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Discovery of RG7112: A Small-Molecule MDM2 Inhibitor in Clinical Development

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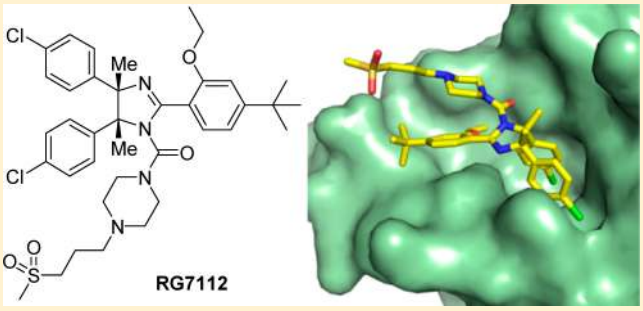
Supporting Information

ABSTRACT: The p53 tumor suppressor is a potent transcription factor that plays a key role in the regulation of cellular responses to stress. It is controlled by its negative regulator MDM2, which binds directly to p53 and inhibits its transcriptional activity. MDM2 also targets p53 for degradation by the proteasome. Many tumors produce high levels of MDM2, thereby impairing p53 function. Restoration of p53 activity by inhibiting the p53-MDM2 interaction may represent a novel approach to cancer treatment. RG7112 (**2g**) is the first clinical small-molecule MDM2 inhibitor designed to occupy the p53-binding pocket of MDM2. In cancer cells expressing wild-type p53, RG7112 stabilizes p53 and activates the p53 pathway, leading to cell cycle arrest, apoptosis, and inhibition or regression of human tumor xenografts.



KEYWORDS: *MDM2, p53, RG7112, protein−protein interaction, cancer*

p53 is a potent tumor suppressor that activates the transcription of a subset of genes controlling cell-cycle progression and apoptosis.[1−3] Dysregulation of the p53 pathway, including mutation or deletion of the p53 gene and changes in downstream signaling molecules, is the most frequent alteration in human cancers.[4] MDM2 is a negative regulator of p53 that binds the transactivation domain of p53 and inhibits its ability to activate transcription.[5−8] MDM2 is also an E3 ubiquitin ligase that targets p53 for proteosomal degradation.[9] In a variety of solid tumors and hematologic malignancies, MDM2 overexpression is one of the mechanisms by which the wild-type p53 function is impaired.[10] Given the central role of MDM2 in regulating p53 activity and stability, developing small-molecule inhibitors of MDM2 could offer a novel approach to treating cancers.[11,12]

The crystal structure of a p53-derived peptide bound to the p53 binding domain of MDM2 revealed the existence of a deep hydrophobic clef on the surface of the MDM2 molecule.[13] Three amino acid residues from the p53 peptide (Phe19, Trp23, and Leu26) play critical roles in the binding between the two proteins by projecting hydrophobic side-chains deep into the cavity of the MDM2 molecule. These structural features of the p53-MDM2 complex suggested the likelihood of identifying small-molecule inhibitors that can successfully block the interaction between the two proteins. Compounds with the ability to inhibit the binding between p53 and MDM2 have been reported.[14−17] We previously reported the discovery of a series of 4,5-dihydroimidazolines called Nutlins. These compounds, exemplified by compound **1** (Figure 1), were discovered through screening and subsequent medicinal chemistry optimization.[18] Compound **1**, also known as Nutlin-3a, has become a tool of choice to study p53 biology and therapeutic applications.[19] Although these early lead compounds have shown good cellular activity and provided the mechanistic proof-of-concept for inhibiting p53-MDM2 interaction for cancer therapy, their pharmacological properties were suboptimal for clinical development. Here, we describe

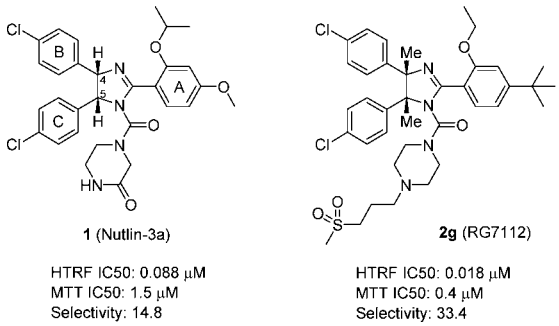


Figure 1. Early lead compound Nutlin-3a and RG7112.

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the optimization efforts that led to the discovery of a new member of the Nutlin family of MDM2 inhibitors, RG7112 (**2g**), which is currently being evaluated in human clinical trials.

