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Pyrazole acids as niacin receptor agonists for the treatment of dyslipidemia

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ABSTRACT

Niacin is an effective drug for raising HDL cholesterol and reducing coronary risks, but patients show low compliance with treatment due to severe facial flushing upon taking the drug. A series of bicyclic pyrazole carboxylic acids were synthesized and tested for their ability to activate the niacin receptor. One analog, **23**, showed improved potency and lacked flushing at doses that effectively altered the lipid profile of rats.

Elevated levels of LDL cholesterol and reduced HDL cholesterol are both strong risk factors for the development of atherosclerotic coronary heart disease. Statins can effectively lower LDL, but they have shown only modest effects on HDL (5–15%).[1] Even when LDL levels are successfully lowered, patients with low HDL still face an increased risk of mortality relative to patients with high HDL. Therefore, the next generation of treatments for dyslipidemia must address the issue of HDL cholesterol levels.

Niacin (**1**, Fig. 1) has been used for over 40 years for the treatment of dyslipidemia. Niacin therapy leads to lowered free fatty acids (20–40%), triglycerides (20–40%), lipoprotein A (30–40%), and LDL cholesterol (20–40%), but also to a significant elevation of HDL cholesterol (20–35%).[2] In addition, long term studies have demonstrated that niacin therapy reduces mortality from coronary heart disease.[3] This profile has made niacin an attractive treatment for low HDL cholesterol, but side effects of the drug have limited its use. Most patients experience an intense and painful facial flushing after taking the drug.[3] In addition, niacin shows hepatotoxicity and reduces glucose tolerance, which limits its use in prediabetic patients.[4]

Early efforts to improve upon niacin proved challenging because its mechanism of action was not known. Renewed interest in HDL raising drugs spurred efforts to identify niacin's cellular target, and a G_i coupled GPCR, GPR109A, in fat cells, also referred to as HM74A, or 19CD, was identified. This receptor showed a high affinity for niacin.[5] In addition, knockout mice of GPR109A did not show the decrease in free fatty acids upon niacin treatment that is seen in wild type mice, making this the likely target of niacin. Unfortunately, niacin receptor knockout mice failed to show a niacin induced flush, indicating that flushing was in fact mediated through the niacin receptor. Thus, the development of a flush free niacin receptor agonist represents a significant challenge.

The niacin receptor agonist **2** (Fig. 1) was developed twenty years ago[6] and was recently reinvestigated using niacin receptor binding and functional assays. Further modifications of **2** led to the discovery of compounds that were partial agonists[7] with equivalent or improved activity compared to niacin. Among them, compound **3** was of interest to us because it was equipotent to compound **2** and seemed amenable to rapid modifications. We

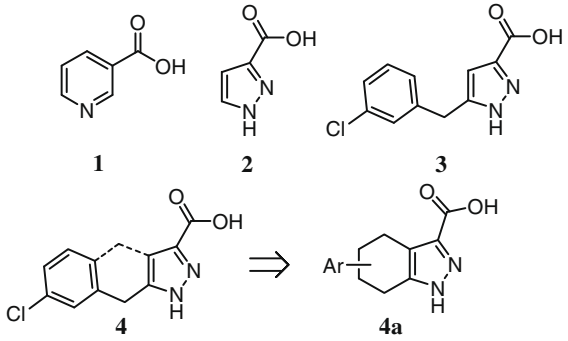

Figure 1. Structure of niacin and lead structures.

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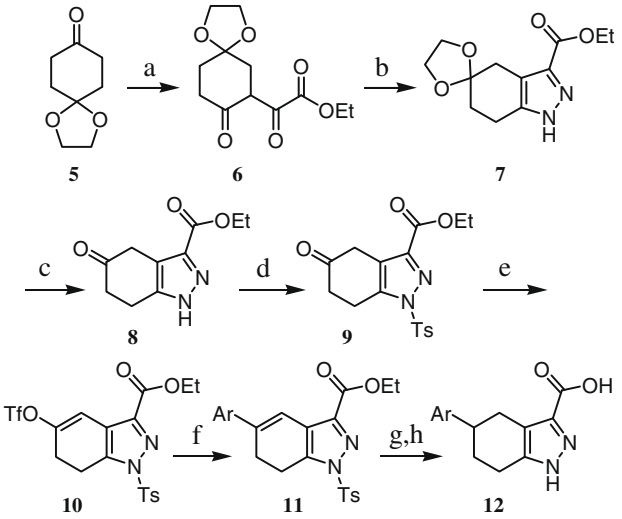


