Supporting Information:

3-(1H-Tetrazol-5-yl)-1,4,5,6-tetrahydro-cyclopentapyrazole (MK-0354) : A Partial Agonist of the Nicotinic Acid Receptor, G-Protein Coupled Receptor 109a, with Antilipolytic but No Vasodilatory Activity in Mice

Graeme Semple,[a]* Philip J. Skinner,[a] Tawfik Gharbaoui,[a] Young-Jun Shin,[a] Jae-Kyu Jung,[a] Martin C. Cherrier,[a] Peter J. Webb,[a] Susan Y. Tamura,[a] P. Douglas Boatman,[a] Carleton R. Sage,[a] Thomas O. Schrader,[a] Ruoping Chen,[b] Steven L. Colletti,[c] James R. Tata,[c] M. Gerard Waters,[c] Kang Cheng,[c] Andrew K. Taggart,[c] Tian-Quan Cai,[c] Ester Carballo-Jane,[c] Dominic P. Behan,[b] Daniel T. Connolly[b] and Jeremy G. Richman.[b]

Departments of a) Medicinal Chemistry and b) Discovery Biology, Arena Pharmaceuticals, 6166 Nancy Ridge Drive, San Diego, CA, 92121, USA; c) Merck Research Laboratories, Rahway, NJ, 07065, USA

