Supplementary data.

Peripheral TPH inhibitors were introduced as a new class of compounds to treat carcinoid syndrome and non-constipating IBS [1, 2, 3]. An early drug in this class, LX1031, has shown efficacy in treatment of non-constipating IBS. LP-920540 ((2S)-ethyl 2-amino-3-(4-(2-amino-6-(2,2,2-trifluoro-1-(3′ -fluorobiphenyl-4-yl)ethoxy)pyrimidin-4-yl)phenyl)propanoic acid) is the more active stereoisomer of the racemic compound, LP-615819, which has been characterized previously [2]. LP-920540 is more potent in vitro and better absorbed than LX1031, which provides greater in vivo efficacy and, in common with other peripheral TPH inhibitors, does not enter the brain. We also studied LX1032 (L-phenylalanine, 4-[2-amino-6-[(1R)-1-[4-chloro-2-(3-methyl-1H-pyrazol-1-yl)phenyl]- 2,2,2- trifluoroethoxy]-4-pryrimidinyl]-, ethyl ester, compounded with *N-benzoylglycine* (1:1); telotristat etiprate; see United States Adopted Names Council listing) independently of LP-920540 because it is a different compound and thus can help to verify that observed effects are due to the shared ability of the two compounds to inhibit TPH. LP-920540 is also being studied in a phase 2 clinical trial in patients with mild-to-moderate ulcerative colitis.

For studies of the effects of peripheral TPH inhibitors on gut and brain 5-HT concentrations, LP-920540 was formulated in 0.1% Tween 80 in 0.25% methylcellulose and administered to mice once daily via oral gavage at 10ml/kg for 4 consecutive days. LX1032 was formulated in 15% cyclodextrin (Captisol™, pH 3-4) or 0.25% methylcellulose and given to mice once daily via oral gavage at 10ml/kg for 4 consecutive days. Whole brain, jejunum and colon (mesentery fat removed, gut lumen

opened and blotted dry) were collected, snap frozen, and stored at -80ºC for future as described below. Investigators at Columbia were blinded to the identity of the compounds under study. LP-920540, LX-1032, LP-778914, LP-778920 and vehicle control were also formulated with 0.5% methycellulose at Lexicon at appropriate doses in coded vials and shipped to Columbia for study. The contents of the coded vials were given by oral gavage in amounts determined by the weights of the recipient mice. After the experiments, results were analyzed at Columbia, codes were broken, and data were shared with investigators at Lexicon.

Experimental colitis. Briefly, 100 µl of TNBS in 30% ethanol was infused into the colonic lumen at a distance of 3.5 cm from the anal verge via a 1-ml syringe fitted with a polyethylene cannula (Intramedic PE-20 tubing; Becton Dickinson). Control mice received infusions of saline in 30% ethanol instead of TNBS. Colitis was recognized in all of the TNBS-treated mice as persistent weight loss and loose stools. Colons were removed, following euthanasia with CO_2 asphyxiation, 5-7 days after infusion of TNBS or 30% ethanol.

Histological scoring: For inflammation, a score of 0 was assigned when only rare inflammatory cells were present in the lamina propria; a score of 1 was assigned when increased numbers of granulocytes were present the lamina propria; a score of 2 was assigned when inflammatory cells became confluent in the mucosa and extended into the submucosa; a score of 3 was assigned when the inflammatory infiltrate extended across the full wall of the gut. For crypt damage, a score of 0 was assigned when crypts were intact; a score of 1 was assigned when the basal third of crypts were lost; a score of 2 was assigned if the two thirds of the basal regions of crypts were lost; a score of 3 was assigned when entire crypts were lost; a score of 4 was assigned when the

epithelial surface was changed and erosions were observed; a score of 5 was assigned when epithelial surface was completely eroded. For evaluation of ulcers, a score of 0 was assigned when ulceration was absent; a score of 1 was assigned when one or two foci of ulcerations were evident; a score of 2 was assigned when three or more foci of ulceration were observed; a score of 3 was assigned when ulcers became confluent and/or extensive. These values were added to give a maximal histopathological score of 11.

RT-PCR and qPCR Primers were purchased from Applied Biosystems (Foster City, CA). The real-time reaction contained cDNA (5 µl), primers for the cytokine/chemokine/standard (250 nmol), PCR master mix (12.5 µl; Applied Biosystems, Foster City, CA) and nuclease-free water (6.25 µl). A GeneAmp 7500 sequence detection system (Applied Biosystems) was used to quantify cDNA levels. Duplicates were incubated for 2 min at 50°C, denatured for 10 min at 95°C, and subjected to 40 cycles of annealing at 60°C for 20 sec, extension at 60°C for 1 min, and denaturation at 95°C for 15 sec. TaqMan 7500 software was used for data analysis.

References

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