Scheme 1. Synthesis of pyrazole acids. Reagents and conditions: (a) LDA, diethyloxalate, THF, −78 °C; (b) hydrazine hydrate, AcOH/EtOH, 65 °C, 68%; (c) 3 N HCl, THF/EtOH, 62%; (d) NaH, TsCl, THF, 0 °C, 71%; (e) LDA, Comins' reagent, THF, −78 °C, 77%; (f) Ar–B(OH)$_2$, Na_2CO_3(aq), $Pd(PPh_3)_4$, THF, 63–100%; (g) Pd/C, formic acid, ammonium formate, MeOH/THF; (h) LiOH, MeOH/THF/H_2O, 10–30%.

sought to restrict the conformation of the aromatic ring in **3** by appending a 6-membered ring onto the pyrazole. Thus, we undertook the synthesis of a series of aryl cyclohexyl pyrazole acids (**4**). Compounds described herein were synthesized using two different routes (Schemes 1 and 2). Commercially available compound 1,4-dioxaspiro[4.5]decan-8-one (**5**) was acylated with diethyloxalate in the presence of LDA at −78 °C to afford **6**. Cyclization with hydrazine afforded the pyrazole **7**. Removal of the ketal protecting group, followed by treatment with sodium hydride and tosyl chloride afforded **9**. Treatment of **9** with LDA at −78 °C followed by Comins' reagent gave vinyl triflate **10** as a single regioisomer.[8] The vinyl triflate **10** was coupled with a variety of boronic acids using standard Suzuki reaction conditions. Finally, hydrogenation of the double bond, followed by tosyl deprotection, and ester hydrolysis gave the final pyrazole acids.

Compound **16** was synthesized using the route described in Scheme 2. Commercially available 3-ethoxy-2-cyclohexen-1-one (**13**) when treated with phenyl magnesium chloride followed by an acidic workup afforded **14**. Deprotonation of **14** with LDA at −78 °C followed by treatment with diethyloxalate provided an α,β-unsaturated ketone that was hydrogenated to give **15**. Compound **15** was subsequently cyclized to the pyrazole with hydrazine hydrate under acidic conditions. Finally, hydrolysis of the ester gave the final compound **16**.

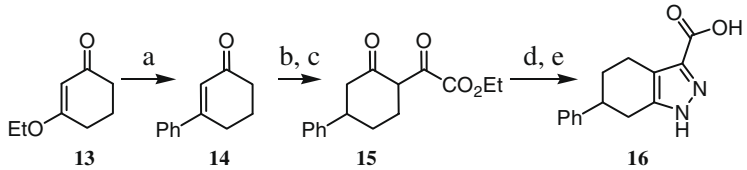
All compounds were evaluated in a binding assay and a functional assay. For the binding assay, membrane preparations were made from CHO–KI cells stably expressing the niacin receptor. Displacement of radiolabelled niacin from the membrane preparation was measured in the absence and presence of human serum. For the functional assay, the same membrane preparations were incubated with compound and then [^{32}S]GTPγS. Retention of [^{32}S]GTPγS on the membrane preparation was measured to determine activation of the G proteins coupled to the niacin receptor.