** corresponding author gsemple@arenapharm.com; tel: +1 858 453 7200; fax: + 1 858 453 7210*

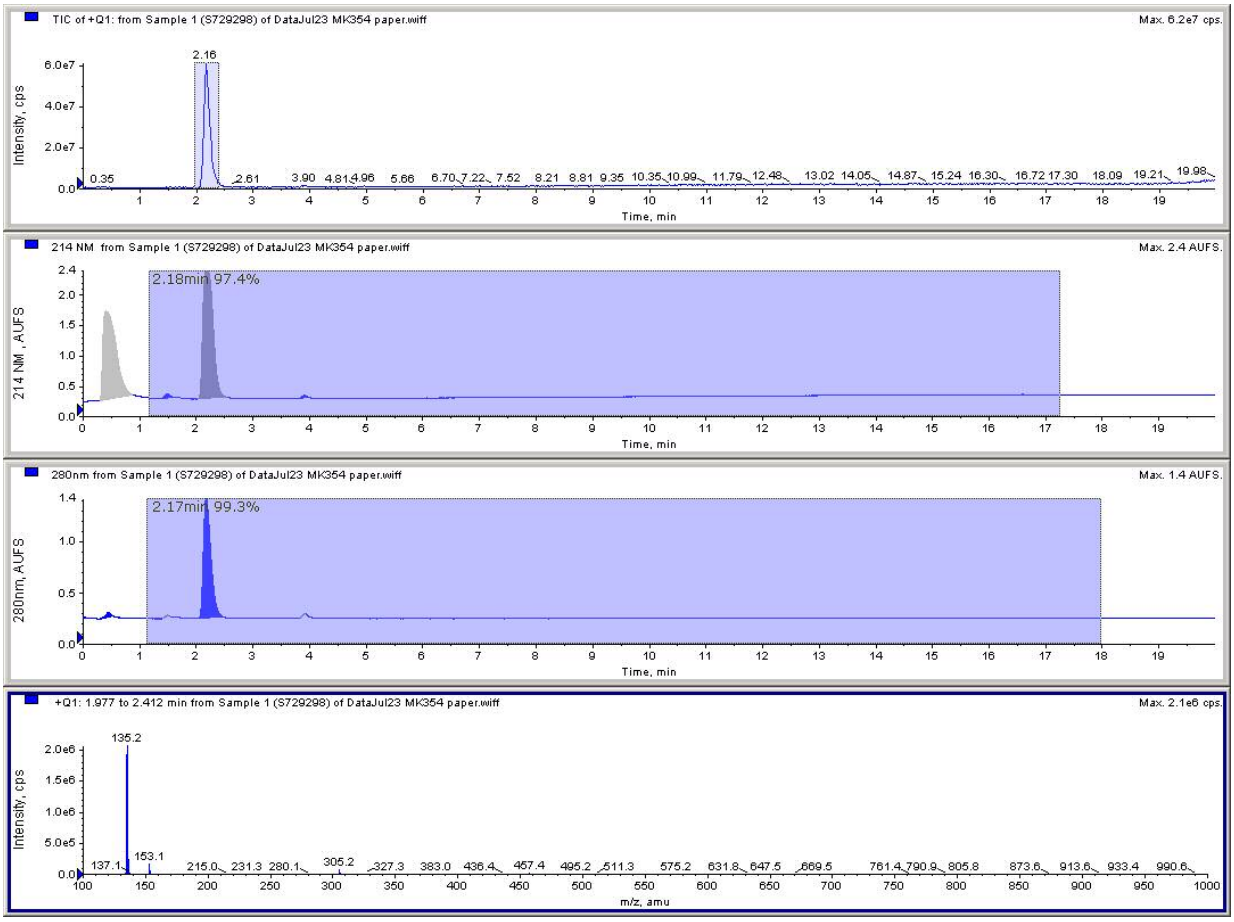
Table of Contents

HPLC-MS Spectra – General Methods

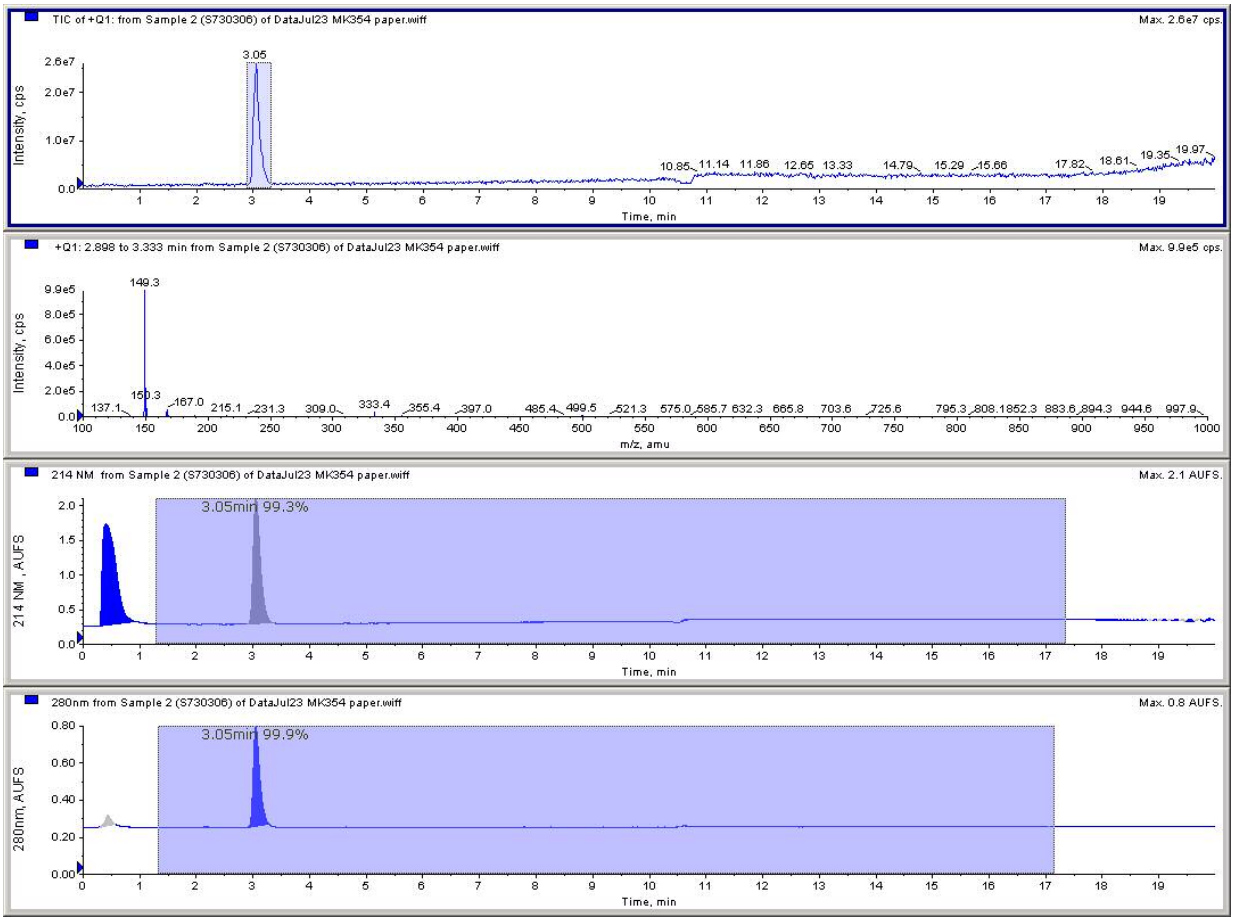
Analytical HPLC/MS was conducted on an AB/MDS Sciex API 150EX mass spectrometer with an electrospray source , using a Shimadzu Inc. LC-10AD *VP* HPLC-pump, Shimadzu Inc. SCL-10A *VP* HPLC system controller, Shimadzu Inc. SPD-10A *VP* UV-Detector, monitoring at 214nm, Leap ScientificCTC HTS, PAL Autosampler, Analyst 1.2 software and a) Alltech Prevail C18 column (5 μ, 50 × 4.6 mm), using a gradient of 5% v/v CH_3CN (containing 1% v/v TFA) in H_2O (containing 1% v/v TFA) (t = 0.0 min) gradient to 100% v/v CH_3CN in H_2O (t = 20.0 min), 3.5 mL/min, or b) Waters® YMC™ ODS-A C18 column (5 μ, 50 × 4.6 mm), using a gradient of 5% v/v CH_3CN (containing 1% v/v TFA) in H_2O (containing 1% v/v TFA) (t = 0.0 min) gradient to 95% v/v CH_3CN in H_2O (t =4.0 min), 3.5 mL/min.

