

Intestinal mucosal barrier repair and immune regulation with an AI-developed gut-restricted PHD inhibitor

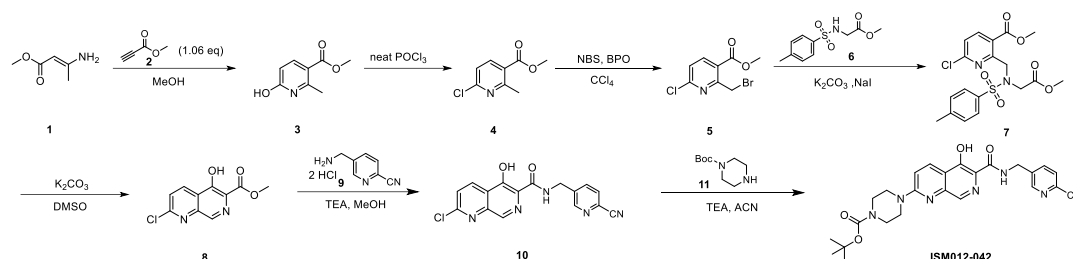
In the format provided by the
authors and unedited

Supplementary information 1

Synthesis and Characterization of ISM012-042 and [^{14}C] ISM012-042

1. Synthesis and characterization of ISM012-042

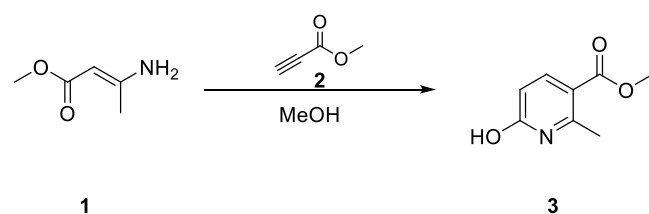
I. Synthetic route of ISM012-042



Scheme 1 Synthetic route of ISM012-042

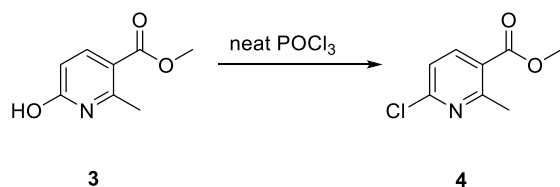
II. Synthesis of ISM012-042

Procedure for preparation of compound 3



To a mixture of **1** (10 g, 87 mmol, 1 eq) in MeOH (100 mL) was added methyl prop-2-ynoate **2** (7.78 g, 92.6 mmol, 1.06 eq). The mixture was stirred at 70 °C for 12 h. Then the mixture was cooled to 5 °C. The precipitate was filtered and triturated with MTBE (50 mL $\times$ 3). **3** (5 g, yield: 34.4%) was obtained as a white solid. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 12.06 (s, 1H), 7.81 (d, J = 9.7 Hz, 1H), 6.20 (d, J = 9.6 Hz, 1H), 3.74 (s, 3H), 2.53 (s, 3H).

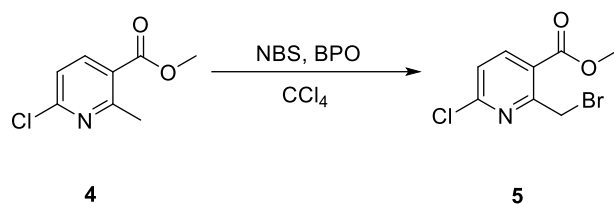
Procedure for preparation of compound 4



The solution of **3** (5 g, 29.8 mmol, 1 eq) in POCl_3 (17.7 g, 115 mmol, 3.85 eq) was stirred at 100 °C for 4 h. The reaction was slowly poured into ice water (100 mL) and then extracted with EtOAc (100 mL $\times$ 2). The combined organic phase was washed with NaHCO_3 (50 mL $\times$ 2), dried over anhydrous Na_2SO_4 , filtered, and concentrated in vacuo. The crude was used directly for the next step. Crude **4** (5 g, yield: 90.07%) was obtained as a yellow oil. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.20 (d, J = 8.3 Hz, 1H),

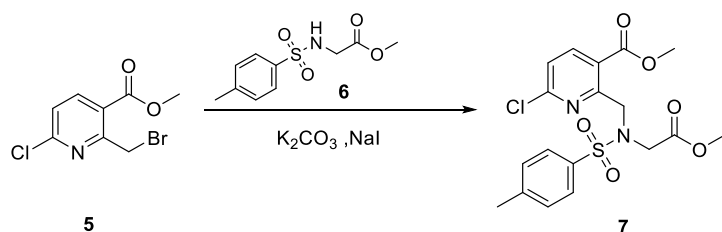
7.49 (d, $J = 8.3$ Hz, 1H), 3.86 (s, 3H), 2.69 (s, 3H).

Procedure for preparation of compound 5



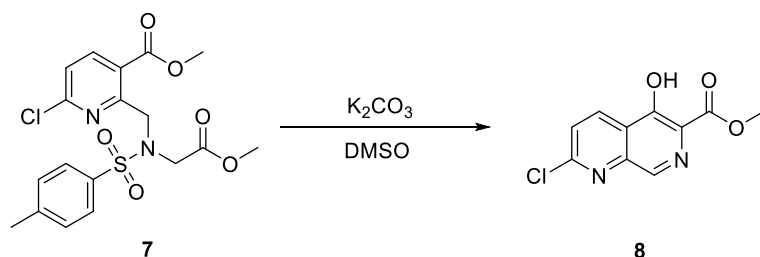
To a solution of **4** (6 g, 32.4 mmol, 1 eq) in CCl₄ (60 mL) was added NBS (6.9 g, 38.8 mmol, 1.2 eq) and BPO (1.56 g, 6.45 mmol, 0.2 eq). The mixture was stirred at 80 °C for 12 h. The reaction was diluted with DCM (60 mL) and washed with H₂O (60 mL×3). The organic layer was dried over MgSO₄ and concentrated to afford crude product. The crude product was purified by silica gel chromatography (ISCO®; 45 g SepaFlash® Silica Flash Column, Eluent of 0~25% EtOAc/Petroleum ether gradient @ 25 mL/min) to afford crude **5** (11 g, 54% purity, yield: 70%) as a yellow solid. LCMS: Rt: 0.981 min, m/z 264.10 [M+H]⁺;

Procedure for preparation of compound 7



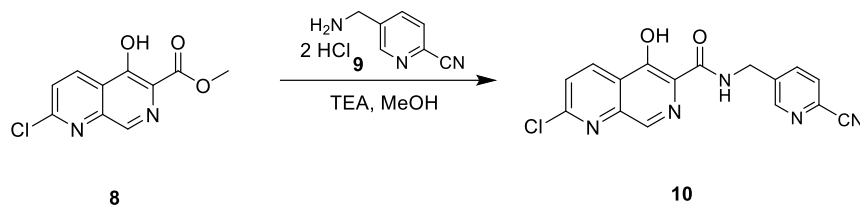
To a solution of **5** (5 g, 18.9 mmol, 1 eq) and methyl 2-(4-methylphenylsulfonylamido)acetate **6** (4.6 g, 18.9 mmol, 1 eq) in DMF (50 mL) was added K₂CO₃ (5.02 g, 36.4 mmol, 1.92 eq) and NaI (0.28 g, 1.87 mmol, 0.1 eq). The mixture was stirred at 50 °C for 12 h under a nitrogen atmosphere. The mixture was diluted with EtOAc (200 mL) and washed with H₂O (80 mL×3) and brine (80 mL×3). The organic layer was dried over MgSO₄ and concentrated to afford the crude product. The crude product was purified by silica gel chromatography (ISCO®; 45 g SepaFlash® Silica Flash Column, Eluent of 0~35% Ethyl acetate/Petroleum ether gradient @ 30 mL/min) to afford crude **7** (6 g, 14.1 mmol, yield: 74%) as a yellow solid. LCMS: RT: 0.544 min, m/z 427.3 [M+H]⁺; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.13 (d, $J = 8.3$ Hz, 1H), 7.70 (d, $J = 8.3$ Hz, 2H), 7.26-7.22 (m, 3H), 5.07-5.05 (m, 2H), 4.35 (s, 2H), 3.95 (s, 3H), 3.66 (s, 3H), 2.40 (s, 3H).

