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Potent tricyclic pyrazole tetrazole agonists of the nicotinic acid receptor (GPR109a)

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ABSTRACT

Tricyclic pyrazole tetrazoles which are potent partial agonists of the high affinity niacin receptor, GPR109a, have been discovered and optimized. One of these compounds has proven to be effective at lowering free fatty acids in vitro and in vivo.

Niacin has been an established treatment for dyslipidemia and cardiovascular diseases for over 50 years due to its positive effects on serum lipids.[1] Niacin lowers very low-density lipoprotein-cholesterol (VLDL-c), low-density lipoprotein-cholesterol (LDL-c), and lipoprotein(a) (Lp(a)) and increases high density lipoprotein-cholesterol (HDL-c) more effectively than other drugs currently in use. Data from the Coronary Drug Project, has shown that use of niacin can effectively reduce the number of cardiac events over a six year dosing period and reduce all cause mortality by 11% after 15 years.[2,3] Subsequently, combinations of niacin with the LDL-lowering statins have been shown to slow the progression of atherosclerosis, decrease the number of cardiac events and provide a therapeutic benefit beyond that of statins alone.[4,5]

At therapeutic doses, a number of adverse side effects are associated with the use of niacin, most notably, a cutaneous flushing effect which limits patient compliance. Since the identification of GPR109a as a molecular target for niacin, interest in developing a therapeutic agent with the lipid modulating properties of niacin but with a reduced flushing effect has intensified.[6] GPR109a is expressed in adipocytes, spleen and Langerhans cells in the skin. The flush side effect has been attributed to vasodilation resulting from the niacin induced release of prostaglandin D2 from Langerhans cells.[7,8] Various strategies have been employed to separate the beneficial effects on lipids from flush induced by niacin such as sustained release formulations and combinations of niacin with aspirin or DP receptor antagonists to suppress the vasodilation effects of prostaglandin release.[9] It has been shown that GPR109a partial agonists can differentially activate downstream signaling pathways thus inducing a decrease in plasma free fatty acids without inducing release of prostaglandin D2 and E2.[10] **MK-0354** (Fig. 1) is an example of a biased agonist that activates the antilipolytic pathways in adipose cells but does not signal via the ERK 1/2 pathway that leads to prostaglandin production and vasodilation.[11] **MK-0354** thus produced a decrease in plasma free fatty acids in mice but did not induce the vasodilation associated with flushing. **MK-0354** behaved similarly in humans, reducing plasma FFAs without an associated flushing response but did not have a statistically significant effect on HDL, LDL or triglycerides.[12] The failure

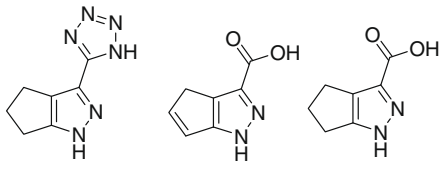

Figure 1. MK-0354 and analogues.

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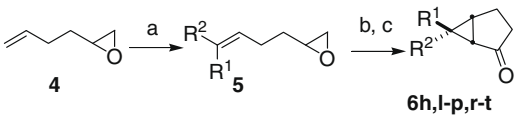
of **MK-0354** to increase HDL cholesterol in the clinic led to two separate hypotheses. The first of these was that more potent analogues would result in beneficial effects on serum lipids and triglycerides. The second hypothesis is that the separation of signaling pathways that inhibit cAMP production from those that activate MAP kinase influences both the effects on lipids and the flushing side effect. This communication describes efforts to address the first of these hypotheses by improving the potency of non-flushing agonists of GPR109A.

During investigations directed at discovery of small molecule pyrazolecarboxylic acid agonists of GPR109a, we found that compound, **1**, demonstrated slightly improved potency in in vitro assays vs the carboxylic acid analogue of **MK-0354**, **2**.[13] However, the inherent tendency of the double bond of **1** to migrate and the propensity of pyrazole acids to cause vasodilation in animal models made it an undesirable candidate for development. In order to explore this improvement in GPR109a agonist potency, we sought a stable double bond surrogate that could be incorporated into the non-flushing tetrazole series. It was reasoned that incorporation of a cyclopropyl ring, which would clearly be fixed in position, may give the requisite increase in receptor potency within a more stable chemical framework. A number of derivatives incorporating a cyclopropyl ring have been synthesized and analyzed in niacin receptor activation assays and in vivo assays of free fatty acid lowering. The results of these studies are reported herein.

