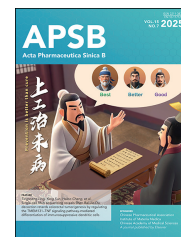




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EDITORIAL

A transformer-based strategy to develop RP20 as a potent RIPK2 inhibitor for acetaminophen-induced acute liver injury



KEY WORDS

CMD-OPT;
Acute liver injury;
RIPK2 inhibitors;
Candidate

Acetaminophen (APAP) overdose remains a leading cause of acute liver injury (ALI) worldwide, with limited therapeutic options except for *N*-acetylcysteine (NAC), whose efficacy is hampered by short half-life and suboptimal bioavailability^{1–3}. Inflammation driven by MAPK and NF- κ B pathways exacerbates hepatocellular damage, positioning receptor-interacting serine/threonine kinase 2 (RIPK2) as an attractive target to attenuate pro-inflammatory signaling and apoptosis^{4,5}. Early RIPK2 inhibitors such as GSK2983559 advanced into clinical trials for inflammatory bowel disease but faltered due to safety and pharmacokinetic drawbacks⁶. Addressing these challenges, Chen et al.⁷ introduce a two-stage, transformer-based CMD-OPT model to refine scaffold design and ADME properties of lead RIPK2 inhibitors, culminating in compound RP20—a selective, orally bioavailable pre-clinical candidate exhibiting robust hepatoprotective effects in APAP-induced ALI models.

CMD-OPT employs a transformer encoder–decoder framework divided into “scaffold hopping” and “property refinement” stages to navigate chemical space under 2D fingerprint and 3D pharmacophore constraints (*e.g.*, desired clearance, half-life, microsomal stability, and QED metrics). Starting from GSK2983559, BRICS-based fragmentation generated diverse scaffolds, from which top candidates were selected by docking scores. Among these, scaffold 8 (pyrimidine-thiophene core)

emerged as novel and synthetically tractable. Subsequent rounds optimized ADME properties *in silico* via ADMET Lab predictions, yielding 20 compounds (**RS1–RS20**) for synthesis and *in vitro* HLM stability assays. Guided by metabolic stability trends, a fluorine atom was introduced at the C-6 position of the benzothiazole moiety to further enhance PK properties, generating **RP1–RP29** series. **RP20** stood out with nanomolar RIPK2 inhibition ($IC_{50} = 13$ nmol/L), >2000-fold selectivity over RIPK1, and improved HLM stability compared to earlier analogues and GSK2983559.

RP20 demonstrated exceptional oral PK in mice ($AUC_{0–t} \approx 41,021$ ng h/mL, $t_{1/2} \approx 3.95$ h, $F \approx 95.9\%$ at 10 mg/kg) and favorable exposure in SD rats, markedly surpassing GSK2983559 in bioavailability and systemic exposure. Kinome profiling against 373 human kinases at 1 μ mol/L confirmed minimal off-target activity outside RIPK2, indicating a selectivity fold >15 against DDR1 and negligible inhibition of major CYP450 isoforms (CYP3A4 and CYP2D6), suggesting a lower potential for drug–drug interactions and systemic toxicity. The 2.26 Å co-crystal structure of RIPK2 bound to **RP20** (PDB: 8X2O) revealed key interactions: hydrogen bonding between the pyrimidine NH and Met98 backbone, engagement of the thiazole moiety with Asp164, and water-mediated entropic stabilization through the amide carbonyl and NH linker. Fragment molecular orbital analysis highlighted strong pair interaction energies with Glu105 and Glu66, corroborating **RP20**’s binding affinity and stability within the ATP pocket.

In MDP-induced peritonitis, **RP20** dose-dependently reduced serum IL-6 levels, underscoring *in vivo* RIPK2 inhibition and downstream suppression of NF- κ B signaling. In APAP-induced ALI models, RP20 significantly decreased serum ALT and AST, reduced hepatic necrosis, and attenuated pro-inflammatory cytokines (TNF- α , IL-1 β , and IL-6) at both protein and mRNA levels

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more effectively than GSK2983559 and NAC at equimolar doses. **RP20** restored hepatic GSH levels, suppressed JNK, ERK, and p65 phosphorylation in liver tissue, and maintained high liver concentrations for up to 8 h post-dose, demonstrating liver-targeted distribution and potent anti-apoptotic effects. Under a lethal APAP challenge, **RP20** (20 mg/kg) achieved 100% survival, whereas NAC only delayed mortality, affirming its superior therapeutic index.

This work represents the first demonstration of RIPK2 inhibition as a therapeutic strategy for ALI, broadening RIPK2's potential applications from autoimmune and cancer contexts to acute liver injury. As a small molecule, **RP20** offers superior tissue penetration, scalable synthesis, and minimal immunogenic risk. By leveraging CMD-OPT's transformer-driven scaffold generation and multi-parameter optimization, the authors establish a reproducible pathway for lead discovery without retraining large models or relying on extensive heuristic rules. **RP20**'s exceptional kinase selectivity and encouraging safety outcomes (Safety47 panel results and 28-day rat toxicology) position it for IND-enabling studies and set a benchmark for future RIPK2-focused drug development.

Despite these advances, certain limitations warrant consideration. First, the reliance on GSK2983559 as a starting scaffold may bias the chemical space; exploration of alternative scaffolds or allosteric binding modes could uncover novel RIPK2 inhibitor chemotypes. Second, while **RP20** demonstrates robust efficacy in APAP-mediated ALI, its therapeutic window in other etiologies (*e.g.*, ischemia–reperfusion, viral hepatitis) remains to be validated. Additionally, long-term toxicity and off-target effects, particularly in RIPK2's roles in host defense, require thorough evaluation in chronic dosing paradigms and higher species. Finally, clinical translation will hinge on biomarker development to stratify patients with RIPK2-driven inflammatory signatures, as has been essential for RIPK2 inhibitors in inflammatory bowel disease.

In conclusion, Chen et al.⁷ present a compelling workflow integrating CMD-OPT with medicinal chemistry to deliver **RP20**—a potent, selective, orally bioavailable RIPK2 inhibitor demonstrating unprecedented efficacy in APAP-induced ALI models. Beyond acute hepatoprotection, preliminary data suggest **RP20**'s potential in ulcerative colitis, multiple sclerosis, and oncology, underscoring RIPK2's multifaceted role in inflammation and

tumorigenesis. CMD-OPT's success in optimizing both target engagement and PK properties exemplifies a next-generation AI-enabled lead discovery paradigm. As **RP20** advances toward clinical evaluation, this work lays the groundwork for broader RIPK2-targeted therapeutics, heralding new avenues for addressing inflammatory and immune-mediated diseases.

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