Procedure for preparation of compound 8



To a solution of **7** (6 g, 14 mmol, 1 eq) in DMSO (60 mL) was added K₂CO₃ (11.6 g, 84.1 mmol, 6 eq). The mixture was stirred at 50 °C for 4 h under a nitrogen atmosphere. The mixture was diluted with H₂O (60 mL), and the aqueous was acidified by 1M HCl to pH=6. The precipitated solid was filtered and dried to afford **8** (1.5 g, 6.29 mmol, yield: 45%) as an off-white solid. **LCMS**: *m/z* 239.1 [M+H]⁺; **¹H NMR** (400 MHz, DMSO-*d*₆) δ 11.69-11.39 (m, 1H), 8.88 (s, 1H), 8.73 (d, *J* = 8.6 Hz, 1H), 7.94 (d, *J* = 8.8 Hz, 1H), 3.99 (s, 3H).

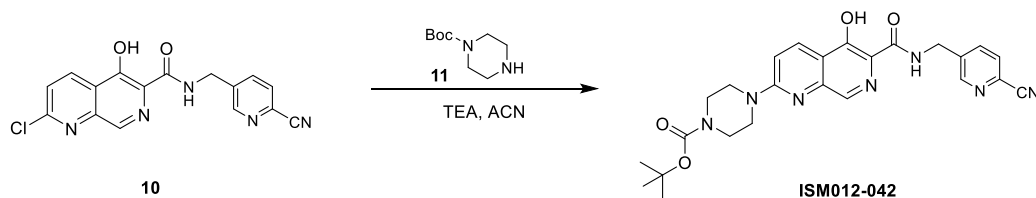
Procedure for preparation of compound 10



To a solution of **8** (200 g, 838 mmol, 1 eq) in MeOH (2 L) were added 5-(aminomethyl)pyridine-2-carbonitrile dihydrochloride **9** (259 g, 1.26 mol, 1.5 eq), TEA (339 g, 3.35 mol, 4 eq), and the reaction was stirred at 60-65 °C for 12 h. The reaction mixture was filtered through the normal funnel, and the filter cake was washed with 1 L MeOH, and dried in a vacuum to afford **10** (200 g, 588 mmol, yield: 70.2%) as a yellow solid.

LCMS: RT: 0.555 min, *m/z* 340.1 [M+H]⁺; **¹H NMR** (400 MHz, DMSO-*d*₆) δ 13.75-13.51 (m, 1H), 10.08 (br s, 1H), 8.86 (s, 1H), 8.78 (s, 1H), 8.69 (d, *J* = 8.8 Hz, 1H), 8.02 (s, 2H), 7.91 (d, *J* = 8.8 Hz, 1H), 4.68 (d, *J* = 6.4 Hz, 2H).

Procedure for preparation of ISM012-042



To a suspension of **10** (17.0 g, 50.0 mmol, 1 eq) in ACN (170 mL) was added **11** (13.0 g, 70 mmol, 1.40) and TEA (7.60 g, 75.0 mmol, 1.50 eq). The mixture was stirred at 80-85 °C for 12 h until the reaction was complete. Adjust the temperature to 20-30 °C and filter the reaction mixture. Then, the solid was stirred in H₂O (200 mL) at 20 °C for 0.5 h and filtered to give a yellow solid. The solid was concentrated under reduced pressure to yield **ISM012-042** (23.0 g, 45.5 mmol, 91.0% yield, 97% purity) as a yellow solid.

HRMS

The high-resolution mass spectrometric analysis was performed on an Agilent G6224A TOF LC/MS using high-performance liquid chromatography with electrospray ionization-time of flight mass spectrometry (HPLC-ESI-TOF LC/MS). Electrospray

ionization was performed in positive ion mode. The sample yielded a strong characteristic positive ion response as demonstrated in the spectra with pseudo molecular ion $[M+H]^+$ observed at 490.2210 m/z corresponding to the protonated ISM012-042 ($C_{25}H_{28}N_7O_4^+$, -2.68 ppm difference compared to the theoretical value m/z 490.2197). The data is consistent with the molecular formula proposed for the drug substance.

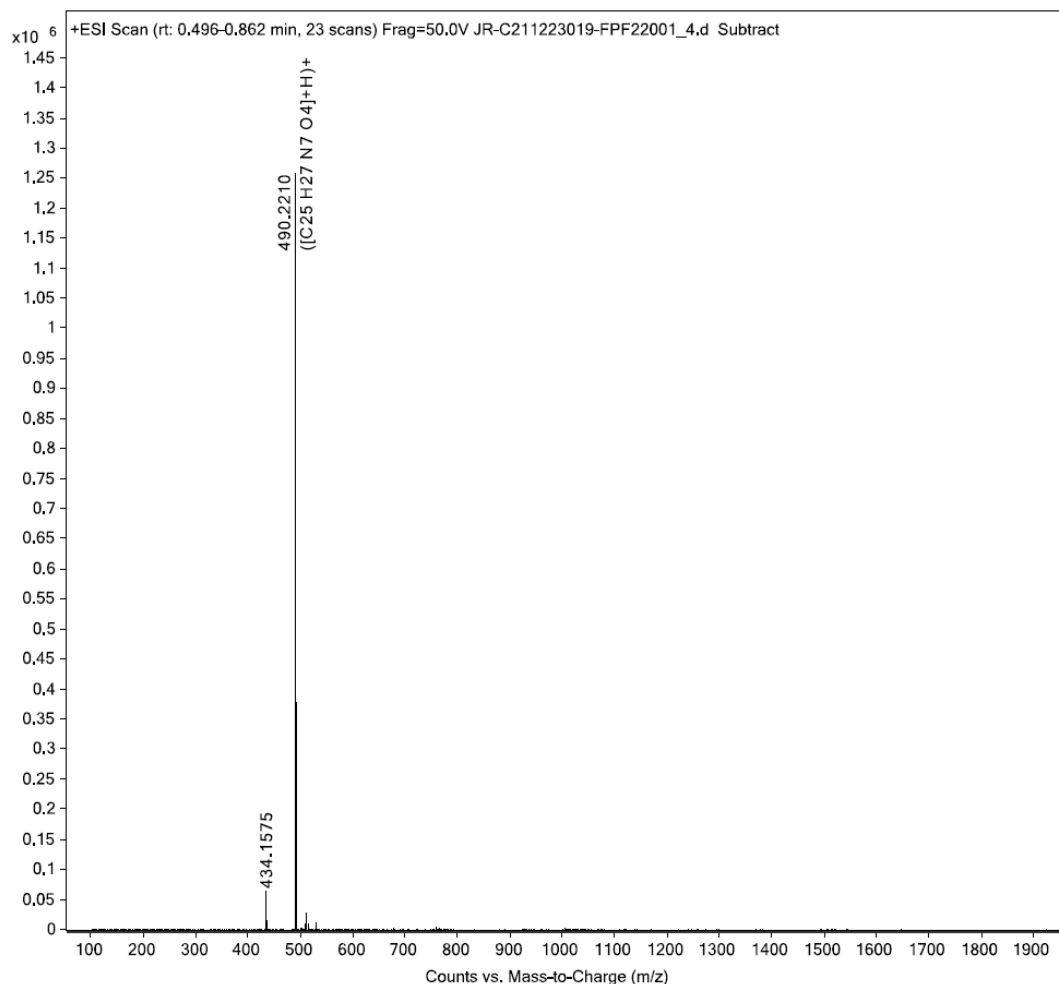


Figure 1 High-Resolution Mass Spectrum for **ISM012-042**

NMR

A Bruker AVANCE III 400 MHz NMR spectrometer equipped with a Bruker 5 mm PABBO BB-400 MHz Z-gradient high-resolution probe, and supported by TOPSPIN software, was used to collect 1-dimensional (1D) proton and carbon data at 27°C.

