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Core modification of substituted piperidines as Novel inhibitors of HDM2–p53 protein–protein interaction

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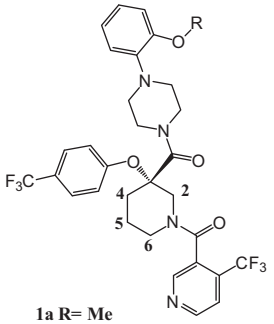
ABSTRACT

The discovery of 3,3-disubstituted piperidine **1** as novel p53–HDM2 inhibitors prompted us to implement subsequent SAR follow up directed towards piperidine core modifications. Conformational restrictions and further functionalization of the piperidine core were investigated as a strategy to gain additional interactions with HDM2. Substitutions at positions 4, 5 and 6 of the piperidine ring were explored. Although some substitutions were tolerated, no significant improvement in potency was observed compared to **1**. Incorporation of an allyl side chain at position 2 provided a drastic improvement in binding potency.

Cancer is a disease of uncontrolled cell growth of various tissues and organs in the body that is a leading cause of death. The International Agency for Research on Cancer recently announced that an estimated 14.1 million new cancer cases and 8.2 million cancer-related deaths occurred worldwide in 2012. According to the American Cancer Society, about 1.6 million new cancer cases are expected to be diagnosed in 2013, with an estimated half million deaths in the US alone. Even with the use of conventional chemotherapy and newly approved targeted therapies, cancer still represents an unmet medical need.[1]

The tumor suppressor p53 plays many critical roles in surveying and responding to various stress and damage signals. It regulates the cell growth, migration, apoptosis, angiogenesis, metabolism, development and stromal matrix cellular environment.[2] The loss of p53 function predisposes cells to a cancerous state.[3] HDM2 is a major negative regulator of p53 activity by repressing p53 transcriptional activity through its binding to p53 and targeting p53 degradation in the proteosome through its ubiquitin E3 ligase activity.

The possibility of targeting the p53 binding pocket of HDM2 with a small molecule antagonist was raised in 1997 when the crystal structure of a p53 peptide binding to HDM2 revealed three surface pockets that bind p53 residues Phe19, Trp23 and Leu26.[4] An inhibitor capable of blocking this HDM2–p53 interaction would free p53 from negative regulation by HDM2, thereby promoting its anti-proliferative and proapoptotic functions. In more than 50% of human cancers, p53 is inactivated due to overexpression of HDM2 protein,[5] making the development of small molecule inhibitors of HDM2–p53 a very attractive cancer therapy.[6] Thus, in 2004, scientists at Roche reported the discovery of a series of 4,5-dihydroimidazolines with Nutlin-3a as the first small molecule interfering with protein–protein interaction of HDM2–p53.[7] Since then, others have started reporting the discovery of additional potent small molecule inhibitors.[8] Similarly, our group identified and recently reported the discovery and optimization of gem-disubstituted piperidines **1a** and **1b** (Fig. 1) as small molecule inhibitors of the HDM2–p53 protein–protein interaction.[9]



1a R= Me
1b R= -$(CH_2)_2$OMe

Figure 1. *gem*-Disubstituted piperidines.

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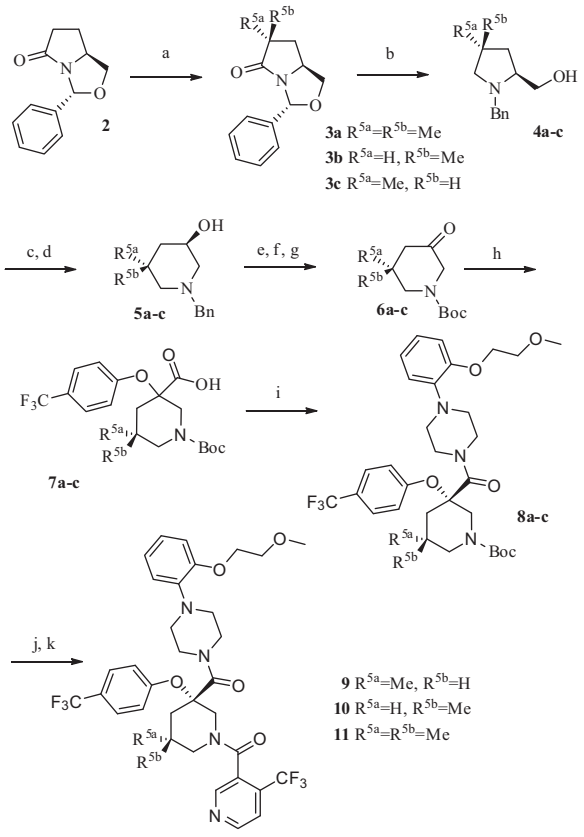