Compound	**R_1**	**R_2**	**R_3**	**Method**	**t_r (min)**	**HPLC-MS purity** (214nM)	**HPLC-MS purity** (280nM)
3a	H	H	H	a	2.18	97.4 %	99.3 %
3b	Me	H	H	a	3.05	99.3 %	99.9 %
3c	H	Me	H	a	2.53	100 %	99.8 %
5a	H	H	H	a	2.28	100 %	98.4 %
5b	Me	H	H	b	2.43	97.4 %	98.9 %
5c	H	Me	H	b	2.44	97.9 %	99.5 %
5d	H	H	Me	b	2.50	99.1 %	99.8 %

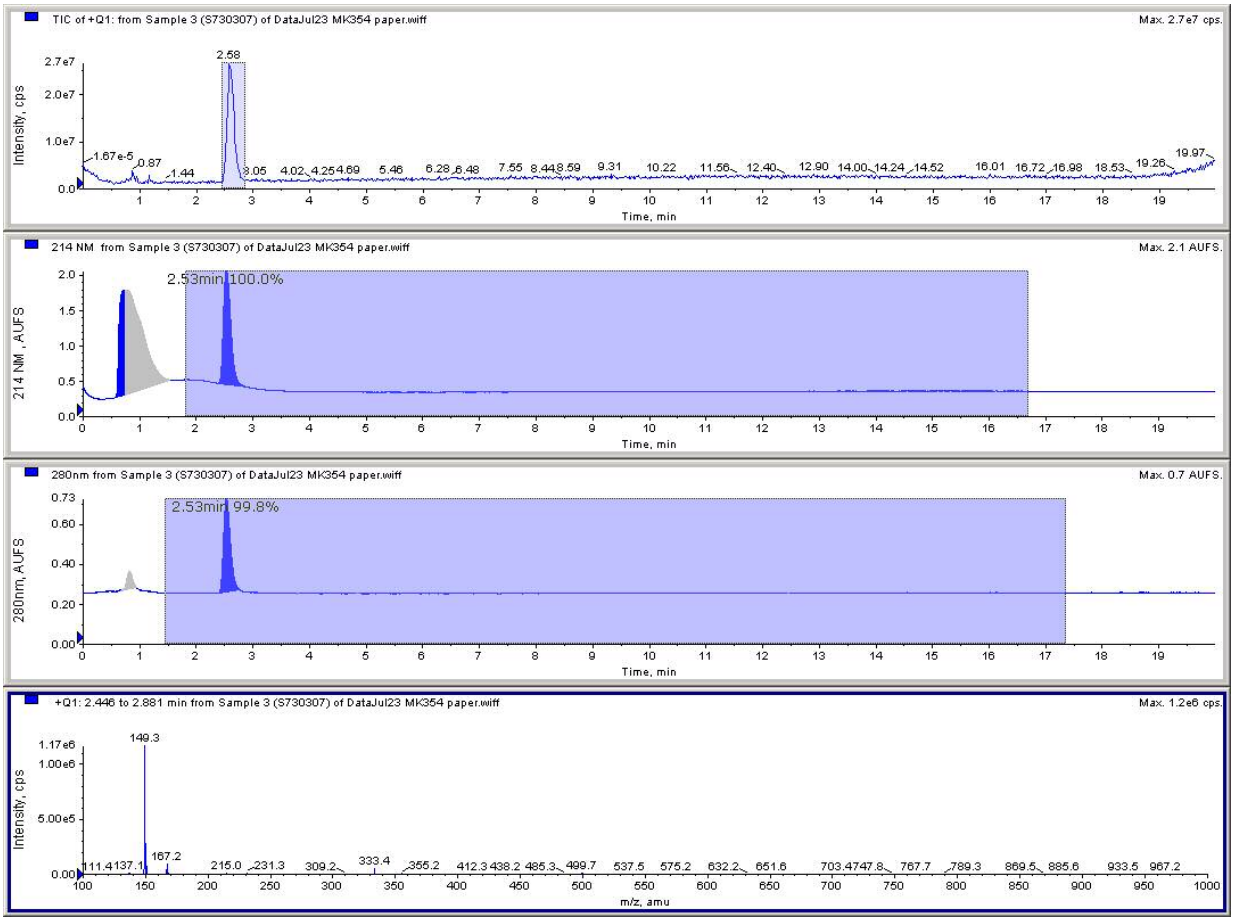
HPLC-MS Spectra – Spectrum for 1,4,5,6-Tetrahydrocyclopenta[c]pyrazole-3-carboxylic acid (3a)



