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Substituted piperidines as HDM2 inhibitors

Yao Ma *, Brian R. Lahue, Gerald W. Shipps Jr., Jeseca Brookes, Yaolin Wang

Discovery and Preclinical Sciences, Merck Research Laboratories, 33 Avenue Louis Pasteur, Boston, MA 02115, United States

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ABSTRACT

Novel small molecule HDM2 inhibitor, substituted piperidine, was identified. Initial SAR study indicated potential for several position optimizations. Additional potency enhancement was achieved by introducing a sidechain off the aromatic ring. DMPK study of one of the active compounds has shown a moderate oral PK and reasonable bioavailability.

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The tumor suppressor protein p53 plays a central role in maintaining the integrity of the genome in a cell. It regulates the expression of a diverse array of genes responsible for DNA repair, cell cycle and growth arrest, and apoptosis.[1–3] As with murine MDM2, the human homologue HDM2 acts to down-regulate p53 activity in an autoregulatory manner in which the cellular level of each protein is controlled by the other.[4–6] Attenuating this negative feedback loop could have a critical effect on cell homeostasis. It was found that in many types of human tumors, HDM2 was overexpressed and p53 function was decreased.[7,8] To restore the function of wt-p53 in tumor cells by inhibition of HDM2 should result in decreased proliferation and apoptosis thus inhibiting tumor growth, which offers great therapeutic potential for the treatment of a variety of cancers.[9,10]

The co-crystal structure of the HDM2 N-terminal domain bound to a p53-derived peptide revealed a major pocket of interaction driven by three nearby amino acid residues on one face of a helix, corresponding to p53 residues Phe19, Leu26, and Trp23.[11] It was anticipated that a small molecule could mimic this interaction and antagonize the HDM2 protein, freeing p53 to perform its downstream oncogenic functions. Despite the large surface area of protein–protein interaction (PPI) interfaces and relative complexity, several potent low molecular weight p53/HDM2 inhibitors have been discovered to date and were recently reviewed.[12,13] Herein, we report a series of novel HDM2 small molecule antagonists that selectively inhibit proliferation of several wild-type p53 human cancer cells. SAR trends identified within this chemotype for substituents correlating to the three aforementioned residues on p53 guided the optimization of this series.

From our in-house high-throughput screening (HTS) platform, several hits (**1**–**3**) were identified which inhibit the p53/HDM2 protein–protein interaction with IC_{50} values in the low single digit micromolar range (Fig. 1). These geminally disubstituted piperidines (**4**) were shown to bind to HDM2's p53 binding pocket based on a fluorescence polarization (FP) peptide displacement assay.[14]

Synthesis of this class of compounds was straightforward (Scheme 1) and has been detailed in previously published patent applications.[15,16] Key intermediates **6** were readily prepared in racemic form through the Bargellini reaction of ketone **5** with the corresponding phenol in the presence of chloroform under basic conditions.[15–17] Extensive optimization of the reaction conditions identified the order of addition of the reagents as a key factor in producing intermediates **6** in the best yields. Depending on the stability of the phenol to the basic reaction conditions, adding it after all the other reagents could considerably improve the yield of desired products **6**. Intermediates **6** were subsequently subjected to amidation, deprotection, and acylation to produce fully substituted final compounds **4**.

Compound **1**, chosen as the starting point for optimization based on the frequency with which its substituents appeared in hits within the initial HTS results, was resynthesized from the corresponding common intermediate carboxylic acid **6**. Palladium-catalyzed hydrogenolysis of **6** in the presence of di-tertbutyl dicarbonate and Hunig's base gave **7** as the diisopropylethylamine salt, which was used directly in an amidation with 1-(2-pyridin-2-

* Corresponding author.
E-mail address: yaoma1@yahoo.com (Y. Ma).