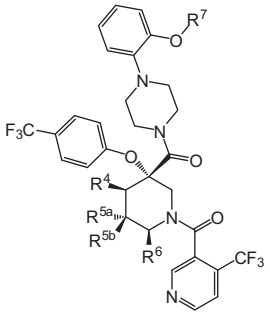
Scheme 1. Synthesis of 5-substituted piperidine inhibitors. Reagents and conditions: (a) LiHMDS, THF, MeI, −78 °C, 58%; (b) LAH, THF, reflux, 100%; (c) TFAA, Et_3N, −78 to 60 °C (up to 4 days); (d) NaOH (2.5 M), 100%; (e) H_2, $Pd(OH)_2/C$; (f) THF, NaOH, $(Boc)_2O$; (g) Dess–Martin, 55–70%; (h) 4-CF_3-phenol, $CHCl_3$, 0–40 °C, 40–72%; (i) HATU, DIPEA, 1-(2-(2-methoxyethoxy)phenyl)piperazine hydrochloride, 41–60%; (j) 4.0 N HCl/dioxane, 100%; and (k) 4-CF_3-nicotinic acid/HATU, 45–50%.

Satisfyingly, leads **1a** and **1b** displayed similar in vitro permeability as Nutlin 3a as well as some evidence of oral absorption in rodents that warranted further investigation.[10] During the course of our SAR investigation, the team envisioned the potential of gaining additional binding affinity by functionalizing the piperidine core. To that end, tolerability for substitutions on the 4, 5, and 6 positions were probed with both small and large groups. As described earlier,[9a] the team capitalized on the Bargellini reaction[11] of protected 3-piperidinone with 4-(trifluoromethyl)phenol to efficiently assemble the gem-disubstituted piperidine core of inhibitor **1**. To exploit the same chemistry, ring expansion methodology of prolinols to 3-hydroxypiperidines reported by Cossy[12] was identified as a very attractive way to prepare the substituted piperidinones required for our SAR exploration.

Probing the 5 position of piperidine with compounds **9**, **10** and **11** required preparation of 5-substituted-3-hydroxy-*N*-benzylpyperidines **5a–c** described in Scheme 1.

The poor selectivity and diastereoselectivity observed during the alkylation of pyroglutamic acid derivative **2** with MeI was beneficial to provide in one step multiple key intermediates. Precursors **3a–c** were easily separated by column chromatography and individually subjected to reductive conditions using LAH. Treatment of 3-substituted prolinols **4a–c** with trifluoroacetic anhydride in THF followed by addition of triethylamine and sodium hydroxide provided 5-substituted-3-hydroxy-*N*-benzylpyperidines **5a–c**.[11] Depending on the steric hindrance of starting prolinols, reaction time of up to 4 days was necessary to get complete consumption of starting material and formation of piperidines **5a–c** in quantitative yield. Extensive optimization of the Barguellini reaction conditions favored the use of N-Boc protected piperidone.[9c] Thus, 3-hydroxy-*N*-benzylpiperidines **5a–c** were subjected to Palladium-catalyzed hydrogenolysis in the presence of di-tert-butyl dicarbonate and subsequently oxidatized with Dess–Martin periodinane[13] to provide the key piperidones **6a–c** in good yield. Bargellini reaction of **6a–c** with the 4-(trifluoromethyl) phenol afforded in one step the 2,2-disubstituted carboxylic acids **7b,c** as