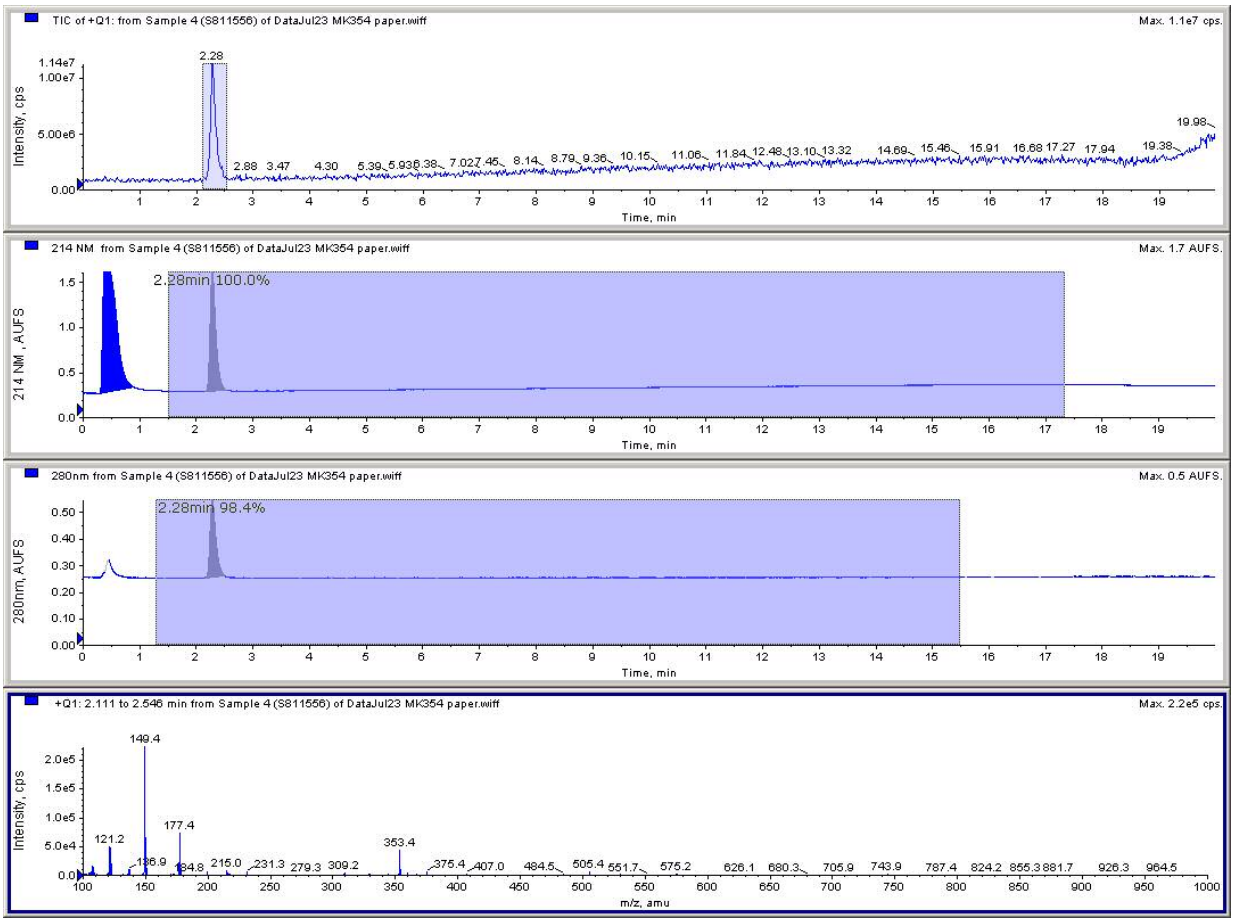
HPLC-MS Spectra – Spectrum for 6-Methyl-1,4,5,6-tetrahydrocyclopenta[c]pyrazole-3-carboxylic acid (3b)