Synthetic routes which utilized ketones **6** as the key intermediate were developed for the assembly of the final products. Many bicyclic pyrazoles were synthesized from cyclopentanones derived from epoxyolefins, **5**, via the intramolecular cyclopropanation described by Hodgson et al.[14] as depicted in Scheme 1. The requisite olefin substitution was introduced via olefin metathesis of the commercially available **4** using the appropriate olefin and Zhang catalyst. Direct cyclization of **4** gave ketone **6a** and cyclization following olefin metathesis produced ketones **6h**, **l–p**, and **r–t**. (for R^1 and R^2 see Table 1). The olefin metathesis reaction produces an equilibrium mixture of *E* and *Z* isomers that results in a mixture of *endo* and *exo* diastereomers following cyclization. The *endo* isomer, corresponding to cyclization of the predominant *E* isomer, was the major product. However, it was convenient to carry the mixture through the synthesis and separate the diasteomers of the final products. The stereochemistry of the final products was confirmed by NOE spectroscopy.

An alternate, and somewhat more versatile, synthetic route was developed which employed the dichlorocyclopropane **8** derived from intermolecular cyclopropanation of commercially available ketal **7** with dichlorocarbene (Scheme 2). Lithium–halogen exchange using *t*-butyllithium and trapping of the anion with methyl iodide gave the chloro methylcyclopropyl compound **9**. A second lithium–halogen exchange using LiDBB followed by quenching with ethanol and hydrolysis of the ketal gave the *exo*-methyl ketone **6b** diastereoselectively. Alternatively, the dichlorocyclopropyl moiety could be mono-dehalogenated using Zn dust to give chlorocyclopropyl compound **10**. Lithium–halogen exchange of **10** using LiDBB and trapping of the anion with an appropriate electrophile followed by ketal hydrolysis gave mono-alkylated bicyclic ketals, **6c** and **6d**, in good yields with a high degree of stereoselectivity favoring the *endo* isomers.

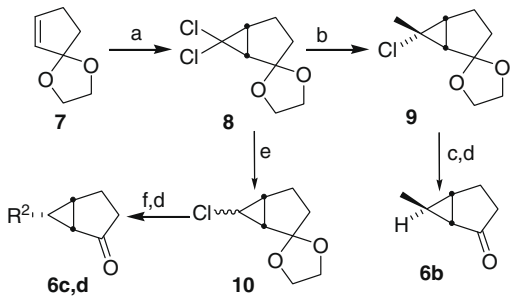


Scheme 1. Reagents and conditions: (a) $R^1R^2C{=}CH_2$, Zhang catalyst, (b) LiTMP, MTBE; (c) cat. TPAP, NMO, 4 Å MS, DCM.

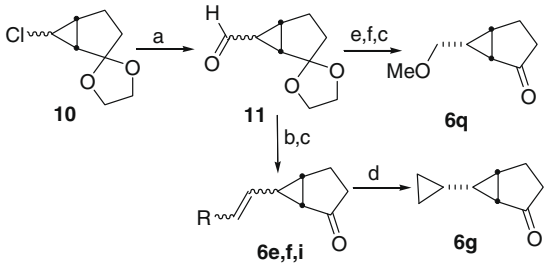
Table 1
Inhibition of forskolin induced cAMP production in cells stably transfected with GPR109a

Compd	R^1 (*exo*)	R^2 (*endo*)	GPR109a EC_{50} μM (*n*)	Efficacy[a]
Niacin			0.027	100
MK-0354			0.286	96
(±)**17a**	H	H	0.210 (3)	65
(+)**17a**	H	H	0.045 (8)	87
(−)**17a**	H	H	n.e.	
17b	Me	H	2.35 (5)	63
17c	H	Me	0.244 (5)	79
17d	H	Et	0.504 (5)	84
17e	$CH{=}CH_2$	H	1.11 (3)	75
17f	H	$CH{=}CH_2$	0.156 (3)	85
17g	H	Cyclopropyl	0.507 (3)	87
17h	H	*n*-Propyl	0.541 (3)	69
17i	H	CH=CHMe	0.276 (2)	66
17j	$CH_2CH{=}CH_2$	H	12.9 (3)	66
17k	H	$CH_2CH{=}CH_2$	1.42 (3)	72
17l	*i*-Butyl	H	23.1 (2)	94
17m	H	*i*-Butyl	2.45 (1)	85
17n	H	*n*-Butyl	3.75 (1)	62
17o	H	*i*-Pentyl	n.e.	
17p	H	*n*-Pentyl	n.e.	
17q	H	CH_2OMe	1.83 (3)	87
17r	Me	Me	2.86 (5)	5
17s	$-(CH_2)_4-$		0.638 (2)	2
17t	$(CH_2)_5$		n.e.	

