Cyclic designer scaffolds for the covalent targeting of proteins

How to target specific proteins at specific locations by exploiting the ”power” of the covalent bond?

Biology–the problem:
Functionally relevant sites are located on proteins at shallow (difficult-to-target) surfaces.

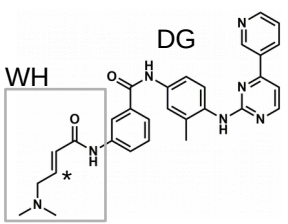
Chemistry–the solution:
α,β-unsaturated ketones react with nucleophilic residues (e.g., cysteines)

Classical composite drugs contain two functional moieties:
Directing group (DG) +
Chemical warhead (WH)

Goal:
To make efficient and safe covalent drugs

State of the art:
Acrylamide-based (open-chain) fragments in composite drugs
Limitations:

- High off-target reactivity
- Limited control on potency (irreversible covalent bond)
- It is a “blunt” tool; specificity must come from the directing group



JNK-IN-8: an example of an acrylamide-based inhibitor; * reactive carbon

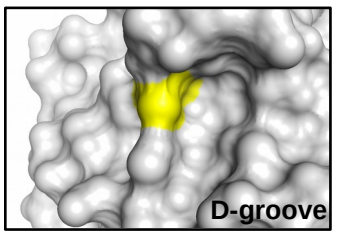
Our solution:
A new cyclic warhead scaffold inspired by biologically active (terpenoid-like) chiral natural products
Advantages:

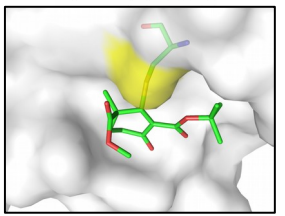
- Resilient to off-target thiols (e.g., GSH)
- Alternative nucleophile choice (e.g., histidine)
- It is a “sophisticated” tool: molecular shape, steric and electronic properties can all be controlled
- Synthetic addition of noncovalent DG at early or late stage

Protenic Kft, Budapest, Hungary
remenyi.attila@protenic.hu
Tel. +36 305680040

Concrete examples:

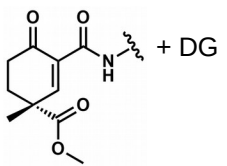
- Targeting the protein-protein interactions (PPI) of mitogen-activated protein kinases (MAPK D-groove)[1]

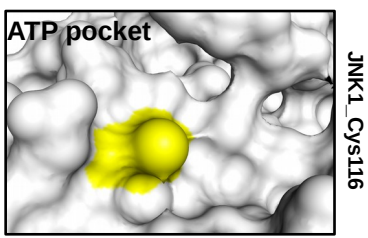


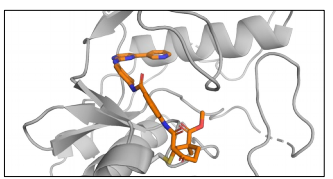


ERK2-adduct

- Targeting specific cysteines next to the ATP-pocket of c-Jun N-terminal kinase (JNK)[2] and other targets



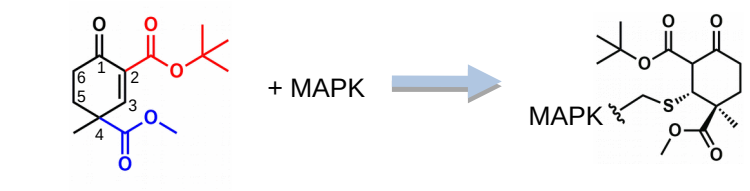




JNK1-adduct

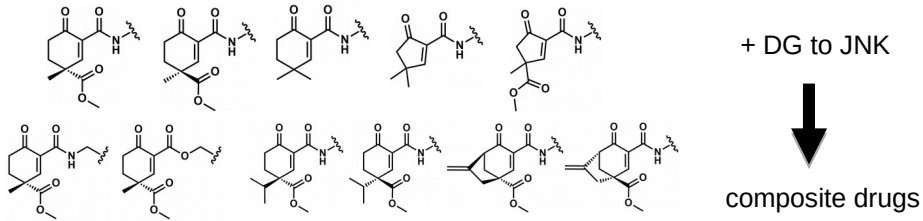
1 Targeting a key protein-protein interaction surface on mitogen-activated protein kinases by a precision-guided warhead scaffold; Póti et al, Nat. Comm. 15. 8607 (2024)
2 Reversible covalent c-Jun N-terminal kinase inhibitors targeting a specific cysteine by precision-guided Michael-acceptor warheads; Bálint et al. Nat. Comm. 15. 8606 (2024)
3 PCT/HU2023/050079: Cyclic designer scaffolds for the covalent targeting of proteins via Michael addition (Europe and US; pending approval)

A sterically crowded, electronically tunable cyclohexenone/pentenone warhead scaffold interferes with Mitogen-Activated Protein Kinase (MAPK)-mediated PPIs[1]



- The electron withdrawing capacity of groups at C2 and C4 modulates binding affinity and make the cysteine adduct reversible due to steric crowding effects.
- Compounds occupy the hydrophobic part of the key MAPK D-groove and thus block protein partner binding to MAPKs.
- Compounds have a stereogenic center through which MAPK-specificity can be modulated.
- Hit compounds were extended by further functionalization of the C4 moiety and these contacted additional PPI surface features.
- New MAPK inhibitors interfere with MAPK-based signaling by directly binding to the MAPK D-groove cysteine and are efficient in perturbing MAPK signaling networks in cells.
- Ester moieties are replaced with alternative substituent groups making the compounds stable (PK studies in rat primary hepatocytes and blood).
- Proteome-wide specificity of the basic designs are explored (~5000 proteins, with native hold-up assay + mass spectrometry).

Demonstration of modularity of the cyclic warhead designs: development of a JNK[2] ATP-pocket binding, cysteine targeting reversible covalent inhibitor



- New reversible covalent inhibitors outperform state-of-the-art irreversible JNK-IN-8 regarding system-level specificity (tested on the human kinome panel; since the cyclic nature of the warhead limits accessibility to the critical carbon).
- New composite JNK inhibitors represent a range of JNK potency in a given cellular setting (which may be beneficial for tuning JNK inhibition; e.g., partial vs complete).
- Residence time can be modulated by tuning the electronic and steric properties around the Michael acceptor carbon atom.
- The reversible covalent inhibitors are resilient to GSH (since the reaction with off-target thiols is very dynamic and has a fast kchem_off).
- Successful replacement of irreversible acrylamide-based warheads with new cyclic warheads showing comparable in vitro potency (starting out from known JNK DGs) but less capacity to bind to ABC transporters (relevance to multidrug resistance).

Biomedical relevance:
- for MAPK PPI interfering drugs: cancer and inflammatory diseases
- for composite inhibitors: new patentable alternatives
- in general: less off-targets and synthetic advantage