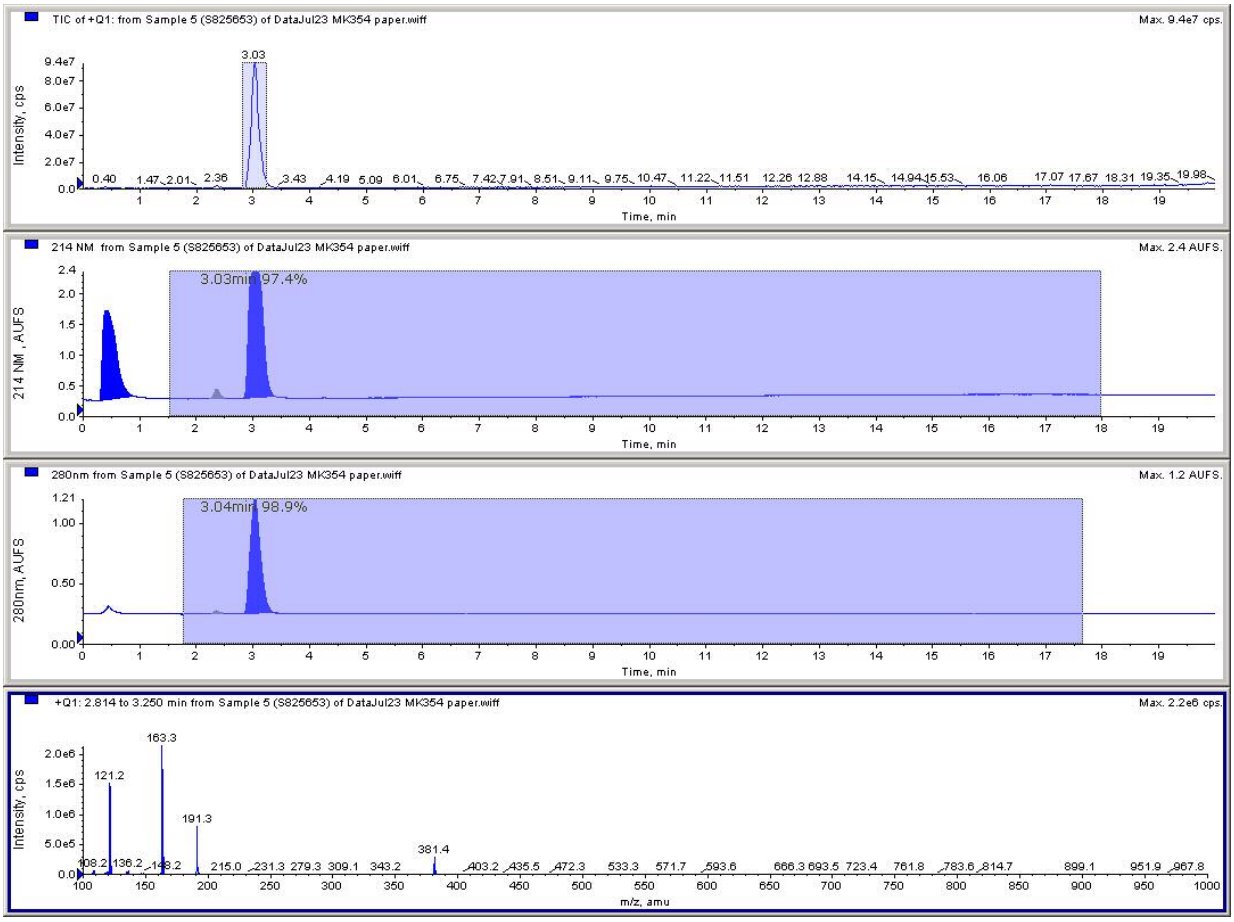
HPLC-MS Spectra – Spectrum for 5-Methyl-1,4,5,6-tetrahydrocyclopenta[c]pyrazole-3-carboxylic acid (3c)



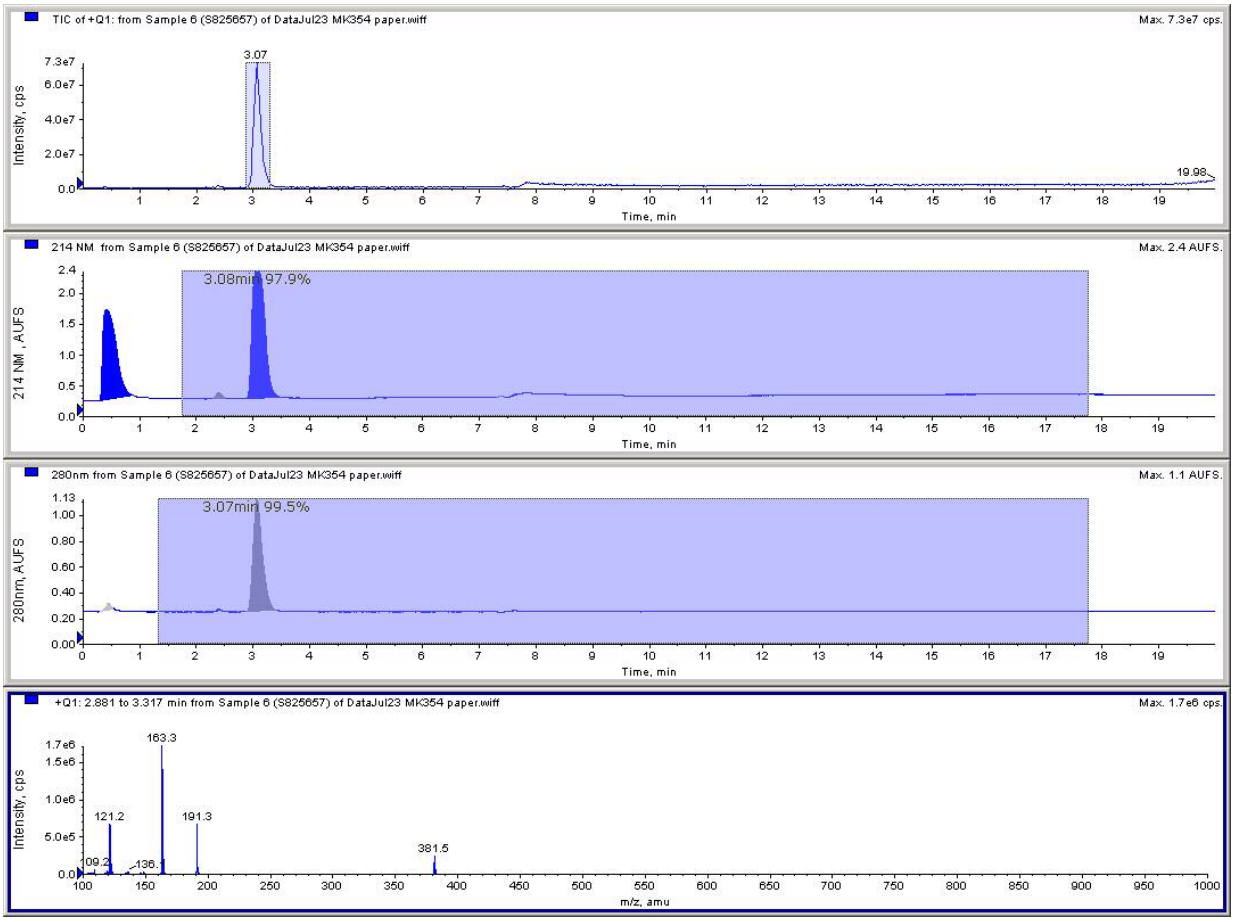
HPLC-MS Spectra – 3-(2H-Tetrazol-5-yl)-1,4,5,6-tetrahydro-cyclopentapyrazole (5a)