As a strategy to optimize the original Nutlin compounds, we chose to preserve the most important structural features needed for binding to MDM2, while exploring the effects of alterations at other sites in the molecules. The crystal structure of Nutlin-3a bound to MDM2 shows that both 4-chlorophenyl groups (ring B and C) perfectly fill the Leu26 and Trp23 pockets, while the isopropoxy group from ring A reaches deep into the Phe19 pocket. These three important elements were maintained in our analogue design. To prevent the oxidation of the imidazoline core to imidazole (inactive against MDM2), methyl groups were substituted at the 4 and 5 positions. In vitro metabolism studies of Nutlin-3 identified the 4-methoxy group of ring A as the most labile site, with the resulting phenol as the major metabolite. Thus, replacement of the 4-methoxy group with a *tert*-butyl group was explored.

On the basis of these considerations, compounds with the general structure of **2** were explored (Figure 2). To reduce

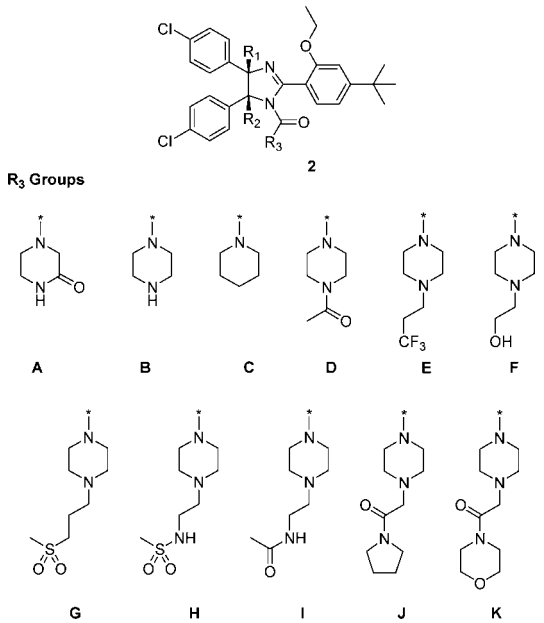


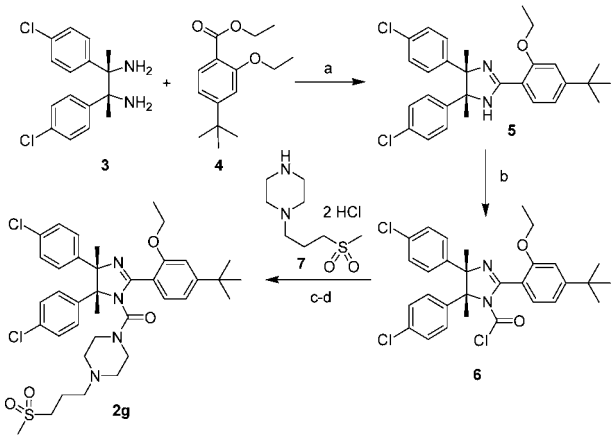
Figure 2. New imidazoline analogues.

molecular weight, we chose the ethoxy group on ring A instead of the isopropoxy group since it is also comparable to isopropoxy as seen in the crystal structure of Nutlin-2 with MDM2.[18] Since R_3 groups project toward the solvent, we used a variety of polar groups to explore their effects on binding and pharmacokinetic (PK) properties.

The synthesis of these compounds is exemplified by compound **2g**, as shown in Scheme 1. The diamine **3**[20] was reacted with the benzoate ester **4** using trimethylaluminum, conditions previously reported by Neef et al.,[21] to give the imidazoline core **5**. The racemic compound **2g** was then obtained by phosgenation of **5** and coupling of the resulting carbamoyl chloride intermediate **6** with the piperazine **7**. The enantiomers of **2g** were then separated by chiral chromatography. Representative compounds in this series and their biological data are reported in Table 1.