We first synthesized the cyclohexyl compound **17**, which showed no activity at 25 μM (Table 1). Next, we examined the effect of an aryl substituent at the C5 and C6 position. Towards this end, compounds **16** and **18** were synthesized. Compound **16** was inactive. Interestingly, compound **18** showed weak activity in the functional assay. Encouraged by this result, we decided to explore the SAR of this series further. We first examined the effect of placing a fluorine atom in the *ortho*, *meta*, and *para* positions. The *meta*- and *para*-fluorophenyl compounds had modest activity (data not shown). However, the *ortho*-fluoro compound **19** showed a sixfold improvement in activity compared to **18**. Next the effect of methoxy, methyl, and chloro groups on the phenyl ring was investigated. Among them, only the *ortho*-chloro substituents, **20**, had activity similar to **19**. Based on this data, we focused on fluoro substitution. A series of di- and trisubstituted fluorine compounds was synthesized. The 2,6-difluoro compound **21** showed poor activity, indicating that only one ortho position could be substituted. The 2,5-difluoro phenyl compound **22** had similar activity compared to **19** in the binding assay, but showed a threefold improvement in the functional assay. The 2,3,5-trifluoro compound **25** showed further enhancement in in vitro activity compared to **19** (threefold improvement in the binding assay; a fourfold improvement in the functional assay). Furthermore, **22** and **25** showed a 25-fold increase in functional activity compared to the unsubstituted phenyl **18**. Compound **22** was selected for evaluation in pharmacokinetic studies in mice. It showed a good half-life and low clearance with oral bioavailability of 53% (Table 2).

The compounds described thus far were racemic mixtures. Compound **22** was resolved to provide **23** and **24**. Compound **23** showed a twofold improvement in the binding assay compared to the racemic mixture, while the other enantiomer, **24**, showed weak activity. Compound **23** represented an improvement on compound **18** of over 250-fold in the binding assay and 25-fold improvement in the functional assay.

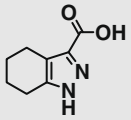
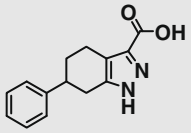
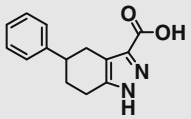
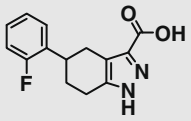
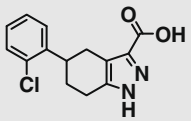
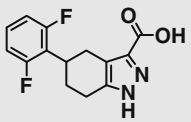
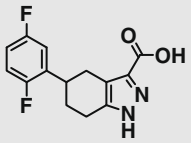
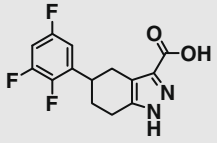
The fluoro compounds were active in the binding and functional assays in the absence of serum. However, in the presence of serum the activity decreased 5–6-fold. As this was a concern, we sought to replace the phenyl ring with more polar groups with the goal of reducing serum shift (Table 3). Towards this end, we examined substituted pyridines. Two fluoropyridine compounds, **26** and **27**, were synthesized. Compound **27** had good binding activity comparable to compound **19** and this activity was retained in the presence of serum (Table 3). Compound **27** when dosed in mice showed threefold higher clearance than compound **22** with a similar half-life (2.6 h) and oral bioavailability (47%) (Table 2).

All compounds synthesized thus far were full agonists. Previous work had indicated that replacing the carboxylic acid with a tetrazole resulted in compounds that were partial agonists and did not cause flushing in a mouse.[9] Therefore, we synthesized the corresponding tetrazole analogs of **22** and **27** (**30** and **31**). Compounds **30** and **31** were synthesized in a similar fashion to compound



Scheme 2. Synthesis and reagents. Reagents and conditions: (a) PhMgCl, THF, −78 °C to rt 70%; (b) Pd/C, H_2, MeOH; (c) LDA, THF, −78 °C then diethyloxalate 90%; (d) hydrazine hydrate AcOH:EtOH (1:10), 65 °C 72%; (e) LiOH, MeOH/THF/H_2O 90%.

Table 1
Binding and functional activity of compounds for niacin receptor

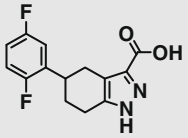
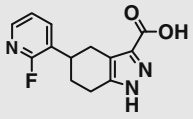
	Compounds	h-^{3}H-niacin IC_{50} (μM)	h-^{3}H-niacin with 5% serum IC_{50} (μM)	hGTPγS EC_{50} (μM)
	NIACIN	0.14	0.14	1.0
17		No activtiy at 25 μM		No activtiy at 100 μM
16		63% at 25 μM		No activtiy at 100 μM
18		19% at 25 μM	35% at 25 μM	28
19		0.27	18% at 25 μM	4.4 (100%)
20		0.46	20% at 25 μM	2.5 (100%)
21		>25.0	>25.0	30% at 100 μM
22 rac **23** end A **24** end B		0.22 0.09 56% at 25 μM	1.4 (sixfold serum shift) 0.4 84% at 25 μM	1.3 (90%) 1.1 (90%)
25		0.067	0.37 (fivefold serum shift)	1.1 (100%)