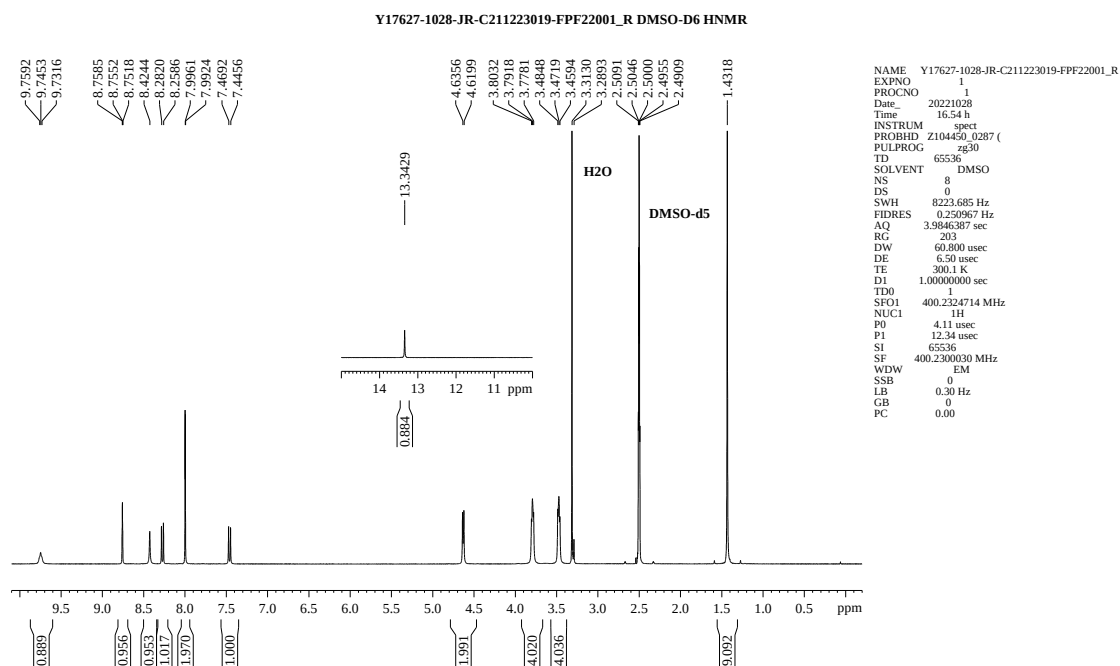


Figure 2 ^1H NMR Spectrum for ISM012-042

^1H NMR (400 MHz, DMSO- d_6) δ 13.34 (br s, 1H), 9.75 (br s, 1H), 8.76 (t, J = 1.3 Hz, 1H), 8.42 (br s, 1H), 8.27 (d, J = 9.4 Hz, 1H), 7.99 (m, 2H), 7.46 (d, J = 9.4 Hz, 1H), 4.63 (d, J = 6.3 Hz, 2H), 3.80-3.78 (m, 4H), 3.51-3.45 (m, 4H), 1.43 (s, 9H).

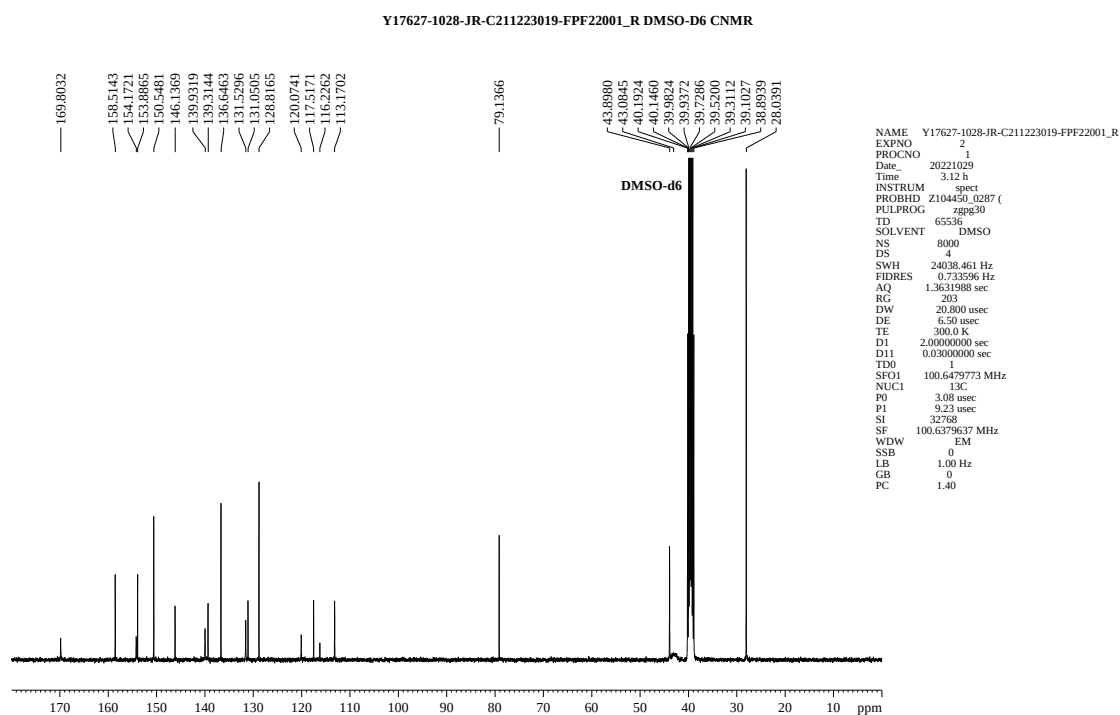
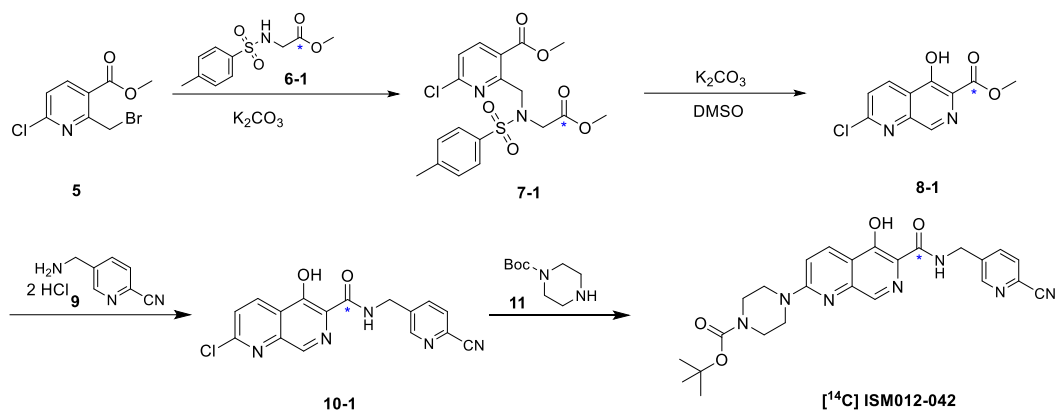


Figure 3 ^{13}C NMR Spectrum for ISM012-042

^{13}C NMR (101 MHz, DMSO- d_6) δ 169.8, 158.5, 154.2, 153.9, 150.6, 146.1, 139.9, 139.1, 136.7, 131.5, 131.1, 128.8, 120.1, 117.5, 116.2, 113.2, 79.1, 43.9, 43.1, 39.5, 28.0.

2. Synthesis and Characterization of [^{14}C] ISM012-042

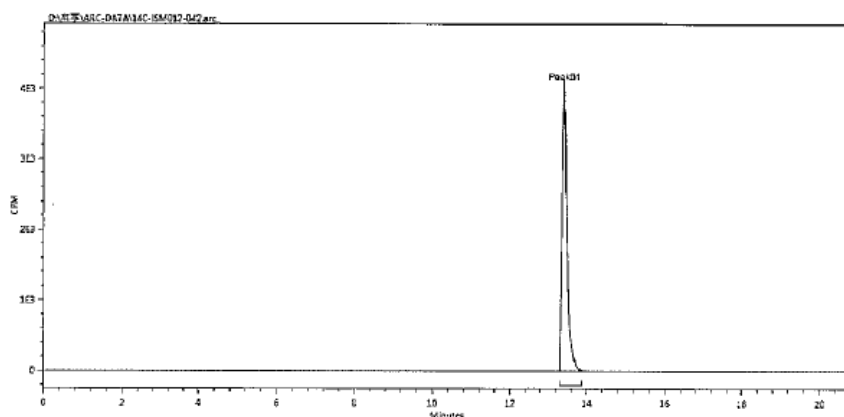
I. Synthetic route of [^{14}C] ISM012-042



Scheme 2 Synthetic route of [^{14}C] ISM012-042

II. Synthesis of [^{14}C] ISM012-042

[^{14}C] ISM012-042 was synthesized from ^{14}C labelled **6-1** using the similar procedure of ISM012-042. An Agilent 1260 HPLC equipped with v.ARC Radio-LC system was used to determine the purity of [^{14}C] ISM012-042.