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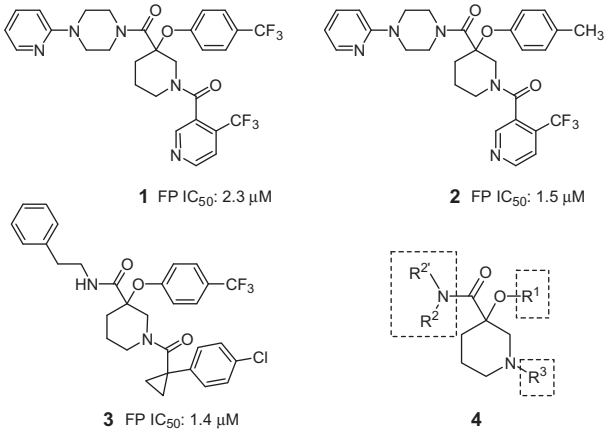
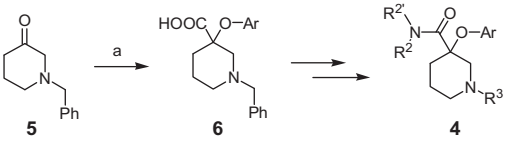


Figure 1. Substituted piperidines as novel HDM2 inhibitors.

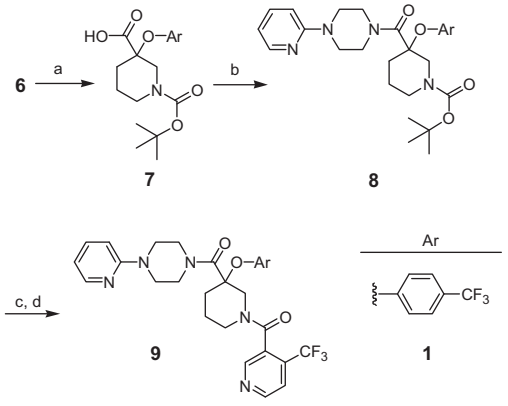


Scheme 1. Synthesis of substituted piperidine **4**. Reagents and conditions: (a) phenol (ArOH), $CHCl_3$, NaOH, 0–40 °C.

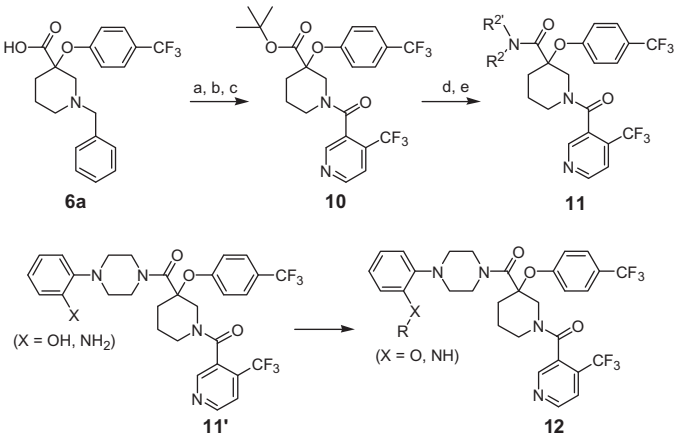
yl)piperazine to give **8** (Scheme 2). Further elaboration by acidic BOC deprotection and subsequent amide formation with 4-(trifluoromethyl)nicotinic acid led to final compounds **9**.

For ease of handling and purification as well as to enable rapid optimization at $R^2/R^{2'}$ (see **4**), intermediate acid **6a** was esterified[18] which, after hydrogenolysis of the benzyl group and amide formation with 4-(trifluoromethyl)nicotinic acid, gave intermediate **10** (Scheme 3). Ester hydrolysis of **10** with HCl produced the corresponding acid which underwent EDC-promoted amidation with various amines to generate diamides **11**. Further $R^2/R^{2'}$ optimization was carried out through extension of a sidechain off the *ortho* position of the piperazine-linked phenyl ring (**11′**), most effectively by O-alkylation or N-acylation leading to final compounds **12**.