Binding to niacin receptor determined by displacement of [^{3}H]niacin in the presence or absence of 5% human serum. Functional assays were run using binding of hGTPγS to membrane preparations. Number in parentheses represents curve height as a percentage of the niacin curve height. Data are an average of two independent titrations having calculated standard errors below 15%. The assay-to-assay variation was generally ± twofold. See Ref. 11 for assay protocols. All compounds containing a chiral center are racemic unless otherwise indicated.

18.[10] Tetrazole compounds **30** and **31** showed activity similar to the carboxylic acid analogs.

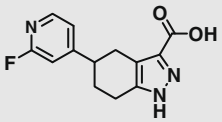
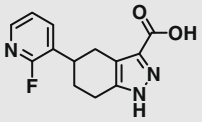
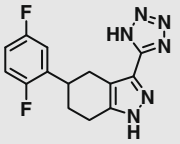
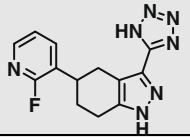
We next sought to test compound **23** in vivo. Free fatty acid levels in the blood decrease in rats upon treatment with niacin. It is

Table 2
Pharmcokinetics of compounds in mouse

Compound		Clp (mL/min/kg)	V_{dss} (L/kg)	$t_{1/2}$ (h)	AUC (μM h kg/mg)	C_{max} (μM)	*F* (%)
22		8.3	1.4	2.9	7.8	3.3	53
27		21	2.4	2.6	2.9	2.3	47

For IV, dose =\ 1 mpk $n = 2$; For po, dose = 2 mpk, $n = 3$.

Table 3
Binding and functional activity of compounds for niacin receptor

Compounds		h-^{3}H-niacin IC_{50} (μM)	h-^{3}H-niacin with 5% serum IC_{50} (μM)	hGTPγS EC_{50} (μM)
	NIACIN	0.14	0.14	1.0 (100%)
26		60% at 25 μM	69% at 25 μM	0% at 100 μM
27 rac		0.60	0.85	4.5 (100%)
28 end A		0.20	0.37	2.3 (100%)
29 end B		90% at 25 μM	88% at 25 μM	67% at 25 μM
30		0.15	17% at 25 μM	1.3 (78%)
31 rac		0.50	1.8 (fourfold serum shift)	3.8 (88%)
32 end A		0.15	0.15	5.5 (92%)
33 end B		40% at 25 μM	75% at 25 μM	28% at 100 μM

See legend of Table 1.

currently postulated that a decrease in free fatty acids leads to the improved lipid profiles observed in humans.[6] Hence, a decrease in free fatty acids was used to measure the effectiveness of our compounds in vivo.

Rats were treated orally with 3, 10, 30, and 100 mpk of **23**. Blood samples were collected at 0 min, 20 min, 1, 2, and 4 h post dose, and free fatty acid levels were measured. The data are summarized in Figure 2. Compound **23** showed a maximal effect on free fatty acid levels at 20 min post dose with effects comparable to niacin at 100 mpk. A comparison of free fatty acid decrease and drug levels led to a determination that the EC_{50} for **23** was 3 μM. Furthermore, free fatty acid decreases of 50% were seen at doses as low as 3 mpk (Fig. 2).

For the side effect of facial flushing, a separate experiment in a rat model was used. Laser Doppler effects were used to test changes in blood perfusion of the rat's ear after administration of compound.[11] Compound **23** was dosed in rats subcutaneously at 3, 10, and 30 mpk. Higher doses could not be achieved in the