Table 1
FP and antiproliferative assay results for compounds **1a**, **1b**, **9–11**, **15–17** and **24**[a]



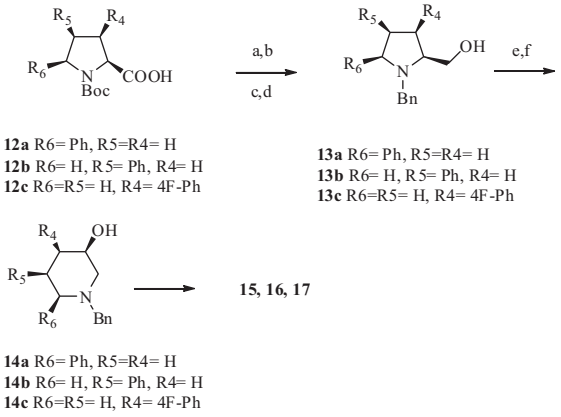
Compds	R^4	R^{5a}	R^{5b}	R^6	R^7	FP IC_{50} (μM)	SJSA-1 IC_{50} (μM)
1a	H	H	H	H	Me	0.6	5.5
1b	H	H	H	H	–$(CH_2)_2$–OMe	0.3	4.7
9	H	Me	H	H	–$(CH_2)_2$–OMe	4.0	–
10	H	H	Me	H	–$(CH_2)_2$–OMe	2.7	–
11	H	Me	Me	H	–$(CH_2)_2$–OMe	1.0	–
15	H	H	Ph	H	Me	3.0	–
16	4F-Ph	H	H	H	–$(CH_2)_2$–OMe	3.9	–
17	H	H	H	Ph	–$(CH_2)_2$–OMe	>10	–
24	H	H	Cyclopropyl		Me	5.6	–

[a] Fluorescence polarization (FP) peptide displacement assay value were determined as described in Zhang et al.[15] For comparison Nutlin-3a fluorescence polarization (FP) peptide displacement IC_{50} assay value obtained in house was 0.07 μM. In our antiproliferative assay, Nutlin-3a had an IC_{50} value of 1.9 μM using osteosarcoma SJSA-1 cells.

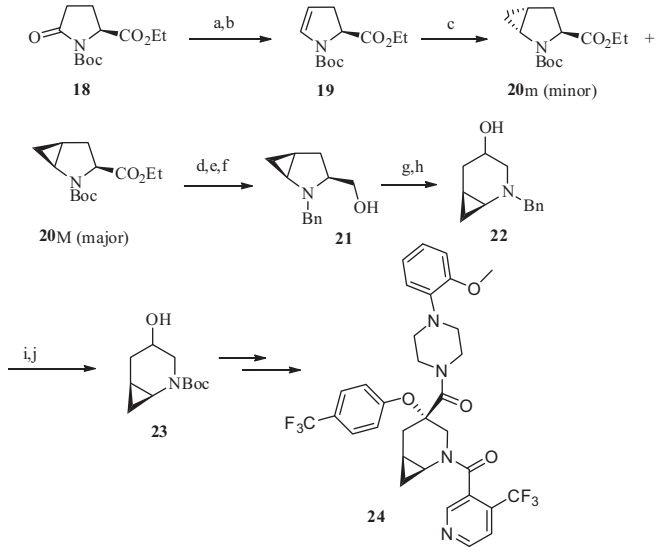
a mixture of diastereomers and **7a** as a racemic mixture. Intermediates **7a–c** were directly reacted with 1-(2-(2-methoxyethoxy)phenyl)piperazine hydrochloride under standard peptide coupling conditions to afford compounds **8a–c**. Further elaboration to **9**, **10** and **11** was achieved via acidic BOC deprotection and subsequent amide formation with 4-(trifluoromethyl)-nicotinic acid. Reverse phase and chiral HPLC conditions were developed to isolate each of the diastereomers or enantiomers.