HPLC File Name: IMHK-20220105		Run Time: 2022-09-14	
Injection Vol.: 8 μL		Sample Name: ^{14}C - ISM012-042	
ID	RT(min)	Area(CPM)	%Total
1([^{14}C]ISM5411)	13.4	17937	100
Total			100

Figure 4 HPLC-Radio Chromatogram of [^{14}C] ISM012-042

Supplementary Figure and Table Legends

Supplementary Figure 1. (a) PHD1/2-HIF-targeted IBD therapy proposed by the Pharma AI platform and its development history. The discovery and development timeline from PHD target identification (2021) to its current status in Phase 1 clinical trial testing that began recruitment in 2023. Our AI-powered PharmaAI suite streamlined the hit identification, potency optimization compound synthesis, and preclinical evaluation of ISM012-042, resulting in preclinical candidate nomination (PCC) in less than 2 years. Preclinical evaluation from 2021 to PCC in 2022 included *in vitro* safety, toxicity, and mechanistic studies, after which ISM012-042's *in vivo* efficacy, safety, and pharmacokinetic profiles were rigorously profiled in multiple murine IBD models. ISM012-042's favorable safety, toxicity, and PK profiles were determined from studies carried out in mice, rats, dogs, and monkeys. **(b)** Alchemy (FEP calculation) guided potency optimization of PHD2 inhibitors by computing binding free-energy estimates for PHD2 protein-ligand complexes. **(c)** ADMET Profiling was applied to predict properties for gut restriction and rank the designed molecules. The predicted results correlated well with the experimentally determined data. The low solubility, high clearance, and moderate permeability features were prioritized to design a gut-restricted compound.

Supplementary Figure 2. ISM012-042 inhibits PHD1/2/3, stabilizes HIF1 α , and maintains ZO-1 at cell-cell contacts. (a) PHD3 hydroxylase inhibition of ISM-012-042 relative to DMSO control. **(b-c)** PHD1 hydroxylase inhibition in the presence of Fe(II) sulfate at indicated concentrations of **(b)** ISM-012-042 or **(c)** GB004 treatment relative to DMSO control (mean $\pm$ SD; n=2 biological replicates at each experimental condition for **a-c** per independent experiment). Representative plots were shown from three independent experiments. **(d)** Immunoblot showing dose-dependent HIF-1 α stabilization in Caco-2 cells treated with ISM012-042 or DMSO. GAPDH was used as a loading control. Blots were representative of three independent experiments **(e)** Immunoblot showing dose-dependent inhibition of hydroxy-HIF-1 α level. Caco-2 cells were pre-treated with DMSO, ISM012-042, or Roxadustat at 2 hours prior to 4-h treatment by MG-132 (20 μ M). Total HIF-1 α protein was shown with GAPDH as a loading control. **(f)** Immunofluorescence staining of ZO-1 in Caco-2 cell monolayers. 8 non-overlapping fields of view per sample were quantitated for mean fluorescence intensity; scale = 50 μ m (mean $\pm$ SD; P values calculated using post-hoc Tukey's test following one-way ANOVA). **d-f**, Representative images were shown from 3 independent experiments.

Supplementary Figure 3. Body weight of male and female SD rats treated once daily with ISM012-042 (0, 100, 300, 1000 mg/kg/day) by oral gavage for 28 days (mean $\pm$ SD for males **(a)** and females **(b)**; n = 10 per group per sex).

Supplementary Figure 4. Prophylactic ISM012-042 administration reduced pathological colitis progression in acute TNBS model. TNBS-induced colitis mice were orally administered 1, 3, or 10 mg/kg of ISM-012-042 once daily from day -1 till day 7 post colitis induction. **(a)** Body weight curves during the study period. The arrow indicates the first dosing of ISM012-042, mesalamine or vehicle (mean $\pm$ SD; n=8 mice/group for sham, vehicle and mesalamine treatment, 14 mice per ISM012-042-treated group) **(b)** Representative macroscopic imaging of dissected colon collected on day 7 post-induction. **(c)** Colon length and weight measured on day 7 post-induction

(mean $\pm$ SD; n = 8 mice per group). P values at day 7 were calculated by Dunnett's test following two-way ANOVA for **a** or one-way ANOVA for **c**. **(d)** LC-MS/MS-based quantitation of ISM012-042 presence in the colon and plasma at 4 h post last dosing. (n=3 mice per time point/dose level). **(e)** Representative H&E-stained Swiss-rolled colon sections from the vehicle and ISM012-042 (3 mpk)-treated mice. scale = 100 μ M **(f)** Histological scoring of Swiss-rolled colon sections from vehicle and ISM012-042 (3 mpk)-treated mice on day 7 post-TNBS induction (mean $\pm$ SD; n = 8 mice/group; P values of comparison in total scores calculated by two-sided unpaired t-test; **(g)** Dot plot showing the top 20 significantly changed KEGG pathways, as determined from enrichment analysis of differentially expressed genes (DEGs) in the colon and the p-value was adjusted using the Benjamini-Hochberg correction method. Bulk RNA sequencing was conducted for the colon tissues collected on day 2 post-colitis induction (n=6 mice per group). **(h)** IL-17 downstream target proteins based on mass spectrometry analysis (mean $\pm$ SD; n = 8 mice/group; P values calculated by two-sided unpaired t-test) **(i)** Plasma IL-6 and TNF- α quantitated by ELISA on day 7 post-TNBS induction (mean $\pm$ SD; n=8-10 mice/group). P values calculated by Dunnett's test following one-way ANOVA. Comparisons with no P value are NS.

Supplementary Figure 5. ISM012-042 treatment mitigated colitis pathology and reduced pro-inflammatory immune cell subsets. **(a)** Body weight loss curves of the study in which ISM012-042 or mesalamine was administered once daily to TNBS colitis mice starting on days 2 post-induction. The arrow indicates the first dosing of the intervention. Data represent mean $\pm$ SD; n=8 mice/group; P values at day 7 calculated by Dunnett's test following two-way ANOVA. **(b)** Representative macroscopic imaging of dissected whole Swiss-rolled colon. **(c)** Colon length and weight from indicated groups at day 7 post-TNBS induction (mean $\pm$ SD; n = 8 mice per group). P values at day 7 were calculated by Dunnett's test following two-way ANOVA for **a** or one-way ANOVA for **c**. **(d)** Flow cytometric analysis and quantitation of the following immune cell subsets in colonic lamina propria isolated on day 7 (mean $\pm$ SD; n=4 mice/group). P values were calculated by two-sided unpaired t-test. **(e)** Fecal calprotectin and LCN2 quantitated by ELISA at 6 h post dosing on day 3 post-TNBS induction (mean $\pm$ SD; n=8 mice/group. P values calculated by Dunnett's test following one-way ANOVA. Comparisons with no P value are NS.

Supplementary Fig 6. ISM012-042 demonstrated anti-colitis activity in the oxazolone-induced colitis model without increasing systemic EPO and VEGF levels. **(a)** Body weight curves of the study during which ISM012-042 or vehicle treatment was initiated at one day before intracolonic challenge. The left arrow indicates the pre-sensitization by a topical application of 2% oxazolone solution. Arrowhead indicated the day of dosing initiation. The right arrow refers to intracolonic oxazolone induction. Data represent mean $\pm$ SD; n = 11 mice/group for sham and vehicle group, 26 mice per ISM012-042 treated group. P values calculated by Dunnett's test following two-way ANOVA. **(b)** Representative macroscopic imaging of colon dissected on day 4. **(c)** Colon length and weight measured on day 4 post-intracolonic induction (mean $\pm$ SD; n = 11 mice/group for sham and vehicle group, 26 mice per ISM012-042 treated group; P values calculated by Dunnett's test following one-way ANOVA **(d)** Plasma EPO and VEGF-A levels measured at the indicated timepoint post dosing on day 4 by ELISA (mean $\pm$ SD; n = 5 or 7 mice per timepoint per group). **(e)** Body weight curves of the head-to-head comparison study during the oral daily treatment by ISM012-042 or GB-004 initiated on day -1 of intracolonic challenge (mean $\pm$ SD; n = 8 mice/group;

P values calculated by Dunnett's test following two-way ANOVA). (f) Histological scoring of colon Swiss-roll sections from vehicle, ISM012-042 (10 mpk, QD) or GB004 (10 mpk, QD)-treated mice on day 4 post intracolonic induction. (mean $\pm$ SD; n = 8 mice/group; P value of comparisons in total scores calculated by Dunnett's test following one-way ANOVA) (g) Body weight curves of the study during the treatment by ISM012-042 or vehicle initiated on day 2 post intracolonic challenge (mean $\pm$ SD; n = 8 mice/group; P values calculated by Dunnett's test following two-way ANOVA). (h) Representative macroscopic imaging of colon dissected on day 7. Comparisons with no P value are NS.

