FROM PERK TO RIPK1: DESIGN, SYNTHESIS AND EVALUATION OF NOVEL POTENT AND SELECTIVE NECROPTOSIS INHIBITORS

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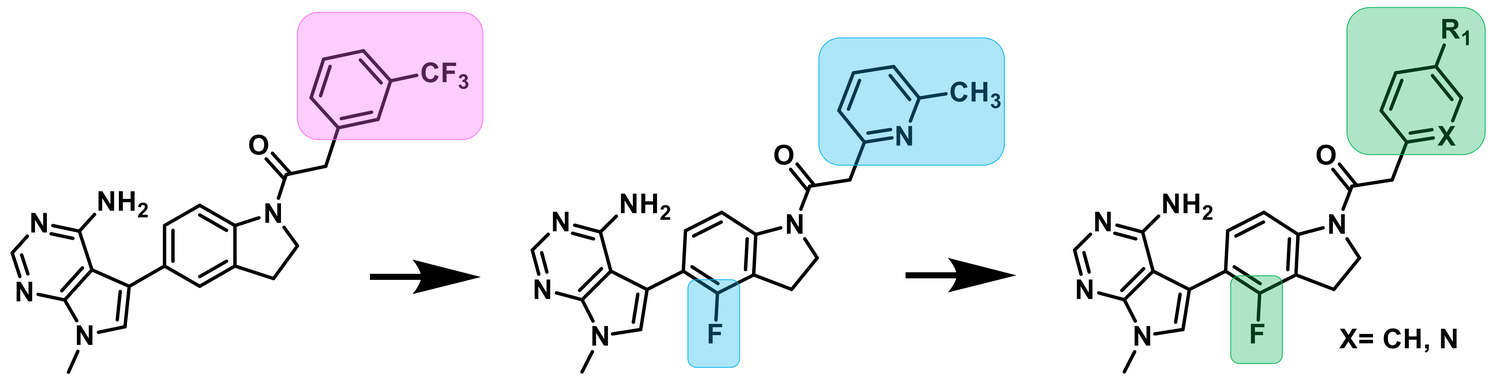
Introduction

Receptor-Interacting serine/threonine-Protein Kinase 1 (RIPK1) emerged as an important driver of inflammation and, consequently, inflammatory pathologies. The enzymatic activity of RIPK1 is known to indirectly promote inflammation by triggering cell death, in the form of apoptosis or necroptosis. RIPK1 inhibitors have therefore recently entered clinical trials for the treatment of a subset of inflammatory pathologies. We previously identified **GSK2656157 (GSK'157)**, a supposedly specific inhibitor of protein kinase R (PKR)-like ER kinase (PERK), as a much more potent type II RIPK1 inhibitor. We now performed further optimisation on the GSK'157 scaffold in order to develop a novel class of selective RIPK1 inhibitors.