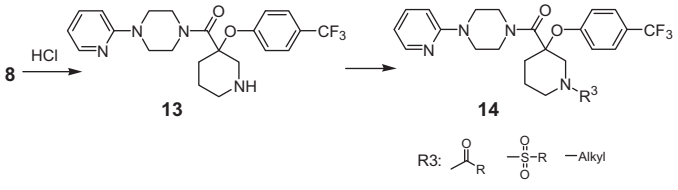
Similarly, common intermediate **13**, used for R^3 optimization, was synthesized by BOC de-protection of the corresponding piperazine amide **8** (Scheme 4). The resulting free amine enabled the broad profiling of R^3 substituents and the generation of SAR



Scheme 2. R^1 (aryl) optimization. Reagents and conditions: (a) 5% Pd/C, H_2, BOC_2O, $^iPr_2NEt$ (b) Carbonyldiimidazole, polymer-supported (PS-CDI), HOBt, 1-(pyridin-2-yl)piperazine, THF (c) 4 N HCl in 1,4-dioxane (d) PS-CDI, HOBt, 4-(trifluoromethyl)nicotinic acid, DMF.

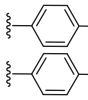
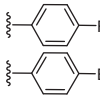
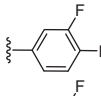
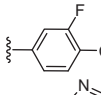
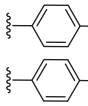


Scheme 3. $R^2/R^{2'}$ (amine and sidechain) optimization. Reagents and conditions: (a) (*Z*)-*tert*-butyl *N,N′*-diisopropylcarbamimidate; (b) H_2, Pd/C; (c) acid, EDC, HOBt; (d) 4 N HCl in 1,4-dioxane; (e) PS-CDI; HOBt, amine.



Scheme 4. R^3 optimization.

Table 1
R^1 SAR: In vitro activity (refer to general structures **9**)

Compound	R^1	IC_{50} (μM)
9a		21
9b		5.2
9c		6.7
9d		6
9e		1.9
9f		2.9
9g		1.4
9h		41
9i		48
9j		33
2		1.5
1		2.3

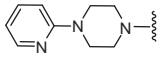
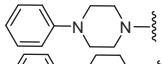
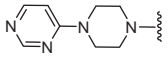
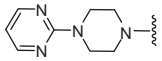
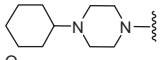
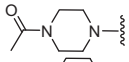
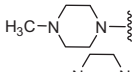
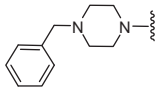
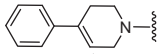
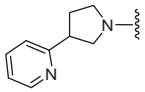
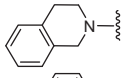
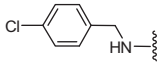
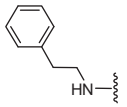
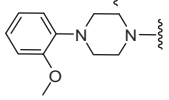
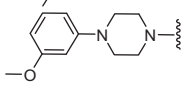
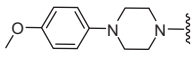
(a) All compounds were tested as HCl salts; (b) Fluorescent polarization was measured by reading the plate using the Analyst AD (Molecular Device). IC_{50} was determined as described in Zhang et al.[14]

through subsequent N-functionalization to give final compounds **14**.

As depicted in the above syntheses, our initial optimization plan centered around various substituents at three motifs attached to intermediate **4** (R^1, R^2, R^3). R^1 variation was derived from original phenols and, although several steps were involved after incorporating R^1, the very mild reaction conditions employed allowed for profiling various phenolic substituents (Table 1).

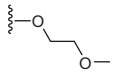
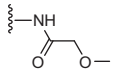
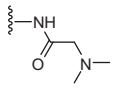
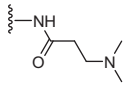
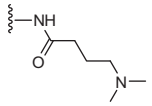
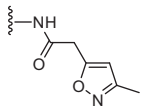
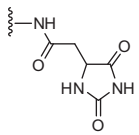
Overall, substitution of the R^1 aryl ring was better-tolerated in the *meta*- and *para*-positions compared to the corresponding *ortho*-substituted analogs as exemplified by chlorinated compounds **9a**–**9c**. Replacement of the *para*-chloro substituent with *p*-fluoro (**9d**) or *p*-bromo (**9e**), respectively, demonstrated that while decreased electron density of the aryl ring was tolerated, a larger group may be preferred in the *para*-position. This trend was confirmed with disubstituted analogs **9f** and **9g**, in which the addition of a *meta*-fluoro substituent was tolerated (compare **9e**–**9f**) or modestly improved (compare **9c**–**9g**) IC_{50} values. However, several analogs disubstituted in the form of heterobicyclic compounds like **9h** exhibited substantial losses in enzymatic activity. The *para*-substituent was further examined in the context of