[a] Efficacy is expressed as a percentage of the niacin response.



Scheme 2. Reagents and conditions: (a) 50% NaOH, $CHCl_3$/DCM, cat. triethylbenzylammonium chloride, 45 °C; (b) (1) *t*-BuLi, THF, −100 °C, (2) MeI, −100 °C to rt; (c) (1) LiDBB, THF, −78 °C, (2) EtOH; (d) cat. TsOH, acetone, H_2O, rt; (e) Zn dust, KOH, EtOH, 80 °C; (f) (1) LiDBB, THF, −78 °C, (2) R^2I, rt.



Scheme 3. Reagents and conditions: (a) (1) LiDBB, THF, −78 °C, (2) $HCONMe_2$, rt; (b) $[(Ph_3)PCH_2R]^+Br^-$, *n*-BuLi, THF, rt; (c) cat. TsOH, acetone, H_2O, rt; (d) $Pd(OAc)_2$, CH_2N_2, Et_2O, rt; (e) $NaBH_4$, EtOH, rt; (f) NaH, MeI, THF, 0 °C to rt.

Alternatively, when the anion was acylated using DMF (Scheme 3, R^2 = CHO) further elaboration of the product, **11**, was possible. Wittig olefination, gave alkenes which were directly deprotected to ketones **6e**, **6f** and **6i**. After separation by HPLC, **6f** was cyclopropanated then hydrolyzed to produce **6g**. Reduction of aldehyde **11** using $NaBH_4$, and etherification by direct alkylation gave ketone **6q** upon deprotection.

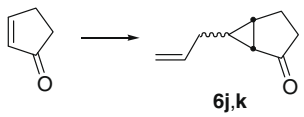
An attempt was made to synthesize a spiro-cyclobutylcyclopropyl analogue by addition of the anion produced from cyclobutyl diphenyl sulfonium triflate (Scheme 4). However, reaction of the anion with cyclopentenone resulted in addition with concomitant ring opening to give the allylcyclopropyl ketones **6j**,**k**.

The synthesis of the final tricyclic pyrazoles is depicted in Scheme 5. Acylation of ketone **6** with diethyl oxalate (**12**) followed by condensation in situ with hydrazine produced pyrazole esters **13**. For ease of handling the products, in most cases the esters were converted to the benzylated pyrazole amides **14** by amidation and benzylation in either order. Dehydration of the amide **14** was accomplished using $SOCl_2$ in DMF to give nitriles **15**. Zn-promoted 3 + 2 cycloaddition of NaN_3 to the nitriles, **15**, and reductive or oxidative debenzylation gave the final tetrazoles, **17**. This sequence could also be accomplished without benzyl protection by an amidation and dehydration sequence employing TFAA giving nitriles **16** followed by the azide cycloaddition to give tetrazoles **17**. Compounds were synthesized as mixtures of diastereomers that were separated by HPLC after the final step.[15] The regioisomer **19** was also synthesized by a similar sequence starting from readily available ketone **18**.[16]