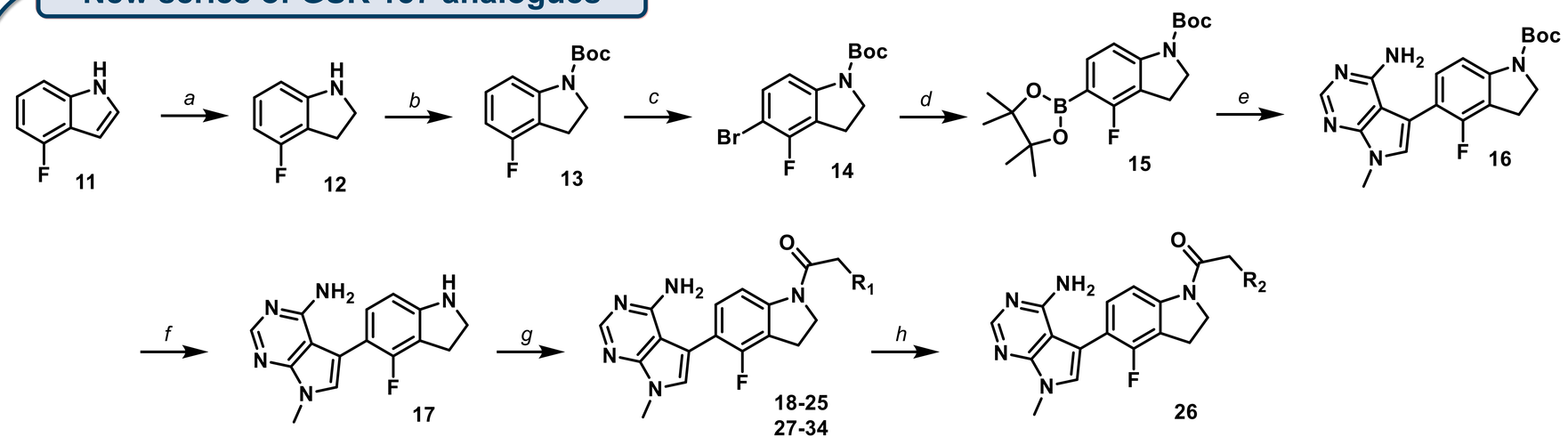
Objective



9 GSK2606414 — **10** GSK2656157 — **Novel analogues**

Based on a structure-activity relationship (SAR) reported in the literature, we anticipated that introducing a substituent on the *para*-position of the pyridinyl ring would decrease the interaction with PERK. Herein, we reported a series of novel GSK'157 analogues with different *para*-substituent with increased selectivity for RIPK1.

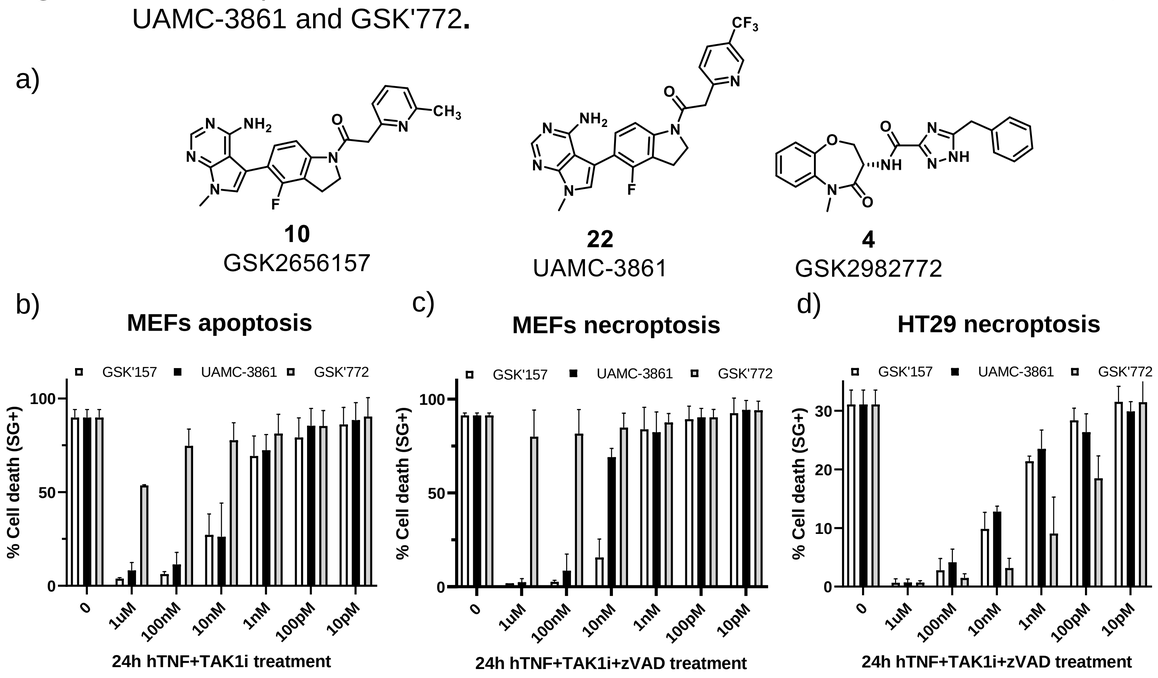
New series of GSK'157 analogues



Scheme 1. Synthesis of novel RIPK1 inhibitors based on GSK'157 synthetic route. Reagents and conditions: (a) 4-fluoro-1H-indole, $NaBH_3CN$, AcOH, 0°C, 10 min, rt, 1 h; (b) Boc_2O, DMAP, DCM, rt, 48 h; (c) NBS, DCM, rt, 1 h; (d) bis(pinacolato)diboron, KOAc, dioxane, water, rt, 5 min (e) 5-Bromo-7-methyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine, t-Bu)$_3$PHBF$_4$, Pd$_2$(dba)$_3$, 90°C, 2 h; (f) HCl in dioxane 4 N, dioxane, rt, 16 h; (g) HATU, corresponding acetic acid, DIPEA, DMF, rt, 16 h; (h), BBr_3, DCM, 0°C, 48 h.