Supplementary Table 1. In vitro ADMET and safety profile of ISM012-042.

Supplementary Table 2. Selectivity of GB004 and ISM012-042 against recombinant forms of human 2OG and Fe (II)-dependent oxygenases. FIH: factor inhibiting HIF, JMJD2D (KDM4D): lysine demethylase 4D, JMJD2E (KDM5B): lysine demethylase 5B, JMJD1B (KDM3B): lysine demethylase 3B, UTX (KDM6A): lysine demethylase 6A. The assay conditions are described in the METHODS section.

Supplementary Table 3. Orally administrated ISM012-042 is enriched in the colon and well-tolerated in multiple preclinical model species. Colon and plasma were collected at multiple time points after a single oral administration of ISM012-042 to healthy C57BL/6J mice, colitis mice 2 days post intracolonic oxazolone induction, or healthy Sprague-Dawley (SD) rats. Samples were also collected after repeated once-daily oral dosing of ISM012-042 for 5 days after intracolonic oxazolone induction followed by LC-MS/MS-based compound quantification. Maximum serum concentration (C_{max}), time to reach maximum serum concentration (T_{max}), the area under the curve/observed exposure at the study endpoint (AUC_{0-t}), and the colon-to-plasma AUC ratio were calculated.

Supplementary Table 4. Blood chemistry summary. Blood chemistry of male and female SD rats treated once daily with ISM012-042 (0, 100, 300, 1000 mg/kg/day) by oral gavage for 28 days. All values are within the corresponding parameter ranges (95% CIs) of normal animals of the same age recorded at the study site. Data presented as mean $\pm$ SD (n = 10 rats per group per sex). The asterisks denote the measurement below the lower limits of detection.

Supplementary Table 5. Hematology summary. Hematology of male and female SD rats treated daily with ISM012-042 (0, 100, 300, 1000 mg/kg/day) for 28 days. All values are within the corresponding parameter ranges (95% CIs) of normal animals of the same age recorded at the study site. Data presented as mean $\pm$ SD (n = 10 rats per group per sex).

Supplementary Table 6. Individual daily DAI score of TNBS-induced colitis mice with prophylactic treatment of ISM012-042.

Supplementary Table 7. Individual daily body weight of TNBS-induced colitis mice with prophylactic treatment of ISM012-042.

Supplementary Table 8. Individual daily DAI score of oxazolone-induced colitis mice with prophylactic treatment of ISM012-042.

Supplementary Table 9. Individual daily body weight of oxazolone-induced colitis mice with prophylactic treatment of ISM012-042.

Supplementary Table 10. Crystallographic parameters and data collection statistics for PHD2 co-crystalized with ISM012-042.

Supplementary Table 11. qPCR primer sequences

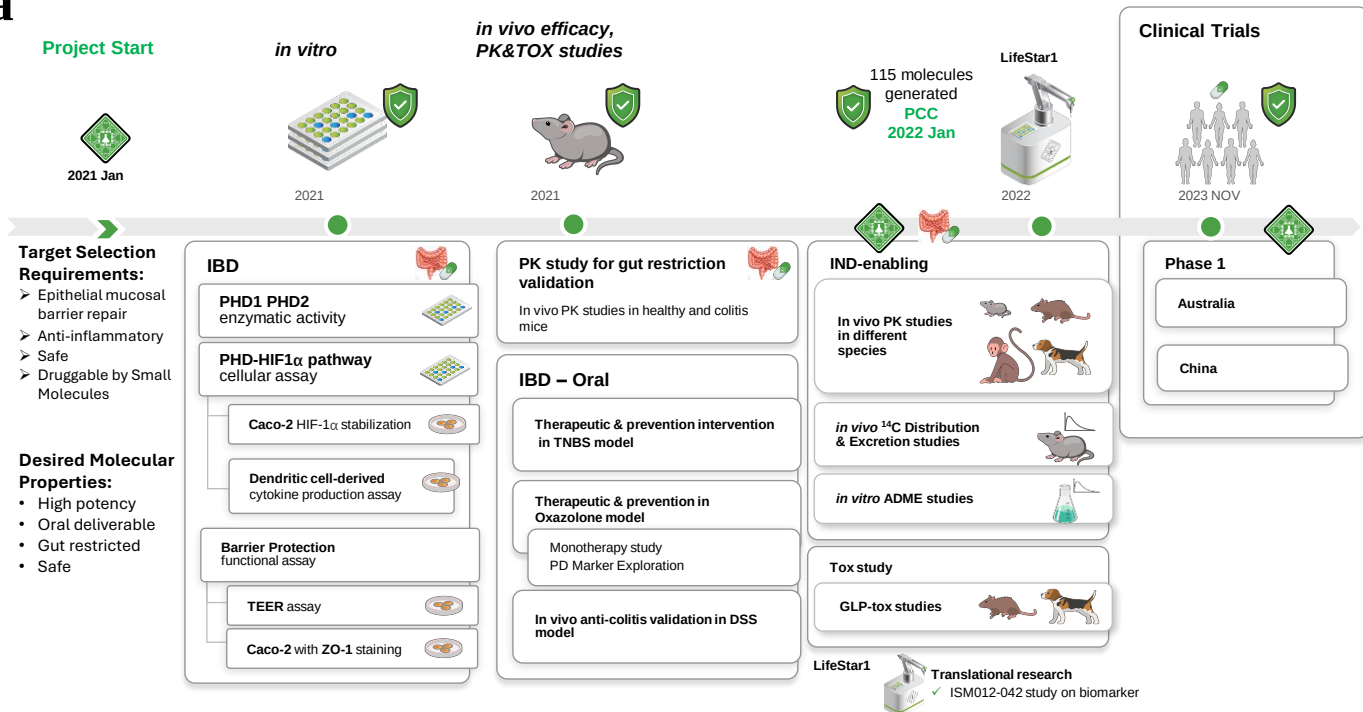
Supplementary Table 12. Disease activity index (DAI) standards for scoring animal IBD

Supplementary Table 13. Flow cytometry reagents and antibodies

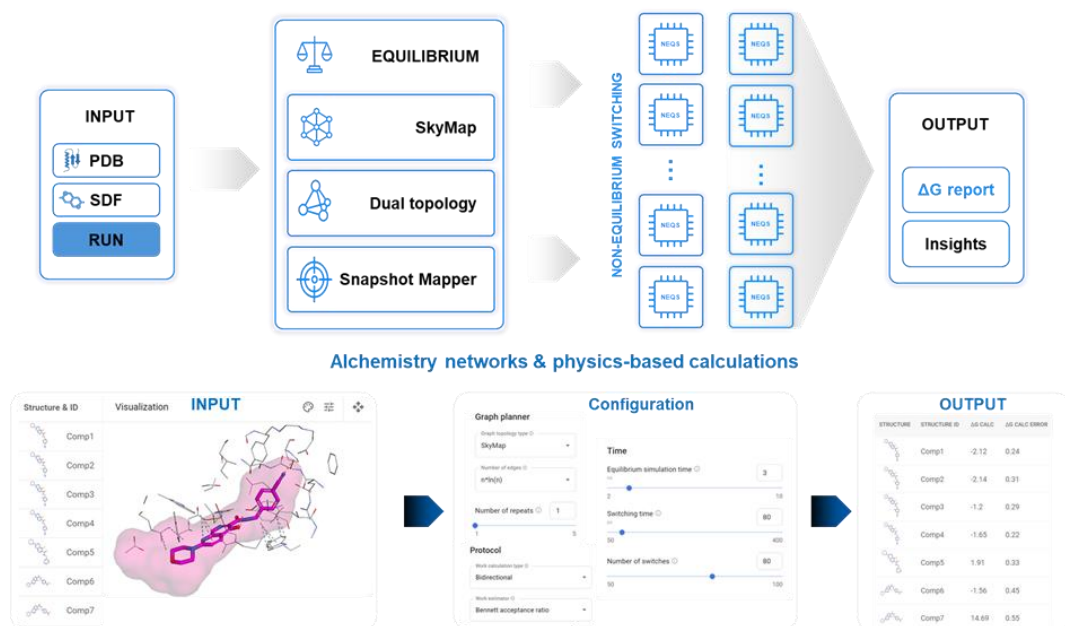
Supplementary Table 14. IBD histopathology scoring criteria