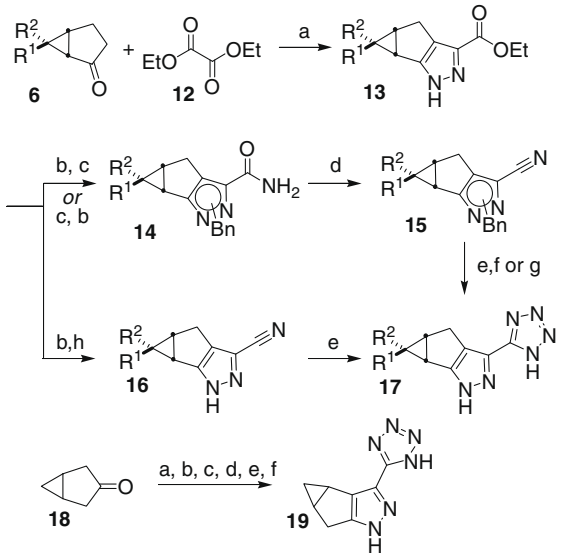
Compounds were assessed for their ability to decrease forskolin promoted production of cAMP in CHO cells stably transfected with GPR109a employing a HTRF assay and the results are summarized in Table 1. Initially, the racemic cyclopropyl pyrazole, (±)**17a**, was synthesized and was, as predicted, slightly more potent than **MK-0354** in GPR109a activation assays. Importantly, (±)**17a** retained partial agonist character at the receptor, producing 65% of the signal produced by niacin. The complete lack of receptor activation observed for the regioisomeric cyclopropane, **19**, confirmed that the receptor activity seen in the olefin was likely due to the 5,6-olefin regioisomer represented by **1** and not from isomerization of the double bond to the 4,5-regiosomer. The series of substituted cyclopropyl analogues revealed a very narrow SAR and a clear stereochemical preference for *endo*- versus *exo*-substitution at the apex of the cyclopropane ring. The unsubstituted compound (±)**17a** was among the most potent although the *endo*-methyl and *endo*-vinyl substituted analogues were nearly equipotent. Larger substituents were not well tolerated leading to significant drops in agonist potency with increasing size. All compounds were also assayed for selectivity over the low affinity niacin receptor, GPR109b. None of the more potent analogues activated GPR109b, however, compounds with more bulky substituents (**17h**, **17m** and **17n**) were weak agonists (5–10 μM) of GPR109b.

The clear preference for the *endo*-diastereomer to activate GPR109a suggested a strong stereochemical bias for activation of the receptor by these compounds. To probe this further, racemic (±)**17a** was separated into individual enantiomers by chiral HPLC. The (+) isomer was found to be a potent activator of GPR109a whereas the (−) isomer had no effect in the cAMP assays. The absolute configuration of the (+) isomer was confirmed by resolution of the starting epoxide enantiomers, **4**, employing the method described by Jacobsen.[17] Using the enantiomerically pure epoxide to synthesize (+)**17a**, we were able to assign the *R*,*R* configuration to the active enantiomer. Compound (+)**17a** was profiled further to assess its potential as a drug candidate.

The pharmacokinetic profile of (+)**17a** was evaluated in several species and was found to be very favorable with good oral absorption and reasonable plasma half lives in all species (Table 2).



Scheme 4. Reagents and conditions: *c*-BuSPh$_2$OTf, *t*-BuLi, THF, −78 °C.



Scheme 5. Reagents and conditions: (a) (1) KO*t*-Bu, EtOH, 0 °C to rt, (2) hydrazine monohydrochloride, H_2O, rt; (b) NH_3/MeOH, 40 °C; (c) KOH, BnBr, dioxane; (d) $SOCl_2$, DMF, rt; (e) NaN_3, $ZnBr_2$, DMF, 0 °C; (f) KO*t*-Bu, DMSO, air; (g) Pd black, MeOH, rt; (h) TFAA, rt.

Table 2
Compound (+)**17a** was dosed at 3 mg/kg iv and 10 mg/kg po Cl (ml/min/kg), V_{dss} (l/kg), $t_{1/2}$ (h), C_{max} (μM), AUC (μM h kg/mg)

Species	Cl_p	V_{dss}	$t_{1/2}$	C_{max}	AUC	%F
Mouse	61.6	3.4	2.4	2.0	1.4	97
Rat	25.5	0.34	1.9	4.9	1.3	37
Dog	12.4	0.53	1.9	36.9	7.5	>100
Monkey	13.9	0.96	7.3	11.7	12.6	>100

