In silico prioritization of novel lactone warheads for aspartic acid covalent reactivity

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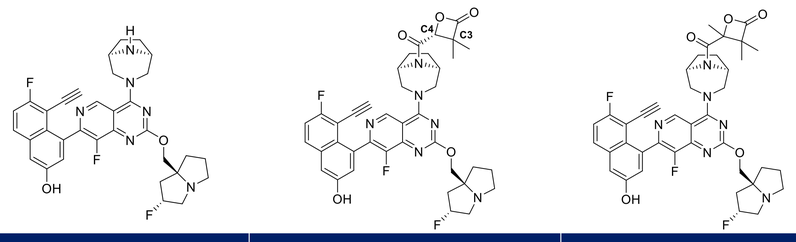
Covalent reactivity between β-lactones and aspartic acid

KRasG12D is a frequent mutation in multiple cancers, including pancreatic ductal adenocarcinoma. Multiple KRasG12D inhibitors have entered clinical trials to meet this high unmet medical need, including MRTX1133,[1] a non-covalent Switch-II pocket binder. MRTX1133 has poor permeability, possessing two basic amines. We sought to build on the finding by Shokat et al,[2] that β-lactones may be a suitable covalent warhead for aspartic acid to understand whether a covalent strategy may remedy the oral profile of MRTX1133:

- Retaining mutant selectivity via covalent reactivity with Asp12 of KRasG12D
- Removing one basic amide via capping with lactone warhead, potentially improving membrane permeability and oral profile
- Balancing on-target reactivity with chemical stability and off-target glutathione attack



Compound ID	MRTX1133	(*R*)-1	2
Covalent modification [%] $KRAS^{G12D}$ GDP / GTP	0 / 0	95 ± 6 / 83 ± 10	0 / 0
Barrier height ΔE_{TS} [kcal/mol]	-	7.70	12.88
pERK SW1990 IC_{50} at 4 h / 24 h [μM]	0.004 ± 0.002 / 0.007 ± 0.005	0.37 ± 0.13 / 0.14 ± 0.08	0.053 ± 0.033 / 0.017 ± 0.009
pERK MKN1 IC_{50} at 4 h / 24 h [μM]	0.38 ± 0.15 / 0.54 ± 0.16	2.56 ± 1.38 / 5.15 ± 2.48	0.061 ± 0.005 / 0.110 ± 0.054
Half-life $t_{1/2}$ in aqueous GSH [min]	>480	9 ± 2	>480
Loss compound [%] in cell media at 4 h / 8 h	n.d.	35 / 60	2 / 6

Table 1 –Structures, covalent reactivity, calculated transition state barrier heights for Asp12 attack, cellular activity and chemical stability in GSH or cell media for MRTX1133, dimethyl β-lactone (*R*)-**1** and with 4-methyl **2**.

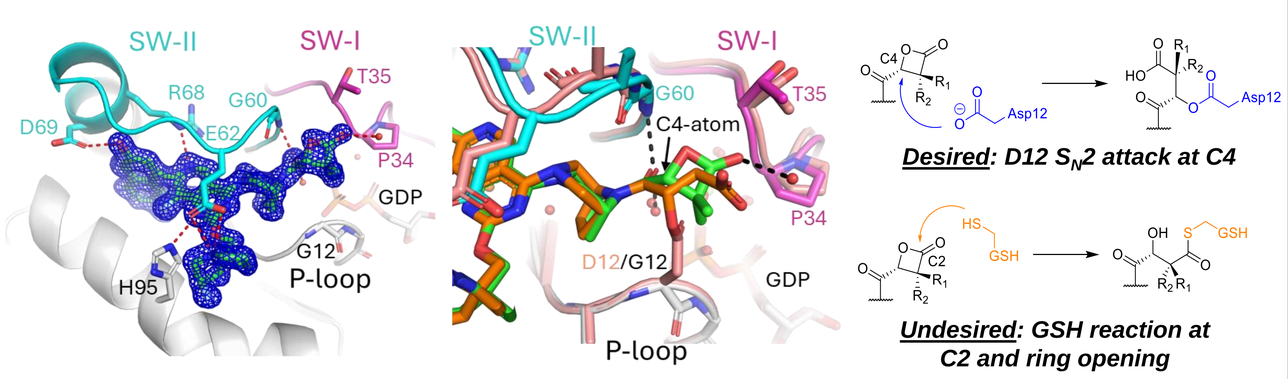
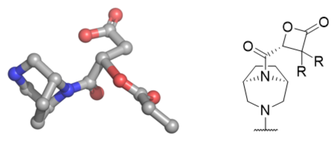


Figure 1 – (*left*) X-ray co-crystal structure of $KRAS^{WT}$/GDP in complex with (*R*)-**1** solved at 1.3 Å resolution (*middle*) x-ray crystal structure of covalent adduct formed between $KRAS^{G12D}$/GDP and (*RS*)-G12Di-**1** [2] at 1.47 Å (*right*) reaction schemes of β-lactone dual reactivity (Asp12 S_N2 attack and glutathione ring opening).

