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Peptide-Guided Design of Mdm2/MdmX Inhibitors with Anticancer Activity

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Introduction

The *N*-terminal domains of MdmX or Mdm2 are highly homologous and have similar conformation (Figure 1a & b). There are three binding pockets for a p53 binding peptide (p53p), defined as F19, W23 and L26 on their surfaces. Current Mdm2 inhibitors including nutlin-3a exhibit weak binding affinity to MdmX. Nutlin-3a (Figure 1c) mimics p53p with high binding affinity to Mdm2 (K_d ~ 20 nM) but very weak to MdmX (K_d ~ 29 μM). The L26 pocket significantly differentiates the binding affinities of nutlin-3a between Mdm2 and MdmX, but detailed mechanism remains unclear.

Results and Discussion

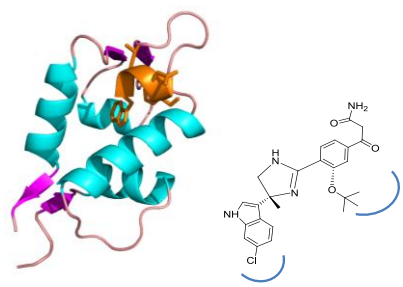
Drug Target Model: A short p53p peptide (p53p^{short}, Figure 2a) can occupy the F19 and W23 binding pockets on MdmX as well as Mdm2. A small molecule compound, HG201 (Figure 2b) adopted from current Mdm2/MdmX inhibitors, mimicking p53p^{short} binding behavior, is used as a positive control.

High-Throughput Screening (HTS) of CTM Compound Library: The Cys76 residues on Mdm2 and MdmX are labeled with fluorescence probe, ANS, respectively.The protein-peptide complexes of MdmX-ANS/ p53p^{short} and Mdm2-ANS/ p53p^{short} are used as targets for HTS.

A natural compound library containing 2400 Chinese Traditional Medicinal Compounds (CTMs) are screened for leads. The compound concentration for screening was set at 20 μM, 5 μM, 1 μM, 0.5 μM and 0.3 μM, respectively.

Identification of Leads: The compound hits are ranked by FRET attenuation between the Trp23 of the p53p^{short} and the labelled-ANS on protein (Figure 3 Left). More than 15 hits were selected from screening at 20 μM, 5 μM, 1 μM and 0.5 μM of each compound (Figure 3 Middle). The three most potent leads selected at above 0.5 μM and defined as HS201, HS202 and HS203 (Figure 3 Right), exhibited significant inhibition to the MdmX-p53 interaction.

Future Work: Resulting scaffold will be used to build a secondary model for searching target-specific functional group



a *b*

Fig. 2. (a). A protein target model is formed by a N-terminal domain in complex with a short p53p peptide. (b) A small molecule compound mimicking p53p^{short} is used as a positive control.

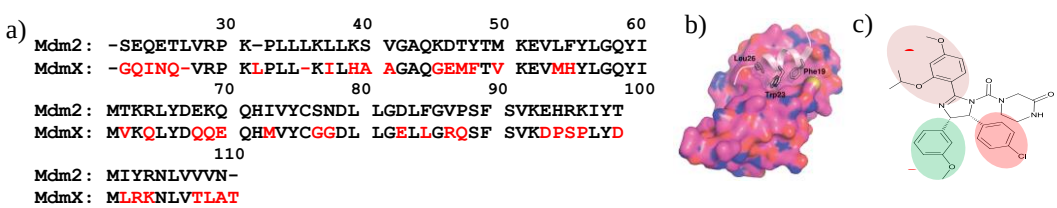


Fig. 1. (a) Alignment of N-terminal domains of Mdm2 and MdmX; (b) Three key residues, Phe18 (L19), Trp23(W23) and Leu29(L29), contribute to the high affinity of p53 peptide (p53p) binding to the domains; (c) A Mdm2 inhibitor, nutlin-3a, mimics p53p.

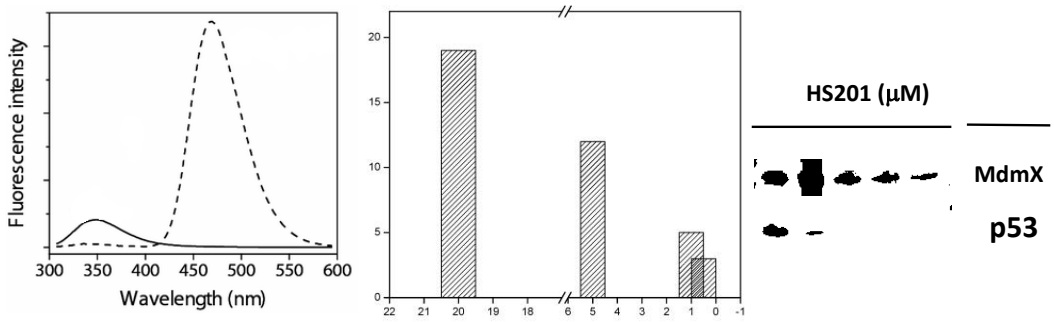


Fig. 3. Summary of HTS Hits. (Left) FRET happens between the Trp23 of p53p and the labelled-ANS on protein. (Middle) Numbers of hits decrease with decreasing threshold values; (Right) One of the three leads exhibits significant inhibition to the MdmX-p53 interaction, evaluated with western blot analysis using GST-MdmX pull-down experiment.

by screening virtual fragment library. Potent Mdm2-specific, MdmX-specific and dual specific inhibitors will be generated through fusing target-specific functional group to the scaffold. New inhibitors will be evaluated for their anticancer activities with cancer cell models.

Acknowledgments

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