Targets **15–17** (Table 1) were prepared according the sequence depicted in Scheme 2. Using phenylpyrrolidine acids **12a–c** as starting materials, esterification followed by reduction and protection group exchange gave prolinols **13a–c**, which were successfully engaged in ring expansion methodology described earlier to generate substituted-3-hydroxy-*N*-benzylpyperidines **14a–c**. Finally, the analogous sequence as the one described in Scheme 1 was followed to prepare compounds **15–17**.

Fused bicyclic ring system **24** was synthesized according to Scheme 3. Cyclopropanation precursor **19** was prepared from pyroglutamic derivative **18** via lithium triborohydride amide reduction and trifluoroacetic anhydride-mediated elimination.[14] Furukawa's



Scheme 2. Reagents and conditions: (a) TMS–CH_2N_2, MeOH, Et_2O (b) 4.0 N HCl/dioxane, (c) BnBr, DCM, DIPEA (d) LAH, THF, reflux, 85–94% from (a) to (d); (e) TFAA, Et_3N, −78 to 60 °C; (f) NaOH (2.5 M); and (f) H_2, $Pd(OH)_2/C$.



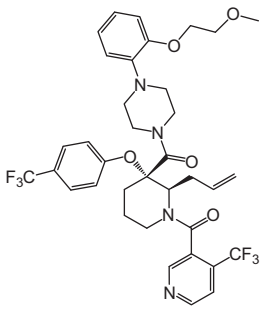
Scheme 3. Synthesis of fused ring on 5 and 6 position of piperidine inhibitor. Reagents and conditions: (a) $LiBHEt_3$, PhMe, −40 °C; (b) DIPEA, DMAP, TFAA; (c) CH_2I_2/Et_2Zn; (d) 4.0 N HCl; (e) BnBr, DMF; (f) LAH, 70% from (d) to (f); (g) TFAA, Et_3N; (h) NaOH, 79% from (g) to (h); (i) H_2, 10% Pd/C, 2 N HCl in ether, 98%; and (j) $(Boc)_2O$, NaOH, THF, 98%.

cyclopropanation conditions provided **20m** and **20M** as a mixture of diastereomers in a 1:11 ratio, respectively.[15] The major isomer **20M** was subjected to the protection group exchange and reduction sequence to generate precursor **21** in 70% overall yield. The highly functionalized prolinol **21** was successfully engaged in ring expansion methodology to afford 3-hydroxy-*N*-benzylpyperidines **22** in 79% isolated yield. Protection group swapping necessary for the Barguellini reaction required a chemoselective hydrogenolysis of the benzyl group of **22** and was achieved using 10% Pd/C in EtOH with one equivalent of HCl (2 N, Et_2O). After treatment with di-tert-tbutyl dicarbonate, Boc-protected hydroxy-piperidine **23** was obtained in 98% overall yield. Target **24** was prepared following a sequence analogous to the one described in Scheme 1.

To assess the potency of these modified geminally disubstituted piperidines, we evaluated their ability to bind to HDM2's p53 binding pocket using a fluorescence polarization (FP) peptide displacement assay.[16]

Most of the substitutions studied at position 5 of the piperidine were tolerated. The dimethyl substituted compound **11** (IC_{50} = 1.0 μM, Table 1) was the most potent and had HDM2 binding potency within 2–3 fold of compounds **1a**,**b**. With the assumption that position 4, 5 and 6 of piperidine were close to the protein surface, the team anticipated that potency enhancements could be further achieved through modification of the hydrophobic interaction of the piperidine moiety with the HDM2 protein. Unfortunately, efforts to functionalize position 5 with a large hydrophobic group was unfruitful. Although the phenyl group in compound **15** was still tolerated, the compound was less active compared to **1a** and **11**. Position 4 and 6 of the piperidine ring seemed less tolerant to modification as incorporation of an aromatic moiety in compounds **16** and **17** resulted in a loss in potency compared to **15**. Rigidification of the piperidine core was also investigated as a strategy to gain additional interactions with HDM2 by locking the conformation of the piperidine ring. Incorporation of a small, fused cylopropyl ring was thought attractive. Chemistry challenges allowed exploration of a fused ring at position 5 and 6 only. Unfortunately, compound **24** showed only moderate activity with an IC_{50} of 5.6 μM.

