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# AM-8553: A Novel MDM2 Inhibitor with a Promising Outlook for Potential Clinical Development

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p53 is a tumor suppressor and plays a key role in preventing tumor development.[1] In p53 wild-type tumor cells, its tumor suppressor function is effectively inhibited by the human murine double minute 2 (MDM2) protein.[2] MDM2 inhibits p53 activity through multiple mechanisms, all mediated through its direct interaction with p53.[2] The crystal structure of MDM2 complexed with p53 shows that its interaction is mediated by a well-defined surface pocket in MDM2 and three key hydrophobic residues (Phe19, Trp23, and Leu26) in p53 (Figure 1),[3]

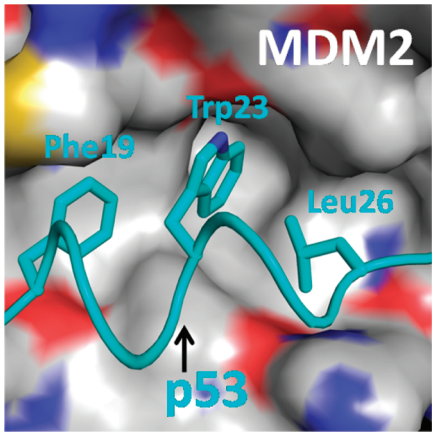


**Figure 1.** Key p53 residues (sticks) interacting with MDM2 (surface). Figure was generated with Pymol based upon the crystal structure of p53 complexed with MDM2 (PDB code 1YCR).[3]

representing an attractive site for the design of non-peptide, small-molecule inhibitors (MDM2 inhibitors). Inhibitors blocking the MDM2–p53 interaction may have therapeutic potential for cancer treatment through reactivation of the powerful tumor suppressor function of p53.[4] Although targeting protein–protein interactions in general has been difficult, several classes of potent and orally bioavailable MDM2 inhibitors have been successfully designed,[4,5] and three such compounds are now in phase I clinical development for cancer treatment.[6]

In the study reported by Rew et al. from Amgen, a new class of potent MDM2–p53 inhibitors bearing a piperidinone scaffold has been successfully designed and optimized using a structure-based de novo design strategy.[6] On the basis of the analysis of known MDM2 inhibitors, which mimic the key p53 binding residues, racemic tetrasubstituted piperidinone **1** was designed (Figure 2), whose $IC_{50}$ was determined to be 2.42 $\mu$M in a competitive MDM2 binding assay. Changing the stereochemistry of one aryl and the C3 acetic acid substituent in **1** and resolution of the active enantiomer yielded compound

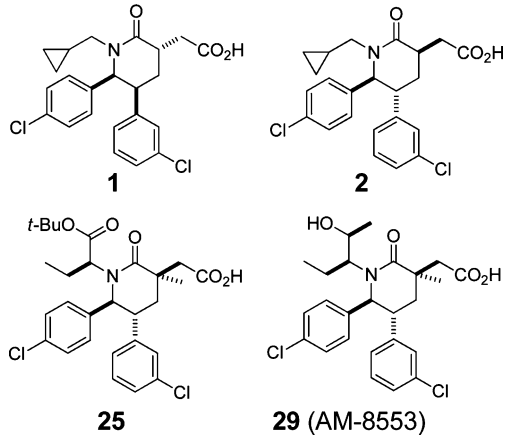


**Figure 2.** New class of MDM2–p53 interaction inhibitors reported by Rew et al.[6]

**2**, whose affinity to MDM2 is 50- to 70-fold better than **1**. Assisted by computer modeling, the *N*-alkyl substituent in **2** was optimized for its interaction with the Phe19 pocket in MDM2. Substitution at C3 with a methyl group led to stabilization of the desired gauche conformation of the designed molecules. These modifications resulted in the discovery of **25**, which has an $IC_{50}$ of 2.2 nM in the same competitive MDM2 binding assay. On the basis of the cocrystal structure of MDM2 with a closely related analogue of **25**, modifications were made on its *tert*-butyl ester group, which yielded **29** (AM-8553). AM-8553 has better binding affinity to MDM2 and cellular activity than **25**. In the surface plasmon resonance spectroscopy binding assay, AM-8553 has a $K_D$ of 0.4 nM. The cocrystal structure of AM-8553 with MDM2 confirmed that the compound not only successfully mimics the three key p53 binding residues but also captures an additional charge–charge interaction with His96 residue of MDM2. The p53-dependent activity of **29** was clearly established using HCT-116 $p53^{wt}$ and $p53^{-/-}$ isogenic cells in both p21 induction and cell proliferation assays. AM-8553 has an oral bioavailability of 100% in rats and 12% in mice. AM-8553 is effective in inhibition of tumor growth in a dose-dependent manner and is capable of achieving partial tumor regression ($R$ = 27%) against SJSA-1 xenograft tumors in mice at 200 mg/kg once daily dosing. On the basis of its low human hepatocyte intrinsic clearance, AM-8553 was projected to have a long human half-life (>12 h).

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Several critical issues need to be addressed before the clinical development of AM-8553 begins. AM-8553 was well tolerated in mice, but additional studies are needed to evaluate its on-target and off-target toxicities in rodents and non-rodents. Although AM-8553 has a very high affinity to MDM2 and potent cellular activity, it fails to achieve complete tumor regression in the SJSA-1 xenograft tumor model, which has an amplified *MDM2* gene and is highly sensitive to MDM2 inhibitors. This may be due to the fact that AM-8553 has only a modest oral bioavailability ($F$ = 12%) in mice. Furthermore, additional studies with a panel of tumor models with wild-type p53 will be useful to further assess the therapeutic potential of AM-8553 and to develop predictive biomarker(s). Finally, although AM-8553 activates p53 in vitro and in vivo and has a clear on-target activity in vitro, its p53-dependent in vivo antitumor activity has not been established. At 200 mg/kg dose, the drug could achieve high concentrations in tumor tissues and its in vivo antitumor activity may be a combination of both on-target and off-target effects. Evaluation of AM-8553 in p53-mutated or deleted tumor models in vivo should be carried out to establish its p53-dependence in vivo and to provide further insights into its molecular mechanism of action.

The study by Rew et al.[6] is a paragon for a structure-based approach for the successful design and optimization of a completely new class of small-molecule inhibitors of the MDM2−p53 interaction. The optimized MDM2 inhibitor, AM-8553, has a promising outlook for potential clinical development.

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