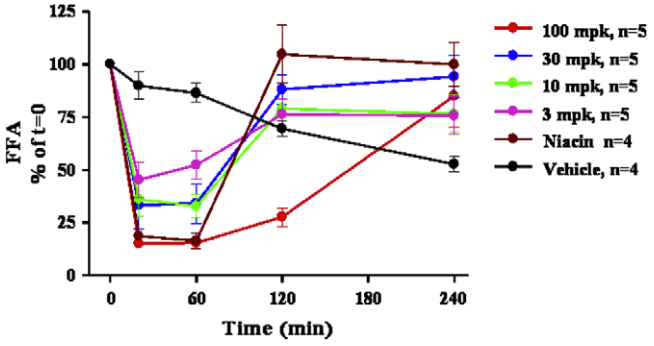


Figure 2. Free fatty acid decrease in rats treated with compound **23**, niacin, or vehicle po in 5% methylcellulose. Five rats were used for compound **23**. Four animals were used for niacin and vehicle experiments. Data are an average of two independent titrations.

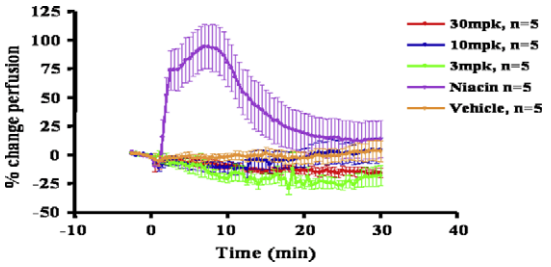


Figure 3. Changes in rat ear blood perfusion when rats were treated with compound **27**, niacin, or vehicle subcuntaneous. in 5% BCD. Five rats were used for each experiment.

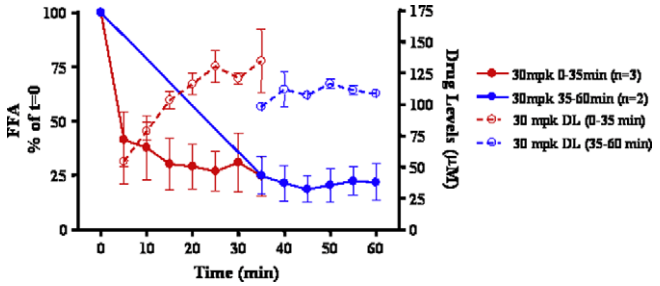


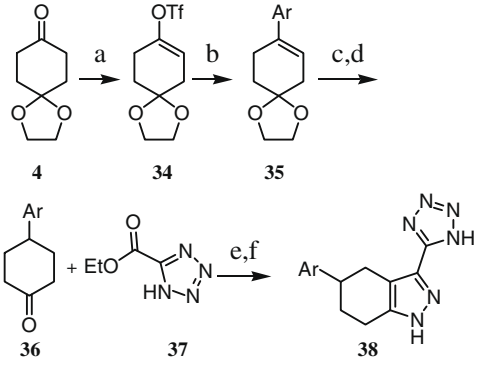
Figure 4. Free fatty acid changes (solid lines) and drug levels (dashed lines) are shown for rats treated with compound **23** subcutaneous. Drug and free fatty acid levels were obtained from 3 animals for early time points (0–35 min) and from 2 different animals for later time points (36–60 min). Data are an average of two independent titrations.

vehicle used for subcutaneous administration. The flushing of the rats' ear was then monitored. No flushing was seen even at the highest dose of compound **23** (Fig. 3). Drug levels could not be monitored in the flushing experiment since removal of blood samples would alter the blood perfusion of the ear, so drug levels were measured in a separate, identical experiment (Fig. 4). At the 30 mpk dose, 135 μM drug levels were achieved. Thus, at drug levels 45-fold over the EC_{50} of the compound, flushing is not observed.

In summary, we synthesized a series of fused cyclohexylpyrazole niacin receptor agonists and discovered that aryl and heteroaryl substitution on the cyclohexyl ring significantly improved binding and functional activity. Among them, compound **23** was evaluated in animal models and was shown to be a full agonist that could separate the free fatty acid effects of the niacin receptor from the flushing effect with a therapeutic index of greater than 45. This result indicates that separation of the two effects can be achieved and this series of compounds represents a promising new class of niacin receptor agonists. Additionally, insight into the biological basis for the observed separation of the flushing and free fatty acid effects may be gained by further study of this class of molecule. It has recently been reported that a related compound activates the adenylate cyclase dependent pathway of GPR109A, but not the MAPK kinase pathway.[12] It will be useful to explore whether compound **23** shows the same effects.

