Identification and characterization of novel small molecule inhibitors of ubiquitin specific peptidase 1 (USP1)

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BACKGROUND

• Deubiquitinating enzymes (DUBs) are an important group of regulators in the ubiquitin-proteasome system, responsible for clearing ubiquitin from substrate proteins.1

• Ubiquitin-specific peptidase 1 (USP1), in complex with USP1-associated factor 1 (UAF1), deubiquitilates substrates involved in key oncogenic pathways.2

• USP1 regulates the DNA damage response by deubiquitilating FANCD2, FANCL, and PCNA, which are major modulators of interstrand crosslink repair and translesion synthesis.3

• In addition, USP1 sustains cancer cell stemness by increasing the stability of ID proteins through deubiquitilation.4

• Previously, inhibition of USP1 has been suggested to be used to increase the effect of DNA damaging drugs on cell viability.5

• As part of Medivir’s DUB drug discovery efforts, we have evaluated the tractability and the feasibility of the USP1/UAF1 complex as a drug target using the Medivir DUB platform, and initiated in-house hit and lead finding activities to identify novel chemical entities for inhibition of this target.

CHARACTERIZATION OF PUBLISHED USP1 INHIBITORS

• Initially, we characterized a set of published USP1 inhibitors in order to identify tool compounds for biological assays, and to evaluate their selectivity as starting points for hit and lead finding activity.6 7 8 9

• Reported USP1 inhibitors such as pimozide, GW67647 and trifluoperazine are shown to be inactive in the K48-deubiquitin cleavage assay.

• While displaying good permeability, M132 and S83-015A are either non-selective, or characterized by low solubility or poor metabolic stability.

EVALUATED LITERATURE COMPOUNDS

- In order to identify a starting point for a chemistry program, we constructed a target-focused library using the BioAssay Compound Cloud.10

- Compounds with putative reactive motifs, unfavorable functional groups or low predicted solubility were filtered out.

- The selection of compounds for inclusion was subsequently based on three different approaches:

  • In silico docking to published DUB structures with the PDB entry 3NHE for USP2 and 2A40 for USP4.11

  • Structural similarity to known DUB inhibitors using the Similarity Ensemble Approach (SEA)™ or a Topological Pharmacophore Search (TPS).12

  • Privileged anchor fragments (PAFs)™, a target independent chemoinformatic approach to enrich bioactive motifs.

- The library of 4500 compounds was screened at 50µM, against a number of in-house DUB targets as well as against MALT-1, a non-DUB cysteine protease.

- 43 preliminary hits (inhibition >50%) were identified in the K48-deubiquitin cleavage assay used for USP1/UAF1.

- The preliminary hits displayed good selectivity over the unrelated cysteine protease HCL-1.

- The preliminary hits were evaluated with a concentration response curve. The majority of the hits could be confirmed and the USP1 IC50 values were in good agreement with the results of the single point screen.

- From the distribution, we could see that the majority of hits were from the Docking or Privileged Anchor inclusion criteria. The most active compounds though, were included based on both the TPS and SEA.

- The most interesting compounds were counter-screened in other USP assays available internally, which showed that the tested compounds were non-selective inhibitors.

IDENTIFICATION OF IMPROVED USP1 INHIBITORS

• In house elaboration led to the identification of multiple compounds with substantially improved biological and physicochemical properties.

• The representative compounds A, B and C showed very good selectivity over a set of established DUBs.

• To further characterize the compounds, we subbed them a selectivity profiling (Ubiquitin DUBProfiler™). The compounds were active against USP1/UAF1 but showed no significant inhibition in a single point screen against 26 different DUBs (data not shown).

• Furthermore, the compounds have high permeability and good solubility in a kinetic solubility assay in phosphate buffered saline (PBS) at pH 7.4.

• Intrinsic clearance in human liver microsomes (HLM) is high and is the current focus of ongoing compound optimization.

SCREENING OF A DUB-TARGETED LIBRARY

• We have described the design and evaluation of a DUB targeted library that rendered µM-potency hits that were suitable for further elaboration.

• Optimization resulted in potent inhibitors of USP1 with excellent selectivity profiles and promising in vivo pharmacokinetics.

• USP2 were confirmed to specific binding to a catalytically active USP1 construct and allowed evaluation of compound binding kinetics.

• The compounds are active in cell-based assays of USP1 function, with potencies correlating with enzyme data, as well as in a viability screen against a panel of cancer cell lines.

• These potent and selective inhibitors are currently undergoing in vivo evaluation in cancer disease models.

METHODS

- USP1/UAF1 (Boston Biochem) was assayed using DUB488-B FITC substrate (Life Sciences). Ubiquitin-proteasome activated USP14 (produced in house) and USP7 (Boston Biochem) were assayed using ubiquitin-methionine 110 (Life Sciences) as substrate. USP14 (produced in house) and USP7 (Life Sciences) were assayed using DUB488-B FITC substrate (Life Sciences).

- A2780 cells were treated with compound B or C for 24 h. The resulting plates were analyzed by Western Blot using antibodies against PCNA (Santa Cruz) and GAPDH (Evan).14

- After treating MOLT-4 cells with compounds for 5 days, cell viability was measured by XTT.

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