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Structure–activity relationship study of 4-substituted piperidines at Leu26 moiety of novel p53–hDM2 inhibitors

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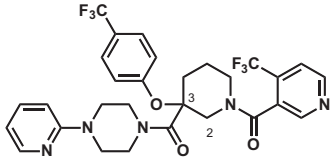
ABSTRACT

Led by the structural information of the screening hit with mDM2 protein, a structure modification of Leu26 moiety of the novel p53–hDM2 inhibitors was conducted. A structure–activity relationship study of 4-substituted piperidines revealed compound **20t** with good potencies and excellent CYP450 profiles.

The p53 tumor suppressor protein plays a critical role in the DNA damage response[1,2] and is defective in more than 50% of human tumors.[3,4] Murine mDM2 (or hDM2, the human isoform) is the main regulator of p53 stability and subjects it to degradation.[5–10] There is a need for effective inhibitors of the hDM2 protein in order to treat or prevent cancer, other disease states associated with cell proliferation, diseases associated with hDM2, or diseases caused by inadequate p53 activity.[11,12] In the past few years, small molecule inhibitors of the hDM2–p53 protein–protein interaction appear to offer an attractive strategy for cancer therapy.[13–16] The crystal structure of mDM2 bound to p53 revealed that mDM2 has a deep hydrophobic cleft on which the p53 peptide binds as an amphipathic α helix.[17] The interface relies on the steric complementarity between the mDM2 cleft and the hydrophobic face of the p53 α helix. In particular, three residues of p53 peptide: Phe19, Trp23, and Leu26 insert deep into the mDM2 cleft. Thus, inhibition of p53–mDM2 interaction could be achieved by introducing a small molecule inhibitor to mimetic p53 peptide, which has the appropriate three dimensional structure to impact these hydrophobic binding pockets of mDM2 target protein. Our research group recently reported the discovery of 3,3-disubstituted piperidine **1** with hDM2 binding activity.[18] To capitalize on this novel chemical structures of p53–hDM2 inhibitors, a series of lead optimization efforts were directed to improve upon the potency and drug properties.[19,20] Herein, we focus on the structure modification on Leu26 binding pockets in our lead series.

The structure of compound **1** in complex with mDM2 protein was solved using X-ray diffraction data (Fig. 1). On the basis of the crystal structure, that the piperidine serves as the central core to hold three subunits in different directions: 4-CF_3-nicotinamide group occupies Phe19 pocket, 4-CF_3-phenoxy group inserts deep into Trp23 pocket and 4-aryl piperizine binds with Leu26 pocket. The conformation of the central piperidine core and the stereochemistry of C3 position are critical to maintain the potency. Trp23 and Phe19 binding pockets are sensitive to ligand modification and 4-CF_3-phenoxy and 4-CF_3-nicotinamide were found as optimal groups for these two binding pockets respectively.



1
FP IC_{50} = 2.3 μM
TdF Kd = 0.5 μM
Cell viab. IC_{50} > 18μM

From the X-ray structure and preliminary SAR study, it was envisioned that two areas could be explored to improve binding affinity for this new chemotype. The first strategy relied on restricting the conformation of the central core;[21] the second was to optimize binding to the Leu26 pocket by targeting interactions