While we anticipated that potency enhancements could be achieved through modification of the hydrophobic interaction of the piperidine moiety with the HDM2 protein, our efforts to functionalize positions 4, 5 and 6 of the piperidine core proved largely unsuccessful. However, our team recently reported that the incorporation of an allyl substituent at position 2 of the piperidine provided compound **25** with four fold gain in potency.[17] Similarly, in antiproliferative assay, **25** displayed similar levels of improvement in activity compared to early leads **1a** and **1b** with an IC_{50} of 1.0 μM in osteosarcoma SJSA-1 cells.



25

FP IC_{50} (uM)= 0.04 ; SJSA-1 IC_{50} (uM)= 1.0

Based on this new finding, the allyl moiety is envisioned to be used as a handle to incorporate functional diversity to probe potential

for additional surface interactions and fine tune physiochemical properties of the lead molecules.

In conclusion, systematic functionalization of position 4, 5 and 6 of the piperidine ring was explored. Although most of substitutions studied were tolerated, incorporation of a gem-dimethyl group at position 5 in compound **11** retained potency level comparable versus **1a**,**b**. Recent reports from our group demonstrated that incorporation of an allyl side chain at position 2 provided a drastic improvement in binding potency; it would suggest that positions 5 and 2 of our newly discovered HDM2–p53 inhibitors can be used for additional SAR optimization. Further work directed towards the incorporation of functional diversity will be reported shortly.