Additional Supplementary Figures. Unprocessed Blots for Supplementary Figures 2b & 2e

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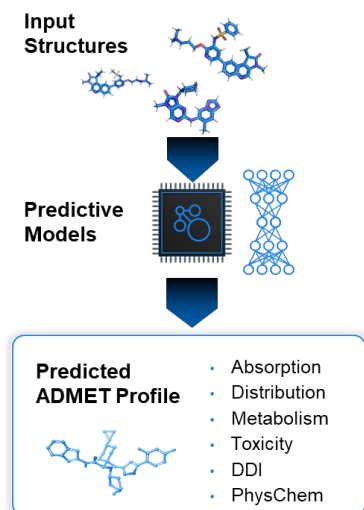


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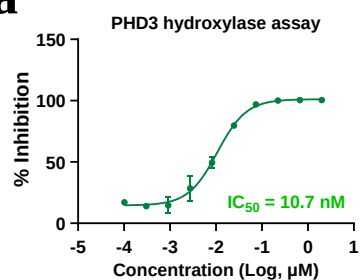
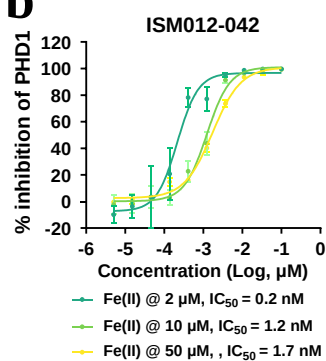
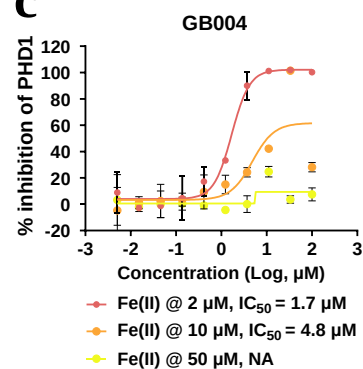
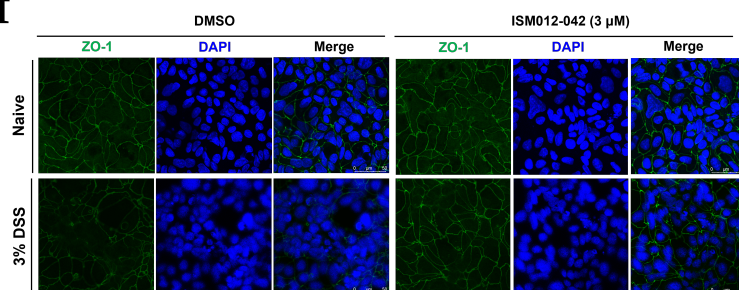
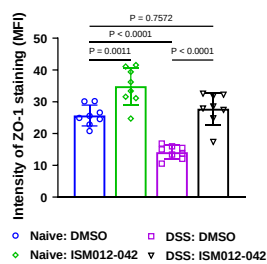
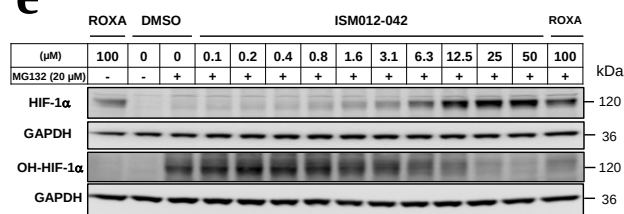


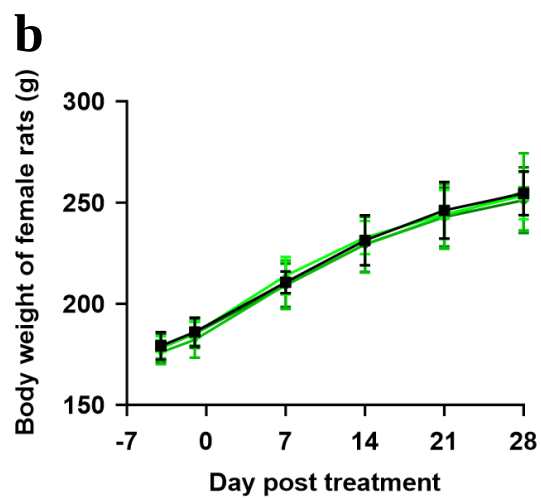
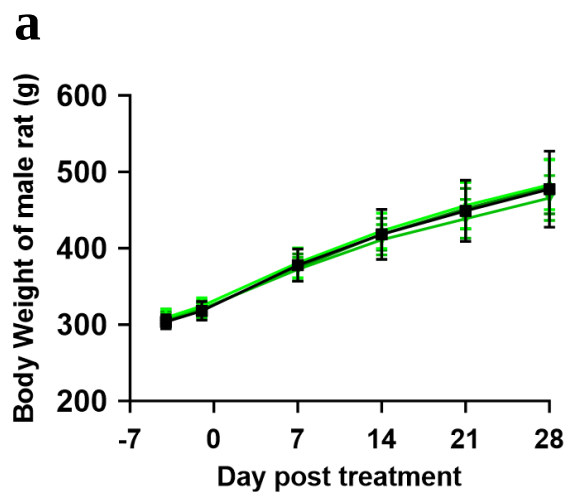
c

ANNOTATE



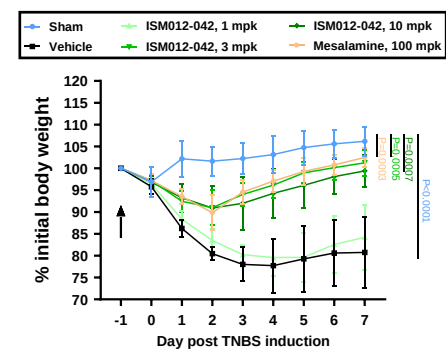
Property	Experiment data	Predicted data
logKs	<-5.8	-4.4
Caco-2 P _{app} (A-B) (10 ⁻⁶ cm/s)	11.2	≥10
HLM CL _(hep) (mL/min/kg)	19.7	> 10
MLM CL _(hep) (mL/min/kg)	34.9	>10
RLM CL _(hep) (mL/min/kg)	14.3	>10

a**b****c****d****f****e**



■ Vehicle ▼ ISM012-042, 300 mg/kg, QD
▲ ISM012-042, 100 mg/kg, QD ◆ ISM012-042, 1000 mg/kg, QD

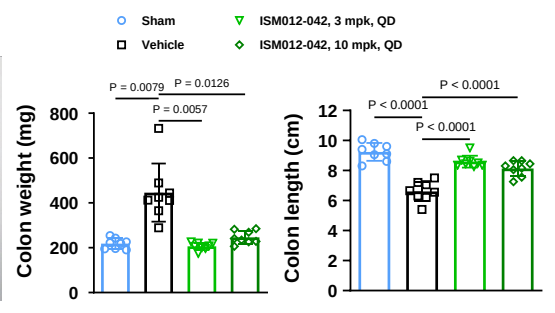
a



b



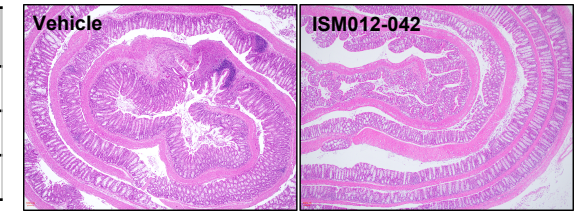
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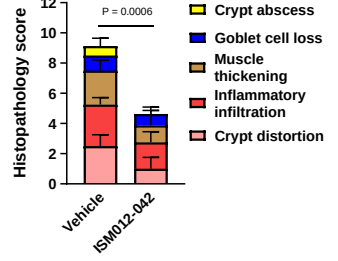
d