To assess the potency of the new analogues as inhibitors of the p53-MDM2 interaction, we used a homogeneous time-

Scheme 1. Synthesis of RG7112 (2g)[a]



[a]Reagents and conditions: (a) $AlMe_3$, toluene, reflux; (b) phosgene, triethylamine; (c) compound **7**, triethylamine; (d) chiral separation

Table 1. Biological Data of the Active Enantiomers of 2a−m

ID	R_1	R_2	R_3	HTRF IC_{50} (μM)	MTT IC_{50} (μM)[a]	selectivity[b]
2a	Me	Me	**A**	0.052	15.9	>1.9
2b	Me	Me	**B**	0.030	0.8	4.5
2c	Me	Me	**C**	0.209	8.3	2.9
2d	Me	Me	**D**	0.023	0.5	35.1
2e	Me	Me	**E**	0.022	1.4	11.9
2f	Me	Me	**F**	0.026	0.4	26.2
2g	Me	Me	**G**	0.018	0.4	33.4
2h	Me	Me	**H**	0.046	0.5	18.1
2i	Me	Me	**I**	0.033	0.3	35.2
2j	Me	Me	**J**	0.014	0.5	23.5
2k	Me	Me	**K**	0.018	0.5	34.4
2l	Et	Et	**G**	2.163	13.1	2.0
2m	Me	H	**G**	0.232	13.4	>2.2

[a]Average IC_{50} in the wild-type p53 cell lines. [b]Ratio of average IC_{50} against mutant p53 cell lines and average IC_{50} versus wild-type p53 cell lines.

resolved fluorescence (HTRF) assay that utilizes the N-terminal domain of recombinant human MDM2 protein and a peptide derived from the binding site of p53.[22] With an exception of compound **2c**, the dimethyl substituted compounds were found to be very potent MDM2 binders, with IC_{50} ranging from 0.014 to 0.052 μM (Table 1). Both methyl groups at the 4 and 5 positions of the imidazoline ring are well-tolerated and prevent oxidation to the corresponding imidazole. As previously discussed, these compounds are stereospecific in their binding to MDM2.[18] The absolute configuration is critical for potency, as the active enantiomer of **2g** (4*S*,5*R*) was about 200-fold more potent than the other enantiomer (4*R*,5*S*) under the same binding assay conditions.

As seen in the poor activity with compounds **2l** and **2m**, substitution with both methyl groups turned out to be optimal. Either bulkier groups such as ethyl or only one methyl group at the 4 position resulted in reduction of MDM2 affinity.

The crystal structure of compound **2g** (PDB code: 4IPF) bound to MDM2[22] showed that it binds in the similar manner as first described for Nutlin-2.[18] Namely, the 4-chloro-phenyl rings occupy the Trp23 and Leu26 pockets, while the ethoxy group projects into the Phe19 pocket (Figure 3). An overlay of the compound **2g** structure with that of compound **1** (Figure

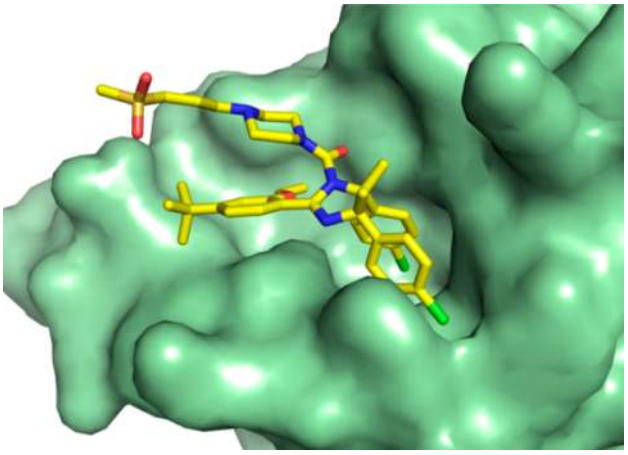


Figure 3. Crystal structure of MDM2 bound to compound **2g** (carbon atoms drawn in yellow, nitrogen in blue, oxygen in red, chlorine in green, and sulfur in orange). PDB code: 4IPF.

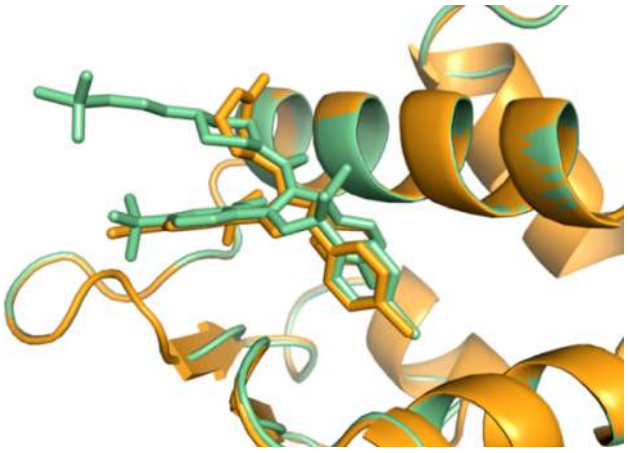


Figure 4. Overlay of the crystal structures of MDM2 bound to compound **2g** (green) and compound **1** (Nutlin-3a, gold; PDB code: 4J3E).