References and notes

1. Barter, P.; Kastelein, J.; Nunn, A.; Hobbs, R. *Atherosclerosis* **2003**, *168*, 195. and references therein.
2. (a) Ashen, M.; Blumenthal, R. *N. Eng. J. Med.* **2005**, *353*, 1252; (b) Alderman, J. D.; Pasternak, R. C.; Sacks, F. M.; Smith, H. S.; Monrad, E. S.; Grossman, W. *Am. J. Cardiol.* **1989**, *64*, 725.
3. Knopp, R. *Am. J. Cardiol.* **2000**, *86*, 51L.
4. Etchason, J. A.; Miller, T. D.; Squires, R. W.; Allison, T. G. *Mayo Clin. Proc.* **1991**, *66*, 23.
5. (a) Tunaru, S.; Kero, J.; Schaub, A.; Wufka, C.; Blaukat, A.; Pfeffer, K.; Offermanns, S. *Nat. Med.* **2003**, *9*, 352; (b) Soga, T.; Kamohara, M.; Takasaki, J.; Matsumoto, S.; Saito, T.; Ohishi, T.; Hiyama, H.; Matsuo, A.; Matsushime, H.; Furuichi, K. *Biochem. Biophys. Res. Commun.* **2003**, *303*, 364; (c) 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. *J. Biol. Chem.* **2003**, *278*, 9869.
6. Seki, K.; Isegawa, J.; Fukuda, M.; Ohki, M. *Chem. Pharm. Bull.* **1984**, *32*, 1568.
7. van Herk, T.; Hartog, A. F.; Schoemaker, H. E.; Wever, R. *J. Med. Chem.* **2003**, *46*, 3945.
8. (a) Comins, D.; Dehghani, A. *Tetrahedron Lett.* **1992**, *33*, 6299; (b) Watanabe, N.; Tanino, K.; Kuwajima, I. *Tetrahedron Lett.* **1999**, *40*, 8133.
9. Semple, G.; Fioravanti, B.; Pereira, G.; Calderon, I.; Uy, J.; Choi, K.; Xiong, Y.; Ren, A.; Morgan, M.; Dave, V.; Thomsen, W.; Unett, D. J.; Xing, C.; Bossie, S.; Carroll, C.; Chu, Z. L.; Grottick, A. J.; Hauser, E. K.; Leonard, J.; Jones, R. M. *J. Med. Chem.* **2008**, *51*, 5101.
10. Commercial ketone **4** was deprotonated by LDA at −78 °C followed by treatment with Comins' reagent to give vinyl triflate **34**. Compound **34** was coupled with boronic acids using standard Suzuki reaction conditions to give **35**. Hydrogenation and deprotection of the ketal protecting group gave ketone **36**. Acylation with the tetrazole ester **37** and cyclization with hydrazine gave the pyrazole tetrazoles.



(a) LDA, Comins's reagent, THF, −78 °C to −20 °C, 100%;(b) Ar–$B(OH)_2$, Na_2CO_3(aq), $Pd(PPh_3)_4$; (c) Pd/C, H_2, MeOH; (d) 3 N HCl, THF/EtOH (3:1), 92%; (e) LDA, THF, −78 °C then 37; (f) hydrazine hydrate AcOH: EtOH (1:10), 65 °C 10–30%; (g) LiOH, MeOH/THF/H_2O 90%.

11. All methods are described in Semple, G.; Schrader, T.; Skinner, P. J.; Colletti, S. L.; Gharbaoui, T.; Imbriglio, J. E.; Jung, J.; Liang, R.; Subharekha, S.; Schmidt, D.; Tata, J. R. WO 2005044816 A1, 2005.
12. Richman, J. G.; Kanemitsu-Parks, M.; Gaidarov, I.; Cameron, J. S.; Griffin, P.; Zheng, H.; Guerra, N. C.; Cham, L.; Maciejewski-Lenoir, D.; Behan, D. P.; Boatman, D.; Chen, R.; Skinner, P.; Ornelas, P.; Waters, M. G.; Wright, S. D.; Semple, G.; Connolly, D. T. *J. Biol. Chem.* **2007**, *282*, 18028.