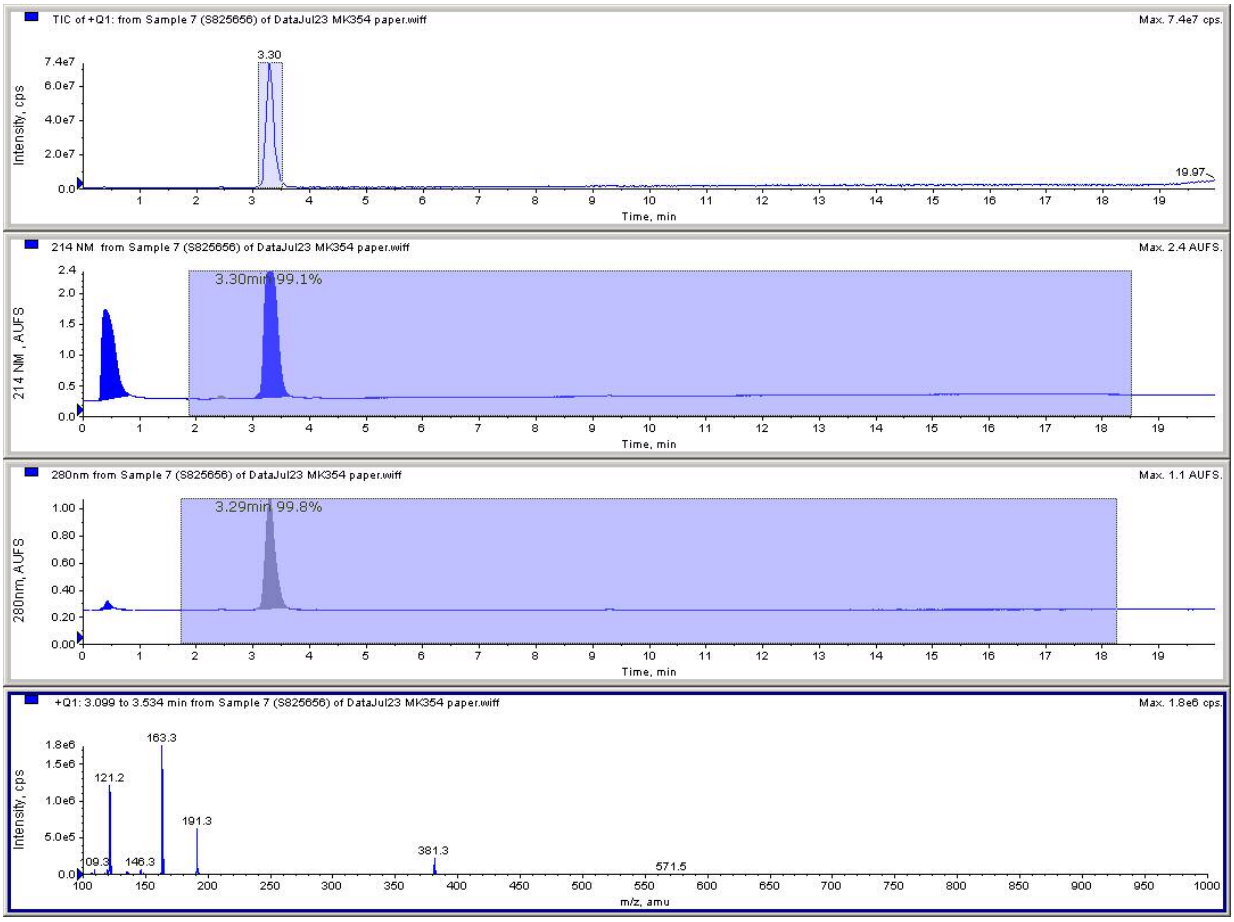
HPLC-MS Spectra – Spectrum for 6-Methyl-3-(2H-tetrazol-5-yl)-1,4,5,6-tetrahydro-cyclopentapyrazole (5b)