4) shows that the dimethyl substitution has not distorted the imidazoline ring or the projection of both 4-chlorophenyl groups in any significant way. This is consistent with the observation that the additional steric bulk has not sacrificed binding.

We then tested these MDM2 inhibitors for their effect on the growth and viability of cultured cancer cells. We used five cell lines, three of which expressed wild-type (HCT-116, SJSA-1, and RKO) and two with mutant p53 (MDA-MB-435 and SW480). They were incubated with compounds for 5 days, and cell viability was measured with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[22] Surprisingly, the potency of compound **2a** in the HTRF assay was not translated in the cellular MTT assay, while most compounds followed the same potency trend. Many of these were more potent than Nutlin-3a (**1**), with $IC_{50} \approx 0.5$ μM. Compounds **2d−k** all have selectivity (defined as a ratio between the average IC_{50} for two mutant p53 and that for three wild-type p53 lines) greater than 10. We focused on compounds with the highest overall selectivity for further optimization. Compound **2g** has been profiled extensively in many cell lines. In 15 cancer cell lines expressing wild-type p53, it showed IC_{50} in the range of 0.18−2.2 μM. However, the inhibition was much less in seven cancer cell lines with p53 mutation, IC_{50} 5.7−20.3 μM. The overall selectivity between the panels of seven mutant and 15 wild-type p53 lines, expressed as fold difference in the average IC_{50} values, was 14-fold.[22]

Single-dose mouse PK studies were used to assess exposures in blood (Table 2). After oral administration of a 50 mg/kg dose, compound **2g** exhibited the best exposure (AUC_{last} = 251.2 μg·h/mL; C_{max} = 15.5 μg/mL). The C_{max} values for compounds **2d**, **2g**, and **2i** were similar at 19.6, 15.5, and 12.0 μg/mL, respectively. However, the apparent half-life of compound **2g** was longer ($t_{1/2}$ = 8.8 h), resulting in a higher AUC. Compound **2g** was also superior to Nutlin-3a. At twice the dose (100 mg/kg), the exposure of Nutlin-3a is about 4-fold less (AUC_{last} = 65.0 μg·h/mL; C_{max} = 12.1 μg/mL; $t_{1/2}$ = 2.6 h).

Table 2. Mouse PK Parameters of Selected Compounds Following Single Oral Dosing[a]

ID	dose (mg/kg)	AUC_{last} (μg·h/mL)	C_{max} (μg/mL)	$t_{1/2}$ (h)
2d	50	64.9	19.6	2.4
2g	50	251.2	15.5	8.8
2i	50	136.5	12.0	2.3
2j	50	8.3	2.3	4.7

[a]Suspension formulation: 1% Klucel LF in water with 0.1% Tween 80.

Given the favorable plasma exposure, compound **2g** was tested for its ability to suppress the growth of established tumor xenografts in nude mice. The human osteosarcoma cell line SJSA-1 was chosen due to its *MDM2* gene amplification and overexpression of MDM2 protein.[23] Daily oral administration of a 50 mg/kg dose of compound **2g** showed 74% tumor growth inhibition, and tumor regression was observed at a higher dose of 100 mg/kg.[22] Similar in vivo efficacy was observed in the MHM osteosarcoma model,[22] which also expresses high levels of MDM2 protein.[18] Comparing to Nutlin-3a (**1**), the efficacious dose of compound **2g** is at least 4-fold lower. With a favorable safety profile in subsequent toxicological studies, compound **2g** was selected for further evaluation in human clinical trials.

In conclusion, combination of dimethyl substitution of the imidazoline core and replacement of the methoxy group by *tert*-butyl group led to the discovery of the first investigational MDM2 inhibitor, RG7112 (**2g**). Oral administration of RG7112 in phase 1 clinical trials has provided evidence that the molecule can activate p53 signaling in human tumors, and there is early evidence of activity in solid tumors[24] and hematologic malignancies.[25] Clinical evaluation of RG7112 monotherapy and combinations are ongoing.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures for synthesis of **2g**, characterization data for all compounds, and individual IC_{50} values for each cell line. PDB codes for crystal structures in this paper are 4IPF and 4J3E (http://www.rcsb.org). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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