	Plasma AUC _{0-24h} (hr*ng/ml)	Colon concentration (ng/g) @ 4 hr	Plasma concentration (ng/ml) @ 4 hr	Colon/Plasma Ratio
ISM012-042 1 mpk	9.62	14.1	BQL	N/A
ISM012-042 3 mpk	12	65.5	1.56	42
ISM012-042 10 mpk	20.0	236	2.5	94.3

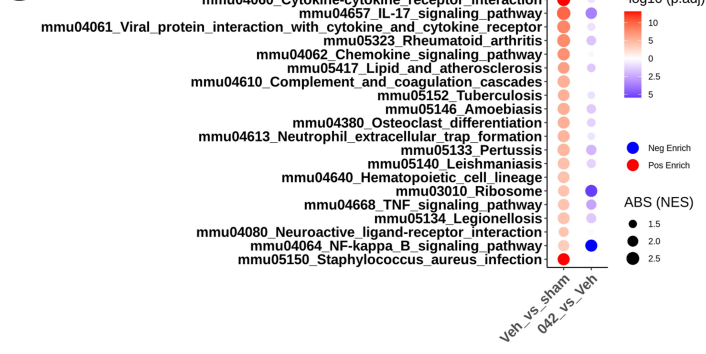
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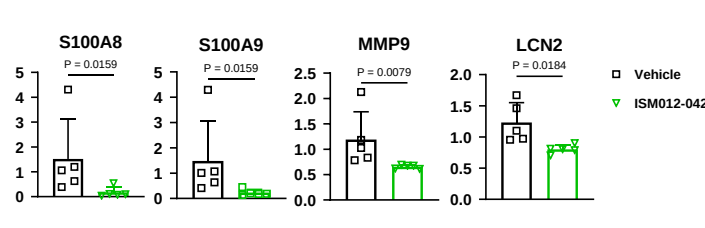
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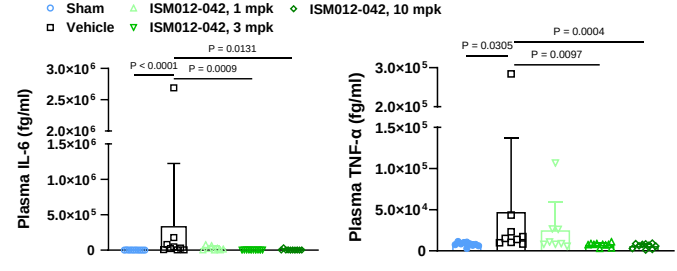
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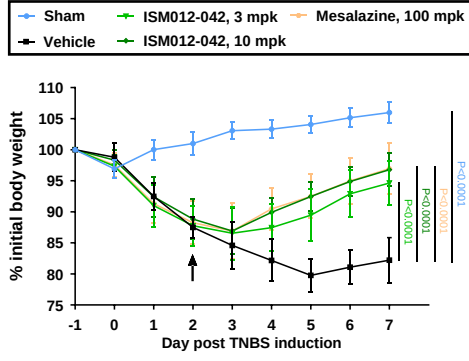
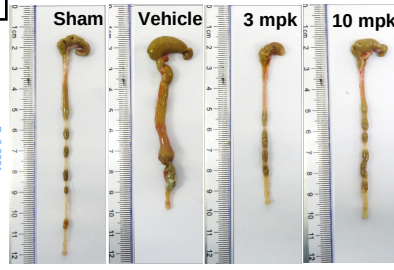
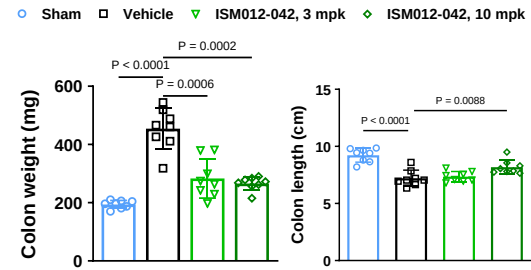
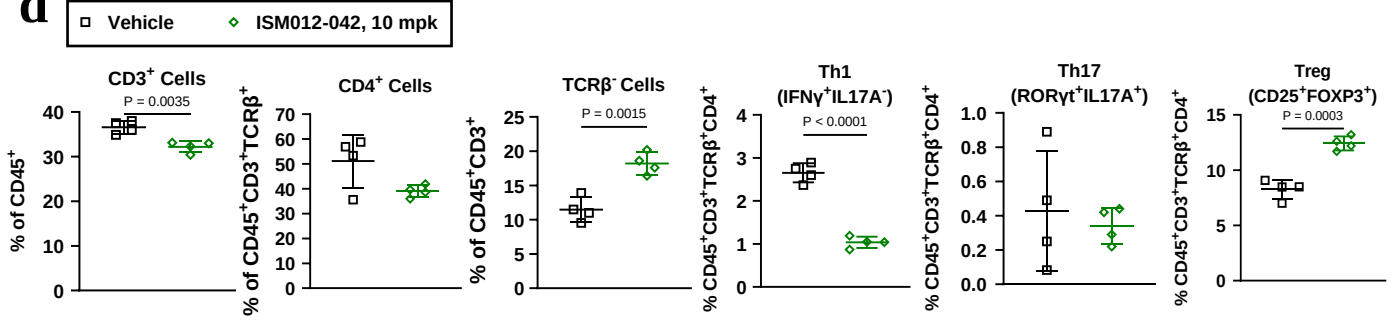
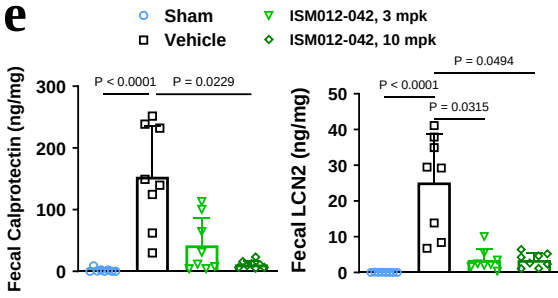