HPLC-MS Spectra – Spectrum for 5-Methyl-3-(2H-tetrazol-5-yl)-1,4,5,6-tetrahydro-cyclopentapyrazole (5c)

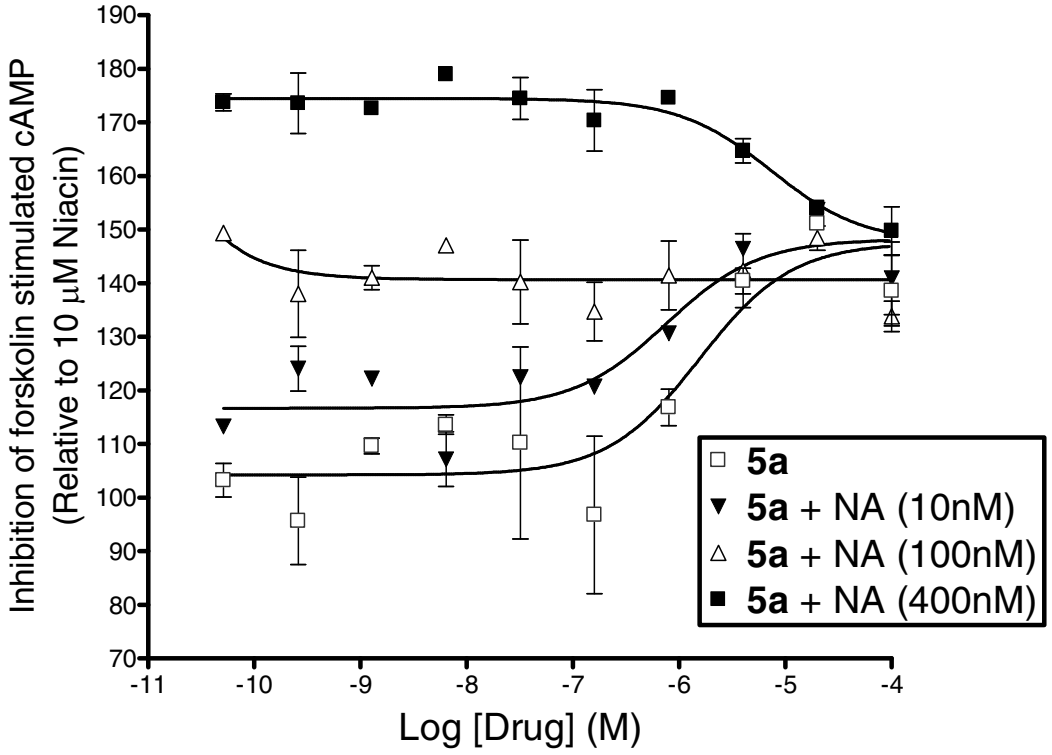


HPLC-MS Spectra – Spectrum for 4-Methyl-3-(2H-tetrazol-5-yl)-1,4,5,6-tetrahydro-cyclopentapyrazole (5d)

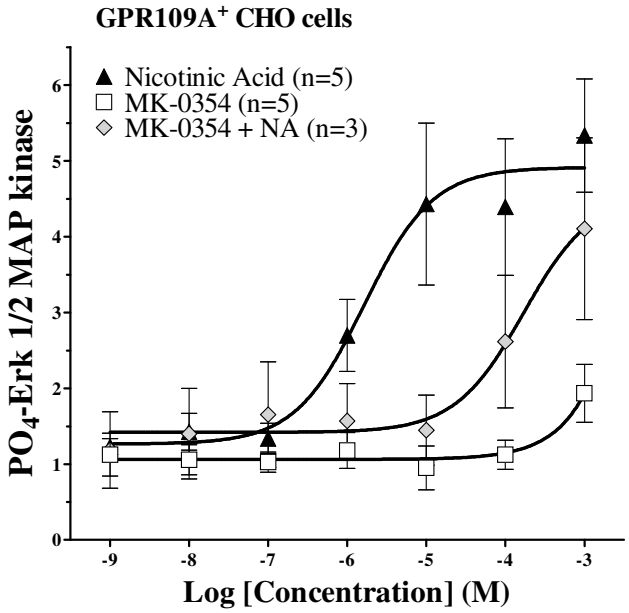


Dose response curves for **5a** in the hGPR109a cAMP assay in the presence of increasing concentrations of nicotinic acid showing the classical competitive behaviour expected of a partial agonist.

The normal dose response for a full agonist (e.g. nicotinic acid, NA) starts at 100 (% response) and increases to 200. For **5a** alone, the efficacy in this experiment is approximately 50% of the maximum. In the presence of increasing concentrations of nicotinic acid, the dose responses for **5a** start at different levels due to the underlying effect of the NA but all end at the same efficacy (~50%, the same efficacy as **5a** alone). In the presence of 400nM NA, the dose response begins above the maximal efficacy of **5a**, and it is reduced by increasing the concentration of **5a** until it comes down to the same efficacy level as **5a** alone. These data are consistent with what would be expected for a partial agonist i.e. behaving as a competitive antagonist in the presence of high concentrations of full agonist.