Automated workflow to estimate lactone reactivity

With up to ~30 novel warheads proposed for synthesis per week, it was important to establish an automated and reliable workflow to prioritize lactones predicted to react with Asp12, yet not having too high chemical instability. We used the lactone position from KRasWT x-ray and Asp12 position from covalent adduct x-ray[2] as a starting transition state geometry estimate.

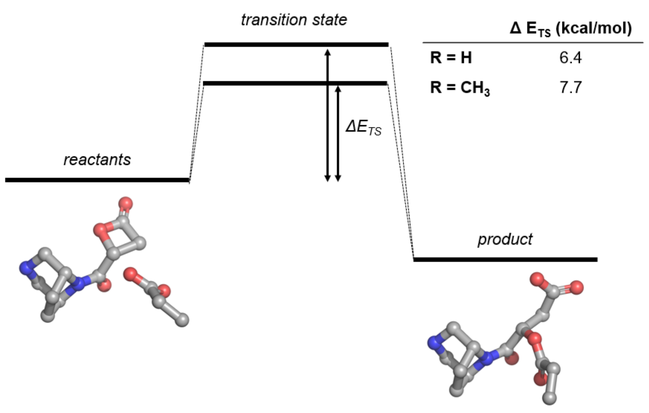


Generate 20 starting geometries (RDKit ConstrainedEmbed)

XTB optimization[3] with dihedral constraints

Progress lowest energy to Gaussian optimization / transition state search[4] (B3LYP/6-31g* PCM water)

Confirm reaction coordinate (imaginary frequency)



$$\Delta E_{TS} = E_{(TS)} - E_{(lactone)} - E_{(aspartic\ acid)} - E_{(proton)}$$

Figure 2 – (*left*) Potential energy diagram for the S_N2 nucleophilic attack of Asp12 at β-lactone C4 position for unsubstituted and di-methyl warheads (*right*) schema of the automatic workflow used to calculate transition state barrier heights for lactone S_N2 attack of Asp12. The workflow was run on the lactone warhead only (reactant energy) and in the presence of Asp12 side-chain chain (transition state).

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Qualitative ranking performance across 45 novel warheads

We analyzed the performance of the transition state barrier heights in predicting the Asp12 reactivity of 45 novel lactone warheads. We find that despite the calculations omitting the pocket environment, the ΔE_{TS} values capture well the unreactive warheads and that a rule-of-thumb of ΔE_{TS} ±1 kcal/mol relative to (*R*)-**1** can be used to prioritize warheads having similar covalent reactivity as (*R*)-**1**.