Results

Figure 1. RIPK1-dependent cell death inhibition in mouse and human cells for GSK'157, UAMC-3861 and GSK'772**.**



(**a**) Chemical structures of the compounds that were tested in vitro for RIPK1-depedent apoptosis and necroptosis. (**b** and **c**) MEFs and (**d**) HT29 were pretreated for 30 min with increasing concentrations of the indicated compounds. RIPK1 kinase- dependent apoptosis was induced by TAK1i+hTNF (100 pg/ml) and RIPK1 kinase-dependent necroptosis by zVAD.fmk +TAK1i+hTNF (100 pg/ml) and hTNF+zVAD.fmk (20 ng/ml). Cell death was measured over time by Sytox Green (SG+) positivity, and the results are presented as mean ± SEM of three independent experiments.

Figure 2. Western blot to compare GSK'157 with compound 22 to inhibit PERK (e) and RIPK1 (f).

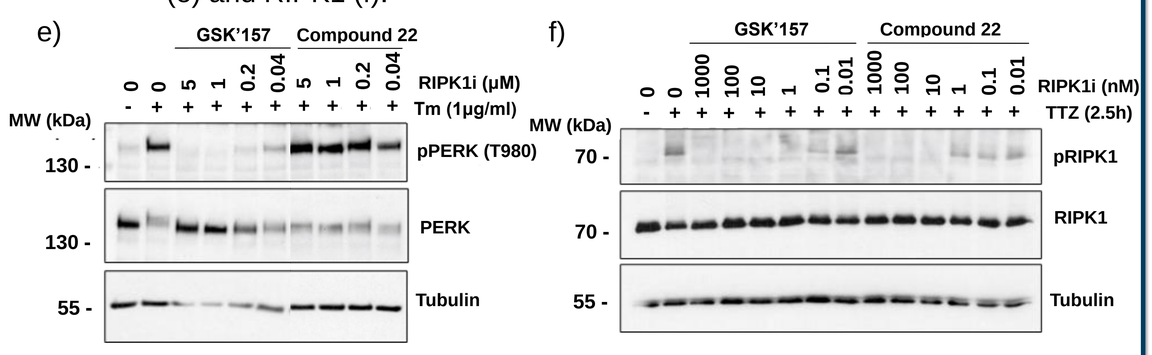
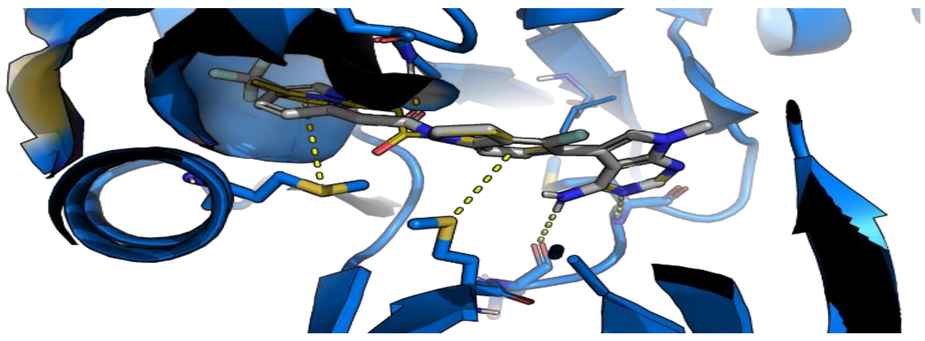
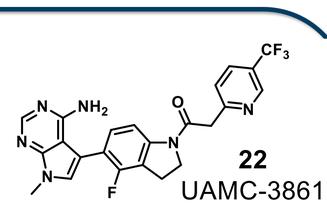


Figure 3. Docking of compound UAMC-3861 (grey sticks) and GSK'157 (yellow lines) in RIPK1.



Conclusions

With this work, we successfully synthesised a novel series of potent and selective type II RIPK1 inhibitors based on the GSK'157 scaffold. The hydrophobic nature of the RIPK1 allosteric pocket accommodating the *para*-substituted pyridine ring was confirmed. To the best of our knowledge, the *para*-fluoro phenyl analogue (**30**) is the most potent necroptosis inhibitor described with an IC_{50} = 0.01 nM. However, since this compound retained some PERK inhibition at higher concentrations, we selected UAMC-3861 (**22**) as the best compound of this series in terms of activity and selectivity for RIPK1 over PERK. This suggest that UAMC-3861 will be an excellent tool compound to study RIPK-1 dependent cell death in mouse models with the potential to translate to human cell lines.



IC_{50} (PERK) > 5000 nM
IC_{50} (RIPK1) = 1 - 10 nM
IC_{50} (necroptosis) = 1.06 nM

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