Table 2
R^2 SAR: In vitro activity of various amides (refer to general structures **11**)[a]

Compound	$R^2R^{2'}N$	IC_{50} (μM)
1		2.3
11a		2.8
11b		18
11c		35
11d		4.2
11e		36
11f		12
11g		39
11h		1.4
11i		3.7
11j		1.4
11k		1.5
11l		3.5
11m		0.56
11n		>50
11o		23

[a] Compounds were tested under similar conditions as described in Table 1.

Table 3
R^2 sidechain SAR: In vitro activity (refer to general structure **12**)[a]

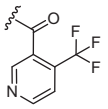
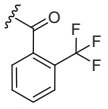
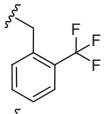
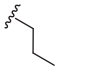
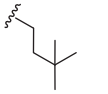
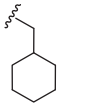
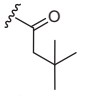
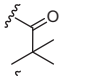
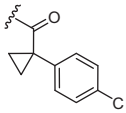
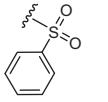
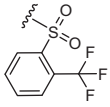
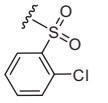
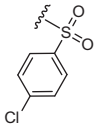
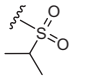
Compound	Side chain	IC_{50} (μM)
11m		0.56
12a		0.97
12b		0.59
12c		0.33
12d		0.38
12e		0.37
12f		0.54
12g		0.67
12h		0.59
12i		1.08
12j		0.63
12k		0.27

[a] Compounds were tested under similar conditions as described in Table 1.

size and polarity–an exercise which revealed the preference for relatively small, hydrophobic groups like methyl (**2**) and trifluoromethyl (**1**) over larger, non-polar phenyl (**9i**) or small, polar methoxy (**9j**) moieties. Based on its overall profile and likelihood to withstand metabolic degradation, compound **1** was selected for further optimization.

By fixing compound **1**'s substituents at both R^1 and R^3 (refer to general structure **4**) as *p*-trifluoromethylphenol and 4-trifluoromethylnicotinamide, respectively (Fig. 1), a thorough investigation of the $R^2/R^{2'}$ amine substituent was undertaken. While the pyridine nitrogen of **1** did not appear to be critical as illustrated by equipotent phenyl analog **11a**, the addition of a second ring-nitrogen (**11b** and **11c**) led to significant losses in activity (Table 2). Saturation of the terminal ring was tolerated (**11d**) however, acyl (**11e**), small alkyl (**11f**), and one-methylene homologated (**11g**) variants were significantly less active. Modifications to the linking piperazine ring were well-tolerated as partially unsaturated **11h**, ring-contracted pyrrolidine **11i**, and annulated tetrahydroisoquinoline **11j** all maintained the activity of **1**. The complete replacement of the piperazine ring by one or two methylenes (**11k** and **11l**, respectively) led to similarly active compounds. This variety of active substituents at the $R^2/R^{2'}$ position offered considerable opportunities for later DMPK optimization. From an enzymatic activity standpoint, it was not until terminal ring-substitution was explored that a jump in activity was unveiled. 2-Methoxy analog

Table 4
R^3 SAR: In vitro activity of (refer to general structure **14**)[a]

Compound	R^1	IC_{50} (μM)
1		2.3
14a		6.5
14b		14
14c		6.5
14d		6.9
14e		5.1
14f		3.0
14g		22
14h		1.9
14i		26
14j		41
14k		31
14l		50
14m		6.9

[a] Compounds were tested under similar conditions as described in Table 1.

