**Translating Gut Microbial Metabotypes to Therapy: DA Adducts from Mori Cortex as Effective Gut-Restricted GUS Inhibitors Against Irinotecan-Induced Diarrhea in Colorectal Cancer.**

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**Background and aims.** Irinotecan (IRT), a cornerstone chemotherapy for colorectal cancer (CRC), causes severe delayed diarrhea due to intestinal exposure to its toxic metabolite SN-38. This toxicity arises from the reactivation of SN-38 by gut microbial β-glucuronidases (GUS). Current GUS inhibitor screening lacks clinical relevance and overlooks intestinal pharmacokinetics (PK), limiting therapeutic translation.

**Methods.** We identified SN-38G extensive metabolizers (EMs) among CRC patients using in vitro assays and characterized EM-enriched GUS-harboring species (*Faecalibacterium prausnitzii*, *Fp*) via metagenomics. A novel ‘3-in-1’ inhibition system—integrating fecal microbiota from EM patients, *Fp* isolates, and recombinant *Fp*GUS protein—was deployed to evaluate Diels-Alder adducts (DAs) from Mori Cortex. The lead compound, sanggenon G (SGG), was assessed in healthy mice and an AOM/DSS CRC model for efficacy/toxicity. SGG-*Fp*GUS interactions were probed by molecular docking. ADME properties were characterized through rat oral PK studies, Caco-2 cellular uptake assays, and SwissADME predictions.

**Results.** Four DA compounds potently inhibited SN-38G deconjugation by EM fecal microbiota (>50% inhibition at 40 µM vs. amoxapine’s <20% at 100 µM). These DAs also suppressed *Fp* and *Fp*GUS activity at comparable potency. In CRC mice, SGG pretreatment attenuated IRT-induced intestinal toxicity and enhanced antitumor efficacy via dual suppression of microbial SN-38 generation and intestinal SN-38 uptake. Chronic SGG administration (14 days) induced no toxicity or significant metabolic shifts. Computational and experimental data confirmed high GUS affinity and gut-restricted exposure (low systemic/high intestinal), validated by rat PK studies and Caco-2 accumulation.

**Conclusion/Discussion.** Mori Cortex DAs, particularly SGG, are potent gut-restricted GUS inhibitors. Their optimized pharmacokinetic profile and dual mechanism—simultaneously reducing SN-38 production and uptake—effectively mitigate IRT-induced diarrhea while preserving antitumor efficacy. This strategy bridges microbial metabotyping with targeted therapy, offering a clinically translatable approach to improve CRC treatment safety.

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