Synthesis and biochemical evaluation of wild-type and mutant p53 oxazoloisoindolinones reactivators derived from tryptophanol

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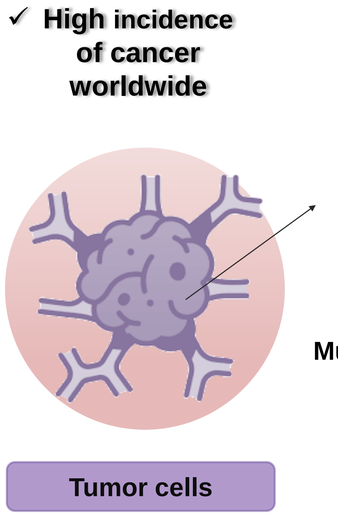
State of the art

✓ **High incidence of cancer worldwide**



Discussion

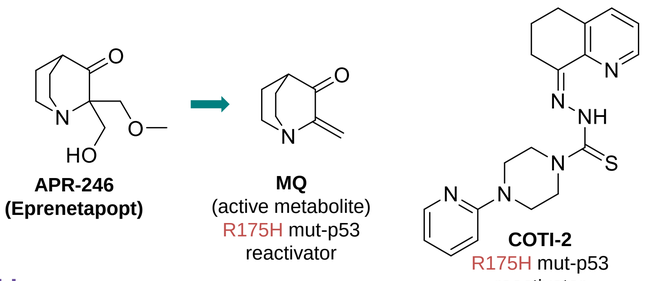
Drug discovery for selected *mut*- p53 with clinical relevance **(R273H and R280K)**

TP53 mutations

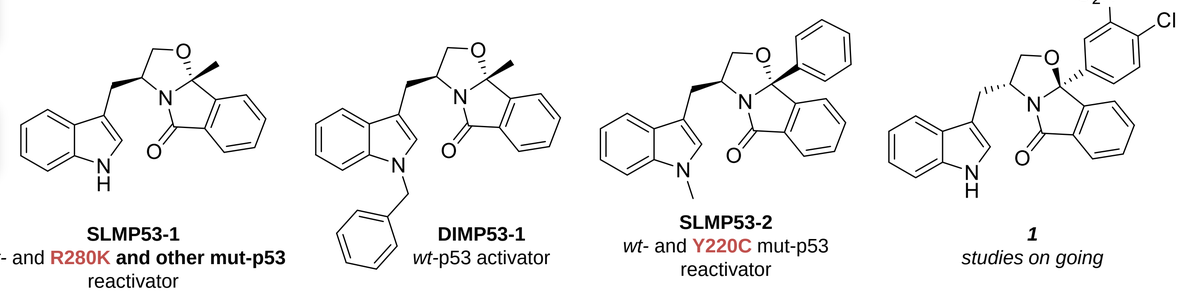
DNA contact mutants (i.e. R248Q, R273H, and R280K)

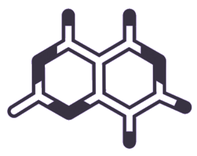
Conformational mutants (i.e.Y220C, R249S, and R282W)

Small molecules in clinical trials:



p53 reactivators previously reported by our group:

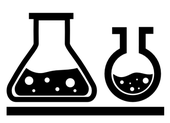




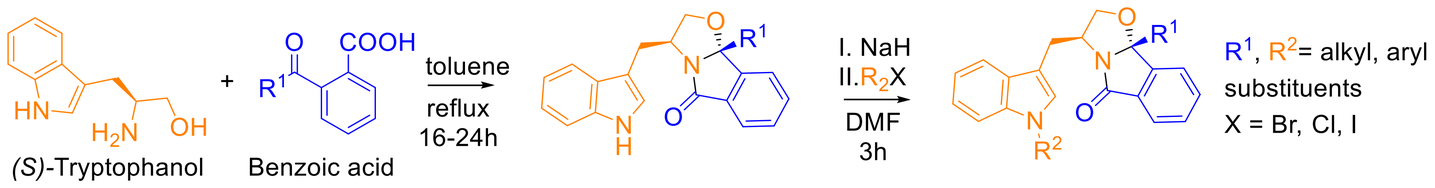
Most mut-p53 reactivators are not acceptable for clinical use due to unpleasant toxic side effects and/or pharmacodynamics properties [1,2].

Development of novel molecules with higher selectivity and lower disadvantageous toxic effects

• SYNTHESIS



✓ **Enantioselective cyclocondensation of *(S)*- or *(R)*- Tryptophanol with adequate benzoic acids [3]**



The respective (*R*)-enantiomers were also synthesized

✓ Tryptophanol-derived oxazoloisoindolinones were obtained in moderate to good yields (48-82%)

✓ Stereochemistry maintenance

✓ All *N*-indole protected tryptophanol-derived oxazoloisoindolinones were obtained in good yields (68-96%)

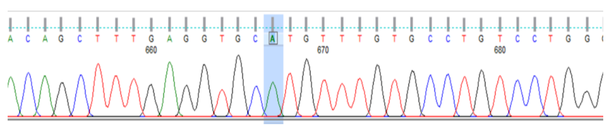
• BINDING TO p53

Protein expression and purification

Tumor supressor p53

Wt-p53

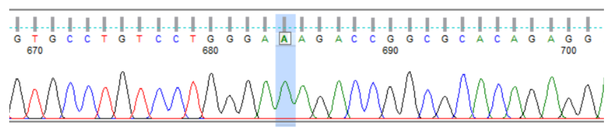
R273H mut-p53



Normal sequence: gc ttt gag gtg cgt gtt tgt gcc
Primer F sequence: gc ttt gag gtg cat gtt tgt gcc

✓ Both mutations were correctly introduced
Substitution of G by A

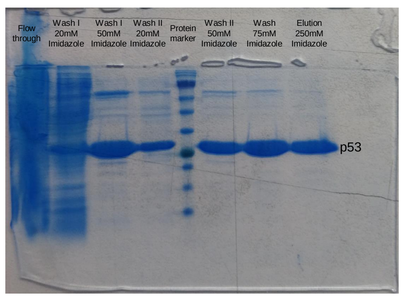
R280K mut-p53



Normal sequence: gcc tgt cct ggg aga gac cgg cgc
Primer F sequence: gcc tgt cct ggg aaa gac cgg cgc

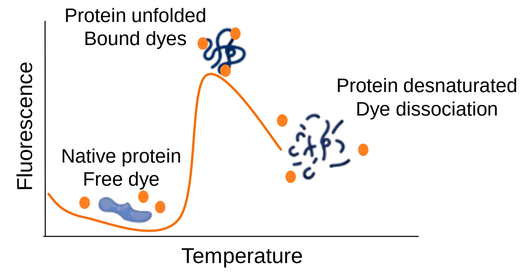
mut-p53 expression and purification

Immobilized Metal Affinity Chromatography



From 500mL of culture (after purification) 490,6484 µg/mL of p53 was obtained in a volume of 5mL

Screening of the compounds **Differential Scanning Fluorimetry (DSF) biochemical assay**



Change in p53 Tm indicates protein binding

Conclusions

1. Development of novel/optimized p53 reactivators is on going with the objective of tackle both *wt*- and *mut*- tumor suppressor p53
2. The entire library will be screened by DSF assay in wt-p53 and both mut-p53 (R273H and R280K)

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3. Barcherini V, Almeida J, Lopes EA, Wang M, Magalhães e Silva D, Mori M, Wang S, Saraiva L, Santos MMM. ChemMedChem, **2021**, 16, 250.