11m represented a 5-fold improvement over pyridyl progenitor **1** and clearly defined an SAR path to follow since the analogous 3-methoxy (**11n**) and 4-methoxy (**11o**) compounds were less active in this assay.

With the preference for a heteroatom-linked sidechain in the *ortho* position on the $R^2/R^{2'}$ phenyl ring determined (see **11m**), the size and polarity of this substituent were profiled in an effort to further improve biochemical activity (Table 3). While shortening of the sidechain to the phenol **12a** led to a twofold loss in activity, longer substituents were tolerated (see **12b**), especially those containing additional heteroatoms (**12c**). Such modifications via a tethered sidechain to pick up additional interactions would be consistent with those of other published HDM2/P53 inhibitors.[19] When the phenolic oxygen atom was replaced with an anilino-nitrogen, amide-based sidechains of analogous lengths were also tolerated (compare **12b** with **12d** and **12c** with **12f**). Basic amine-containing sidechains of similar length were well-tolerated (**12g–12i**) as were select heterocycles like isoxazole **12j** and hydantoin **12j**. This general tolerability for added polarity within this sidechain offered an opportunity to improve the overall physical properties of the compounds without sacrificing enzymatic activity.

Table 5
DMPK of the **12c** active enantiomer

Route	Dose (mpk)	AUC 24 h (μM h)	C_{max} (μM)	T_{max} (h)	Bioavailability (%)
Full Mouse PK					
IV	20	3.4			
PO	20	1.1	0.7	1	32

CYP Inhibition					
Co-incubation (IC_{50}, μM)			Pre-incubation (IC_{50}, μM)		
3A4	2D6	2C9	3A4	2D6	2C9
4.9	>30	>30	0.3	>30	>30

Structural modifications around the R^3 amide in **1** were undertaken to generate SAR trends in an effort to further improve activity (Table 4). Removal of the apparently important R^3 pyridine nitrogen resulted in a 3-fold loss in activity (compare **1** with **14a**). Subsequent replacement of the amide carbonyl with a methylene to give **14b** led to a significant loss in enzymatic activity, indicating the possible importance of non-basic nitrogen in the piperadine core of this series. However, further profiling of basic amine-linked substituents identified smaller aliphatic groups like **14c–14e** could retain the in vitro activity of benzoic acid derivative **14a**, an indication that perhaps the amide carbonyl served more to orient the *ortho* trifluoromethyl moiety than to moderate the basicity of the piperidine nitrogen atom. Attempts to combine the amide linkage of **1** and **14a** with the simple aliphatic sidechains of **14c–14e** revealed that a substituent as simple as a *tert*-butyl group could maintain the enzymatic activity of **14a** when positioned the appropriate distance from the carbonyl (**14f**) but was much less active when just one carbon too short (**14g**). Amides hindered at the alpha carbon to the carbonyl regained their ability to inhibit the HDM2/p53 protein–protein interaction when coupled with an aromatic substituent, as in **14h**. Our efforts to replace the amide functionality with similarly substituted sulfonamides proved largely unfruitful (**14i–14l**, compare **14a** with **14j**). Only moderate activity was observed with smaller aliphatic sulfonamide (**14m**).

Chiral HPLC separation of racemate **12c** cleanly afforded two enantiomers, the active isomer of which displayed an IC_{50} of 160 nM. DMPK analysis of this compound indicated a moderate oral exposure in mice with a reasonable bioavailability (32%, Table 5). While this compound did not inhibit cytochrome P450 2D6 and 2C9 isoforms, it exhibited both direct and mechanism-based inhibition on isoform 3A4. Further optimization of this series is ongoing and will be reported on in the future.

Overall a new chemical series, substituted piperidines, was identified as inhibitors of the HDM2/p53 protein–protein interaction. Initial optimization efforts have generated useful SAR trends at several binding positions. Further improvements to this scaffold should furnish a potent HDM2 inhibitor with an improved overall DMPK profile.

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