Abstract: Ulcerative colitis (UC) affects over a million people in the US and requires long-term treatment. Janus kinase (JAK) inhibition has shown promise in alleviating UC, but safety concerns and black box warnings from the FDA have been associated with approved JAK inhibitors, regardless of their dosage forms or JAK isoform specificity (JAK1/2/3, or TYK2). Accordingly, we designed a GI locally activating JAK inhibitor, i.e., MMT3-72, that properly balanced its tissue selectivity and JAK specificity for effective UC treatment. To expedite the development process with high confidence, we integrated three machine learning models in this study. Our in-house JAK inhibitor classifier (CoGT) identified MMT3-72 as a JAK1 inhibitor, with its major metabolite, MMT3-72-M2, exhibiting inhibitory activity against JAK1, JAK2, and TYK2. This prediction was further supported by the regression model (MTATFP) and diffusion generative model (DiffDock). The cross-validated MMT3-72 and MMT3-72-M2 showed consistent efficacy in the subsequent in vitro and in vivo testing. Notably, MMT3-72 (p.o., 5, 10 mg/kg) demonstrated superior efficacy compared to tofacitinib in the DSS-induced colitis model. In conclusion, our machine learning-guided development approach has enabled us to develop an effective GI locally activating JAK inhibitor with minimal testing efforts.