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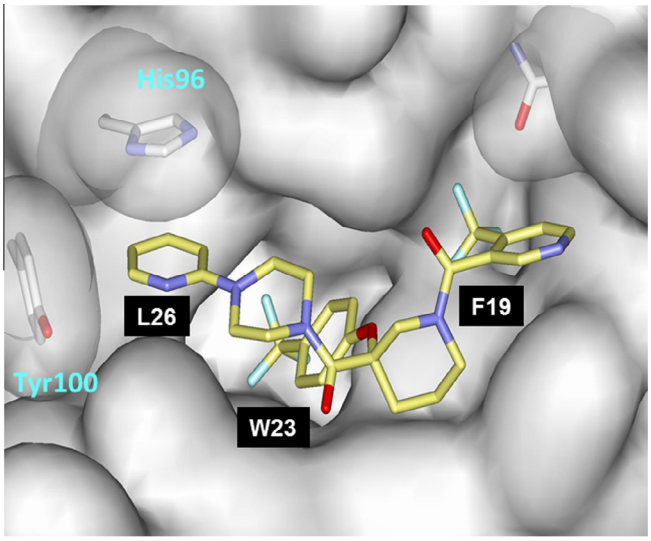
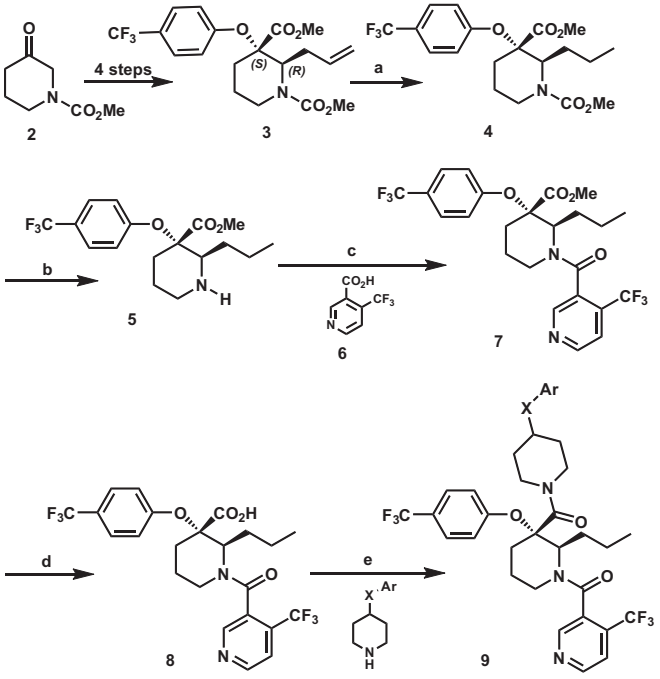


Figure 1. Co-crystal structure of compound **1** with mDM2.

with His96 and Tyr100 residues of mDM2 protein. 4-Pyridyl piperizine group of compound **1** showed potential for π–π stacking with His96 as well as edge-to-face interaction with Tyr100. Thus, our group decided to modify the piperazine linker of compound **1** to probe its effect on such interactions. A comprehensive SAR study of 4-substituted piperidine analogs on Leu26 moiety is described herein.

The syntheses of the target compounds are shown in Scheme 1. Starting from the commercial material 3-piperidone methyl carbamate (**2**), the enantiomerically pure 2-*R*–3-*S*-isomer **3** was synthesized and resolved in 4 steps.[21] Catalytic hydrogenation and deprotection of the methyl carbamate with iodotrimethylsilane yielded compound **5**. Subsequently, amide coupling with 4-trifluoromethyl-nicotinic acid (**6**) followed by demethylation of the sterically hindered ester with NaSEt gave acid **8**. The final target compounds were synthesized through the amide coupling reaction of acid **8** with the corresponding piperidine building blocks.

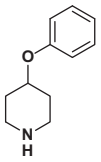
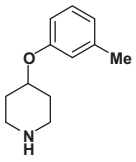
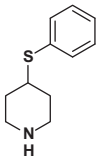
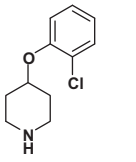
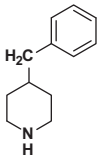
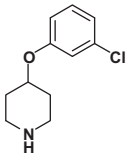
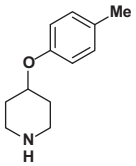
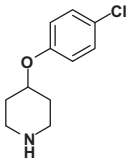
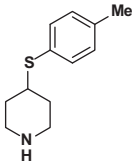
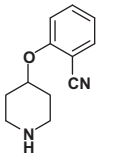
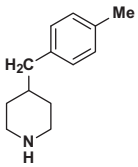
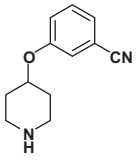
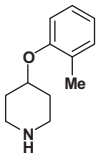
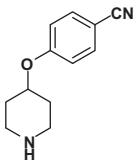


Scheme 1. Reagents and conditions: (a) H_2, Pd/C, MeOH, 23 °C, 93%; (b) TMSI, DCM, 23 °C, 55%; (c) HATU, acid **6**, DIEA, DMF, 23 °C, 82%; (d) NaSEt, DMF, 80 °C, 75%; (e) HATU, DIEA, DMF, 23 °C, 60–85%.

