pike, N. B. Molecular identification of high and low affinity receptors for nicotinic acid. *J. Biol. Chem.* **2003,** *278*, 9869–9874.

(22) Tunaru, S.; Kero, J.; Schaub, A.; Wufka, C.; Blaukat, A.; Pfeffer, K.; Offermanns, S. PUMA-G and HM74 are receptors for nicotinic acid and mediate its antilipolytic effect. *Nat. Med.* **2003,** *9*, 352–355.

(23) Torhan, A. S.; Cheewatrakoolpong., B.; Kwee, L.; Greenfeder, S. Cloning and characterization of the hamster and guinea pig nicotinic acid receptors. *J. Lipid. Res.* **2007,** *48*, 2065–2071.

(24) Carlson, L. A.; Orö, L. The effect of nicotinic acid on the plasma free fatty acid; demonstration of a metabolic type of sympathicolysis. *Acta Med. Scand.* **1962,** *172*, 641–645.

(25) Carlson, L. A. Studies on the effects of nicotinic acid on catecholamine stimulated lipolysis in adipose tissue in vitro. *Acta Med. Scand.* **1963,** *173*, 719–722.

(26) Carlson, L. A.; Hanngren, A. Initial distribution in mice of ^{3}H-labeled nicotinic acid studied with autoradiography. *Life Sci.* **1964,** *3*, 867–871.

(27) Butcher, R. W.; Baird, C. E.; Sutherland, E. W. Effects of lipolytic and antilipolytic substances on adenosine 3′, 5′-monophosphate levels in isolated fat cells. *J. Biol. Chem.* **1968,** *243*, 1705–1712.

(28) Aktories, K.; Schultz, G.; Jakobs, K. H. Regulation of adenylate cyclase activity in hamster adipocites. Inhibition by prostaglandins, α-adrenergic agonists and nicotinic acid. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1980,** *312*, 167–173.

(29) Sprecher, D. L. Raising high-density lipoprotein cholesterol with niacin and fibrates: a comparative review. *Am. J. Cardiol.* **2000,** *86*, 46–50.

(30) Le Goff, W.; Guerin, M.; Chapman, M. J. Pharmacological modulation of cholesteryl ester transfer protein, a new therapeutic target in atherogenic dyslipidemia. *Pharmacol. Ther.* **2004,** *101*, 17–38.

(31) Korfmacher, W. A.; Cox, K. A.; Ng, K. J.; Veals, J.; Hsieh, Y.; Wainhaus, S.; Broske, L.; Prelusky, D.; Nomeir, A.; White, R. E. Cassette-accelerated rapid rat screen: a systematic procedure for the dosing and liquid chromatography/atmospheric pressure ionization tandem mass spectrometric analysis of new chemical entities as part of new drug discovery. *Rapid Commun. Mass Spectrom.* **2001,** *15*, 335–340.

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