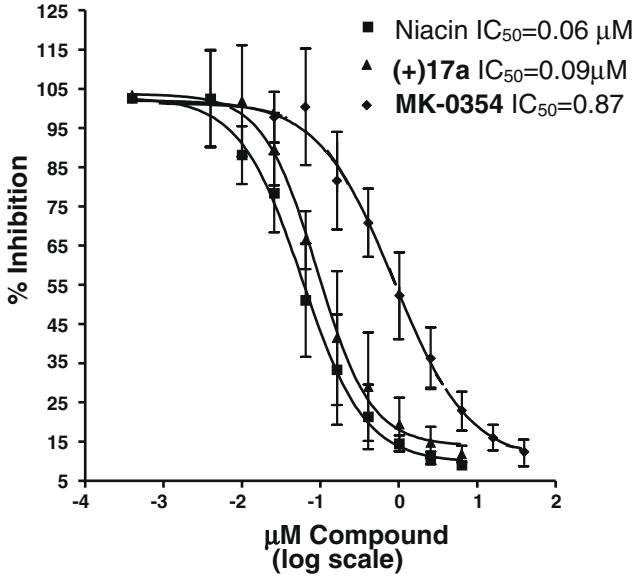


Figure 2. Inhibition of production of free fatty acids in primary human adipocytes. Determinations are averages from four donors performed in duplicate.

Compound (+)**17a** was assessed for its propensity to lower free fatty acid levels both in vitro and in vivo (Fig. 2). In isolated human adipocytes stimulated with isoproteranol, (+)**17a** dose dependently inhibited production of FFAs with an IC_{50} of 0.09 μM which com-

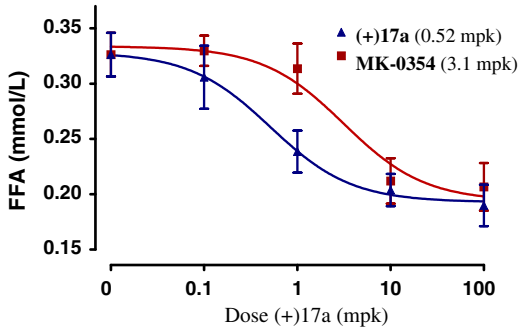


Figure 3. Plasma free fatty acids in mice dosed orally with (+)**17a** and **MK-0354**. ED_{50} in parenthases.

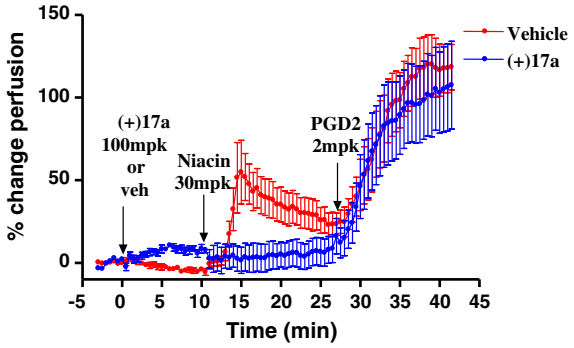


Figure 4. Effect of (+)**17a** on niacin induced flushing.

pares favorably with niacin (IC_{50} = 0.06 μM) and is greatly improved over **MK-0354** (IC_{50} = 0.87 μM).

In mice dosed orally, compound (+)**17a** lowered plasma free fatty acids (Fig. 3, ED_{50} = 0.52 mpk) much more effectively than **MK-0354** (ED_{50} = 3.1 mpk).

Vasodilation induced by (+)**17a** was estimated by measurement of laser doppler changes in ears of anesthetized mice (Fig. 4). A dose of 100 mpk did not induce significant changes in vasodilation in this model. Additionally, (+)**17a** blocked vasodilation induced by niacin demonstrating that compound (+)**17a** was acting at the same receptor site as niacin to reduce free fatty acids. In the same animals, a subsequent 2 mpk dose of prostaglandin D2 induced vasodilation demonstrating that (+)**17a** was not interfering with the normal flushing response.

In conclusion, a number of potent partial agonists of GPR109a have been discovered and their ability to modulate lipids has been examined. Compound (+)**17a** was shown to be a potent partial agonist of GPR109a and subsequently demonstrated to reduce free fatty acids both in vitro in human adipocytes and in vivo in mice. The favorable effects of (+)**17a** on plasma free fatty acids at low doses and its good pharmacokinetic profile suggest that further development of this class of compounds for the treatment of lipid disorders may be warranted.

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