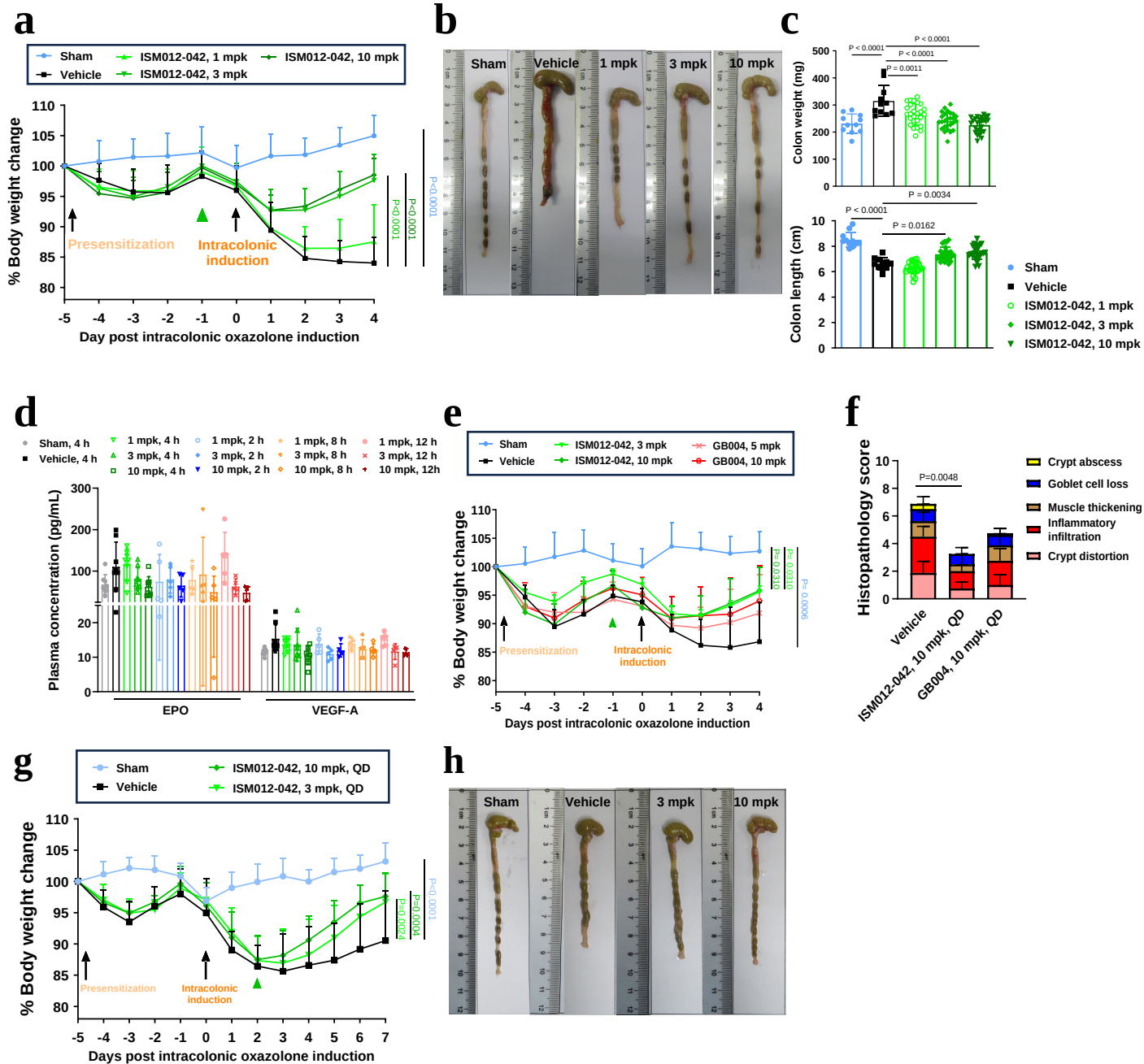
h



i



a**b****c****d****e**



		ISM012-042
In vitro ADME	Solubility (µg/mL)	0.89 (FassIF), 5.45 (FessIF), 2.18 (SGF)
	Caco-2 Permeability (nm/s)	8.29
	MMS(h/c/d/r/m) (mL/min/kg)	17.5/37.5/13.5/<10.8/29.4
In vitro safety	CYP inhibition IC ₅₀ , µM (1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A-M, 3A-T)	ALL>1.0
	hERG IC ₅₀ (µM)	>3.172
	Ames	Negative
	Mini-panel screening @ 10 µM 44 targets	Negative
	KINOMEscan® (@ 1 µM) 468 kinases	No competition binding

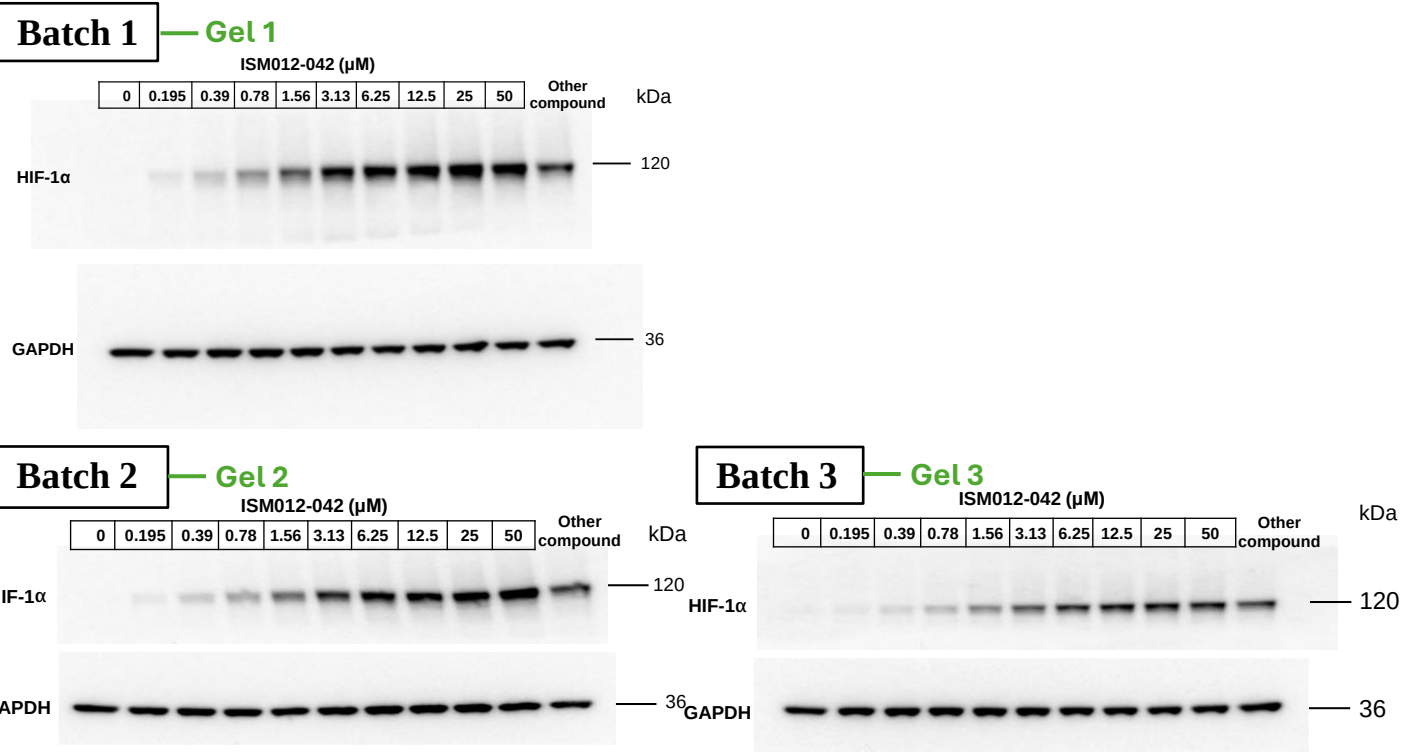
Human Fe (II) & 2OG-dependent oxygenase	Compound	IC50 (μM)
FIH	GB004	7.461
	ISM012-042	>10
JMJD2D (KDM4D)	GB004	3.347
	ISM012-042	>10
JMJD2E (KDM4E)	GB004	6.181
	ISM012-042	>10
JMJD1B (KDM3B)	GB004	4.792
	ISM012-042	>10
UTX (KDM6A)	GB004	5.427
	ISM012-042	>10

Animal condition	Measured Analyte	Matrix	Pharmacokinetic measurements			Colon: Plasma
			C _{max} (nmol/g or nmol/ml)	T _{max} (h)	AUC _{0-t} (nmol*h/mL or nmol*h/g)	(AUC _{0-t} colon/AUC _{0-t} plasma)
Normal mice	ISM012-042 (10 mpk, QD)	Colon	6.33	4.00	19.18	67.08
		Plasma	0.08	1.00	0.29	
Colitis mice (2 day post intracolonic colitis induction)	ISM012-042 (3 mpk, single dose)	Colon	1.74	8.00	9.14	87.99
		Plasma	0.01	8.00	0.10	
	ISM012-042 (10 mpk, single dose)	Colon	2.29	8.00	15.32	93.57
		Plasma	0.02	8.00	0.16	
Colitis mice (4 day post intracolonic colitis induction)	ISM012-042 (1 mpk, QD)	Colon	0.73	4.00	4.61	72.74
		Plasma	0.01	2.00	0.06	
	ISM012-042 (3 mpk, QD)	Colon	1.58	4.00	9.18	111.84
		Plasma	0.02	4.00	0.08	
	ISM012-042 (10 mpk, QD)	Colon	4.57	4.00	17.44	107.52
		Plasma	0.02	2.00	0.16	
Normal Rat	ISM012-042 (30 mpk, QD)	Colon	5.66	8.00	36.16	372.63
		Plasma	0.02	2.67	0.10	

Supplementary Figures of Unprocessed Blots

Supplementary Figure 2d

Original images-HIF-1α stabilization by ISM012-042 in Caco-2 cells



Supplementary Figure 2e

Original images-the effect of ISM012-042 on HIF-1α hydroxylation in Caco-2 cells