Initially, analogs **10**, **11** and **12** with phenyl or substituted phenyl groups as Ar group were tested in FP assay and the biological data (IC_{50}) are summarized in Table 1. From the direct comparison of **10a–c** and **11a–c**, the sulfur and oxygen linker were superior to methylene linker. In terms of substitutions on the phenyl ring, there were no significant electronic effects when methyl-, chloro- and cyano-groups were applied. However, in general, the *meta*-substitutions show better potencies than *ortho*- and *para*-substitutions. For example, compound **12b** was 10 times more potent than its regioisomers **12a** and **11a**, and *meta*-cyano analog **14b** was one of the most potent compounds in this series.

Since the oxygen linker had comparable biological activity and could potentially be more metabolically stable than the sulfur

Table 1
Enzymatic potency of the analogs with substituted phenyl group

Compd #	Piperidine	FP IC_{50} (nM)	Compd #	Piperidine	FP IC_{50} (nM)
10a		2414	**12b**		668
10b		1981	**13a**		2764
10c		5831	**13b**		2095
11a		8766	**13c**		4112
11b		9359	**14a**		8714
11c		25,801	**14b**		399
12a		6599	**14c**		1919

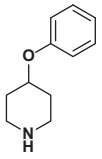
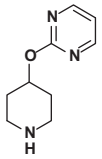
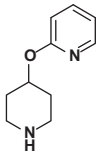
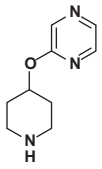
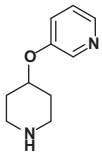
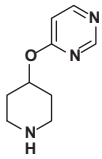
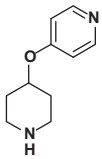
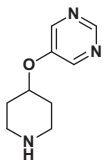
linker, further investigation was directed towards the oxygen linker. Subsequently, the SAR studies were focused on the modification of the aromatic ring occupying the Leu26 pocket. A variety of six-membered-ring aromatics were investigated and the biological data are summarized in Table 2. When phenyl ring was replaced with 2-pyridyl, the potency was dramatically improved from 2.4 μM of compound **10a** to 0.15 μM of compound **15a**. On the other hand, 3 or 4-pyridyl analog **15b** or **15c** were less potent than their regioisomer **15a**. When the second nitrogen was introduced on the pyridyl ring, the enzymatic potency dropped (compounds **16**–**19**).

X-ray structure of compound **15a** reveals that 4-substituted piperidine occupies the Leu26 pocket (Fig. 2). The pyridyl ring has strong π–π stacking with His96 and edge to face interaction with Tyr100 of mDM2 protein. X-ray structure also showed that there additional space in Leu26 pocket could accommodate small substitution on pyridyl ring, especially on 6-position. Thus, an extensive SAR study was conducted (Table 3).

Initially, a set of 6-substituted pyridyl analogs **20a**–**f** were tested and the cyano functionality showed the most promising result. Moving cyano substitution around the pyridyl ring indicated that 4-CN analog **20h** was most potent among four of its regioisomers **20f**–**i**. On the contrary, for H-bond donating polar substituents, the SAR differed. Comparing four regioisomers of aminomethyl analogs **20j**–**m**, which were the reduced products from the corresponding cyano compounds, the 6-position was most active. This SAR was consistent with the corresponding primary amides **20n**–**q**, culminating with 6-carboxamide **20n** (111 nM) and its equipotent monomethyl analog **20r** (114 nM). Not surprisedly, at the 6-position, the less polar carboxylic ester (**20s**) was less potent than the amide group due to the lack of H-bond donor. Among the set of substitutions of pyridyl ring, 6-carboxylic acid was found most effective with compound **20t** being the most potent (Table 3, FP IC_{50} = 42 nM).