References and notes

1. American Cancer Society, Surveillance Research, 2013.
2. (a) Mandinova, A.; Lee, S. W. *Sci. Transl. Med.* **2011**, *3*, 64rv1; (b) Raycroft, L.; Wu, H. Y.; Lozano, G. *Science* **1990**, *249*, 1049.
3. Hainaut, P.; Hollstein, M. *Adv. Cancer Res.* **2000**, *77*, 81.
4. (a) Kussie, P. H.; Gorina, S.; Marechal, V.; Elenbaas, B.; Moreau, J.; Levine, A. J.; Pavletich, N. *Science* **1996**, *274*, 948; (b) Chen, J.; Marechal, V.; Levine, A. *J. Mol. Cell. Biol.* **1993**, *13*, 4107; (c) Bottger, A.; Bottger, V.; Garcia-Echeverria, C.; Chene, P.; Hochkeppel, H. K.; Sampson, W.; Ang, K.; Howard, S. F.; Picksley, S. M.; Lane, D. P. *J. Mol. Biol.* **1997**, *269*, 744.
5. (a) Vousden, K. H.; Lane, D. P. *Rev. Mol. Cell Biol.* **2007**, *8*, 275; (b) Bond, G. L.; Hu, W.; Levine, A. J. *Curr. Cancer Drug Targets* **2005**, *5*, 3; (c) Wade, M.; Wang, Y. V.; Wahl, G. M. *Trends Cell Biol.* **2010**, *20*, 299.
6. (a) Poyurovsky, M. V.; Prives, C. *Genes Dev.* **2006**, *20*, 125; (b) Brown, C. J.; Lain, S.; Verma, C. S.; Fersht, A. R.; Lane, D. P. *Nat. Rev. Cancer* **2009**, *9*, 862.
7. Vassilev, L. T.; Vu, B. T.; Graves, B.; Carvajal, D.; Podlaski, F.; Filipovie, Z.; Kong, N.; Kammlott, U.; Lukacs, C.; Klein, C.; Fotouhi, N.; Liu, E. A. *Science* **2004**, *303*, 844.
8. (a) Hu, C.-Q.; Hu, Y.-Z. *Curr. Med. Chem.* **2008**, *15*, 1720; (b) Patel, S.; Player, M. R. *Expert Opin. Invest. Drugs* **2008**, *17*, 1865; (c) Zhao, Y.; Yu, S.; Sun, W.; Liu, L.; Lu, J.; McEachern, D.; Shargary, S.; Bernard, D.; Li, X.; Zhao, T., et al *J. Med. Chem.* **2013**, *56*, 5553; (d) Gonzalez-Lopez de Turiso, F.; Sun, D.; Rew, Y.; Bartberger, M. D.; Beck, H. P.; Canon, J.; Chen, A.; Chow, D.; Correll, T. L.; Huang, X., et al *J. Med. Chem.* **2013**, *56*, 4053; (e) Zak, K.; Pecak, A.; Rys, B.; Wladyka, B.; Domling, A.; Weber, L.; Holak, T. A.; Dubin, G. *Expert Opin. Ther. Patents* **2013**, *23*, 425; (f) Vu, B.; Wovkulich, P.; Pizzolato, G.; Lovey, A.; Ding, Q.; Jiang, N.; Liu, J.; Zhao, C.; Glenn, K.; Wen, Y.; Tovar, C.; Packman, K.; Vassilev, L.; Graves, B. *ACS Med. Chem. Lett.* **2013**, *4*, 466; (g) Ding, Q.; Zhang, Z.; Liu, J.; Jiang, N.; Zhang, J.; Ross, T. M.; Chu, X.; Bartkovitz, D.; Podlaski, F.; Janson, C.; Tovar, C.; Filipovic, Z.; Higgins, B.; Glenn, K.; Packman, K.; Vassilev, L.; Graves, B. *J. Med. Chem.* **2013**, *56*, 5979.
9. (a) Ma, Y.; Lahue, R. B.; Shipps, W. G., Jr.; Brookes, J.; Wang, Y. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 1026; (b) Wang, Y.; Zhang, R.; Ma, Y.; Lahue, B.; Shipps, G. U.S. Pat. Appl. Publ. 2008, US2008004286.; (c) Ma, Y.; Lahue, B.; Shipps, G.; Wang, Y.; Bogen, S.; Voss, M.; Nair, L.; Tian, Y.; Doll, R.; Guo, Z.; Strickland, C.; Zhang, R.; McCoy, M.; Pan, W.; Siegel, E.; Gibeau, C. U.S. Pat. Appl. Publ. 2008, US2008004287.
10. *Nutlin 3a:* Caco2 permeability = 126 nM/s. *Compound **1a**:* Caco2 permeability = 158 nM/s, mouse PK (PO/IV, 20/20 mpk) AUC = 1.9 μM h; *F* = 19%. *Compound **1b**:* Caco2 permeability = 203 nM/s, mouse PK (PO/IV, 20/20 mpk) AUC = 1.1 μM h; *F* = 32%.
11. Cvetovich, R. J.; Chung, J. Y. L.; Kress, M. H.; Amato, J. S.; Matty, L.; Weingarten, M. D.; Tsay, F.-R.; Li, Z.; Zhou, G. *J. Org. Chem.* **2005**, *70*, 8560; Bargellini, G. *Gazz. Chim. Ital.* **1906**, *36*, 329; Rychnovsky, S. D.; Beauchamp, T.; Vaidyanathan, R.; Kwan, T. *J. Org. Chem.* **1998**, *63*, 6363.
12. Cossy, J.; Dumas, C.; Michel, P.; Pardo, D. G. *Tetrahedron Lett.* **1995**, *36*, 549.
13. Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155.
14. Yu, J.; Truc, V.; Riebel, P.; Hierl, E.; Mudryk, B. *Tetrahedron Lett.* **2005**, *46*, 4011.
15. Furukawa, J.; Kawabata, N.; Nishimura, J. *Tetrahedron* **1968**, *24*, 53.
16. Zhang, R.; Mayhood, T.; Lipari, P.; Wang, Y.; Durkin, J.; Syto, R.; Gesell, J.; McNemar, C.; Windsor, W. *Anal. Biochem.* **2004**, *331*, 138.
17. Ma, Y.; Lahue, R. B.; Gibeau, R. C.; Shipps, W. G., Jr.; Bogen, S. L.; Guo, Z.; Guzy, T.; Wang, Y. *ACS Med. Chem. Lett.* **2013**. accepted for publication.