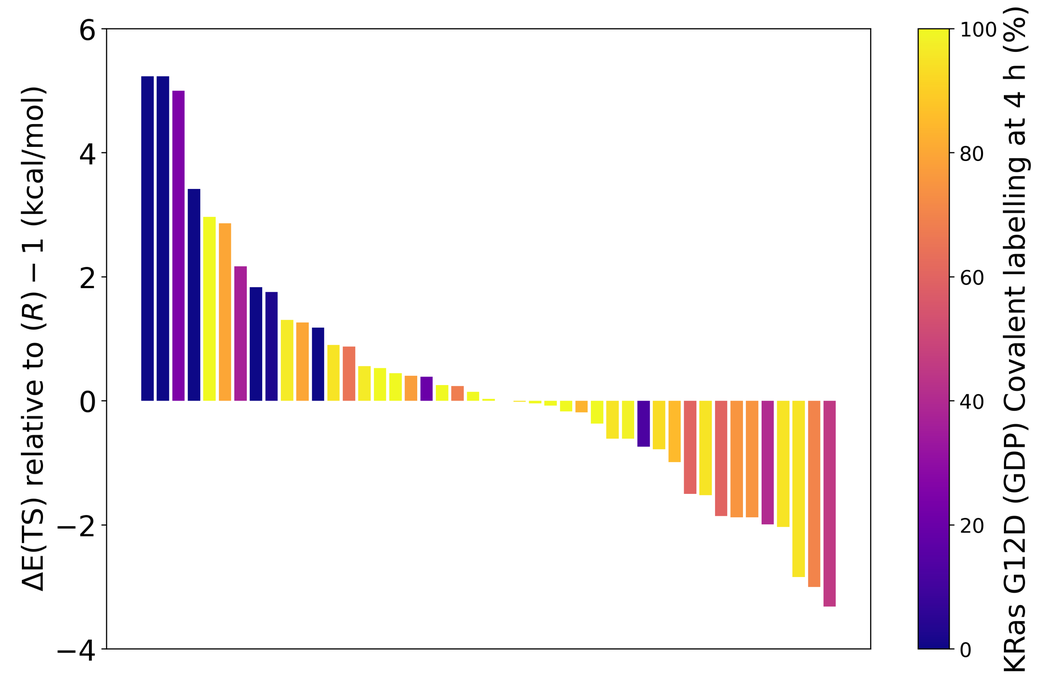
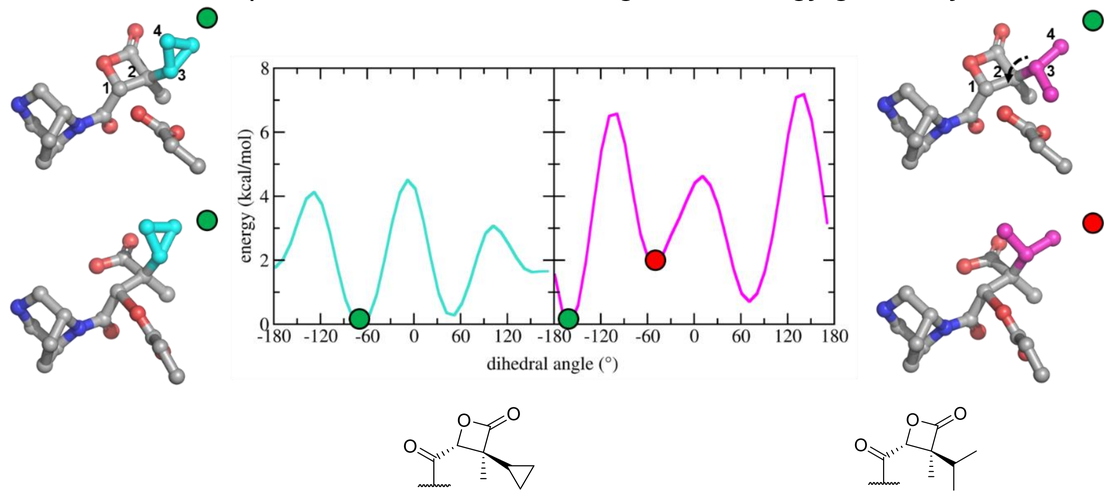


Figure 3 – Transition state barrier heights ΔE_{TS} relative to the dimethyl lactone warhead (*R*)-**1** coloured by $KRas^{G12D}$/GDP covalent labelling at 4 hours as determined by mass spectrometry.

Transition state geometries aid interpretation of SAR

An interesting matched molecular pair are (*3R,4R*)-**10** with cyclopropyl (full labelling of Asp12) and (*3S,4R*)-**6** with isopropyl (<40 % labelling). On inspection of the transition state geometries, we find the isopropyl must rotate from a low energy conformation to allow Asp12 attack, whereas the cyclopropyl can accommodate the Asp12 reaction while retaining a low energy geometry.



Compound ID	(3*R*,4*R*)-10	(3*S*,4*R*)-6
Covalent modification [%] $KRAS^{G12D}$ GDP / GTP	97 / 90	37 / 30
Barrier height ΔE_{TS} [kcal/mol]	7.0	9.8

Figure 3 – (*top*) Quantum mechanical dihedral scans for β-lactone substituents of (*3R,4R*)-**10** and (*3S,4R*)-**6** and transition state geometry positioning along this coordinate (*bottom*) covalent reactivity and ΔE_{TS} barrier heights.

Conclusions

Switch-II pocket inhibitors of KRasG12D may be a viable strategy to target cancers driven by this mutation. However, non-covalent binder MRTX1133 suffers poor permeability. In this work, we explored lactone warheads as a strategy to retain mutant selectivity via covalent targeting of D12 while removing a basic amine, aided by quantum mechanical transition state scans for synthesis prioritization of novel lactones and understanding of SAR. Further, we analyzed descriptors generated by XTB, finding the Fukui indices of carbon C4 trends with covalent reactivity (Pearson R=0.59), yet no descriptors showing a significant trend with off-target GSH instability.

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