5a (MK-0354) antagonizes nicotinic acid induced ERK 1/2 phosphorylation in vitro

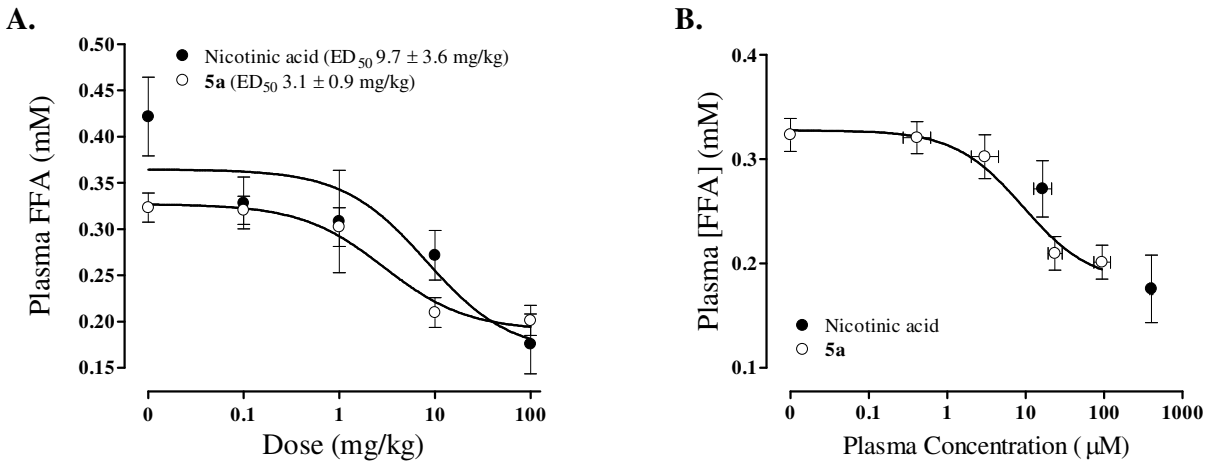


MK-0354 + NA = Concentration-response of nicotinic acid (NA) in the presence of **5a** (10 μM). These data are consistent with **5a** having an antagonistic effect (right shift) on the concentration-response curve for NA.

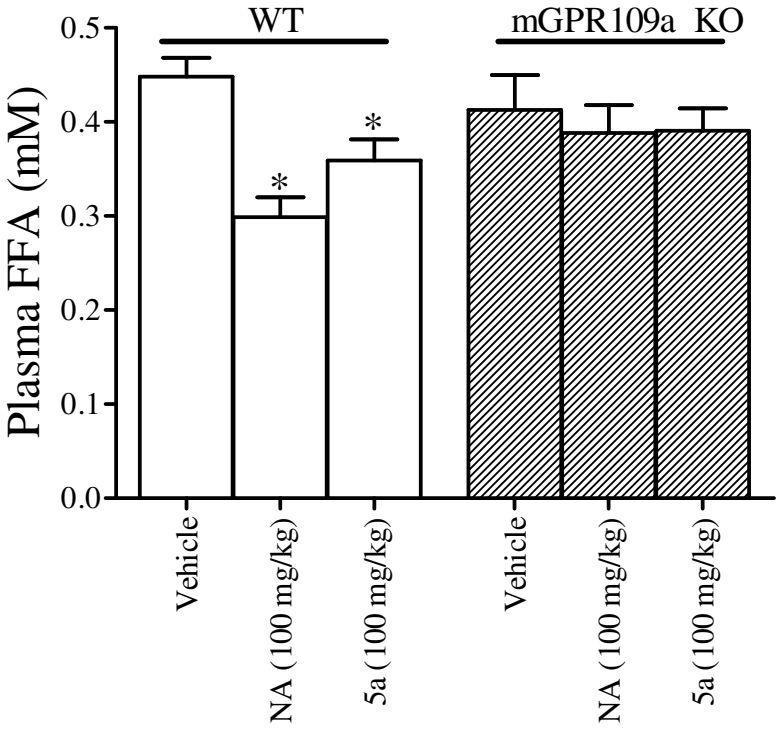
PK-PD relationship for the effect of acute **5a** and nicotinic acid on plasma FFA levels in fasted C57/BL6 mice at t = 20min.

This is a separate experiment from the one depicted in the full body of the paper and the experimental procedure used was as described in the paper except that animals were fasted for 6 h prior to treatment. In this experiment, both FFA and compound concentrations were measured from the same samples.

a) Administered dose-response and b) Plasma concentration-response



Effect of **5a** (MK-0354) and nicotinic acid on plasma FFA in WT and mGPR109a knockout mice, 20 min following p.o. administration.



The experimental procedure was as described in the paper except that animals were fasted for 6 h prior to treatment. mGPR109a knockout mice were obtained with the kind assistance of Dr. K. Pfeffer, University of Dusseldorf. [10]