Compound **20t** was tested in cell proliferation (cell viability) assays using multiple cell lines and was found to have selective inhibition of cell growth in cancer cells expressing wild-type p53

Table 2
Enzymatic potency of the analogs with different aromatic group

Compd #	Piperidine	FP IC_{50} (nM)	Compd #	Piperidine	FP IC_{50} (nM)
10a		2414	**16**		334
15a		148	**17**		1065
15b		267	**18**		376
15c		224	**19**		525

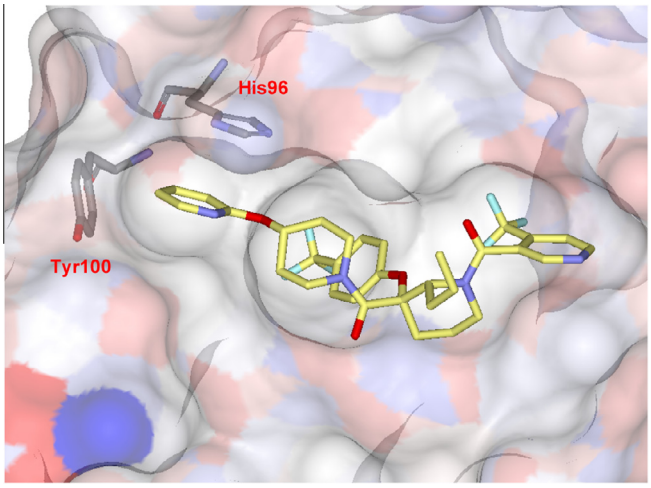
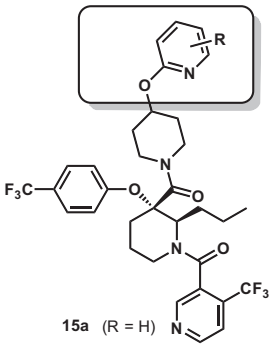


Figure 2. X-ray co-crystal structure of compound **15a** with mDM2 protein.

Table 3
Enzymatic potency of the analogs of **20a**–**t**



15a (R = H)

Compd #	R	FP IC_{50} (nM)	Compd #	R	FP IC_{50} (nM)
20a	6-Me	5027	**20k**	5-CH_2NH_2	801
20b	6-OMe	1146	**20l**	4-CH_2NH_2	4026
20c	6-CF_3	2039	**20m**	3-CH_2NH_2	7744
20d	6-Cl	2359	**20n**	6-$CONH_2$	111
20e	6-Br	1863	**20o**	5-$CONH_2$	144
20f	6-CN	686	**20p**	4-$CONH_2$	8721
20g	5-CN	492	**20q**	3-$CONH_2$	4276
20h	4-CN	293	**20r**	6-CONHMe	114
20i	3-CN	2399	**20s**	6-CO_2Me	392
20j	6-CH_2NH_2	173	**20t**	6-CO_2H	42

(osteosarcoma SJSA-1 cell line IC_{50} = 4.65 μM, colorectal cell line HCT-116 IC_{50} = 8.65 μM), but not cancer cells expressing mutant p53 (SKUT-1 uterine cancer) and null p53-null cells (HCT-116) with IC_{50} > 100 μM. More importantly, compound **20t** was also evaluated for inhibition of a panel of CYP's (3A4, 2D6, 2C9; IC_{50}s > 50 μM) and found to be devoided of CYP liabilities, overcoming a major limitation in our earlier research on this lead series.[21,22]

In conclusion, structure-based design was applied on the SAR development of Leu26 moiety of the lead series of p53–hDM2 inhibitors. Focusing on the ligand–target interaction in Leu26 pocket, 4-substituted piperidines were extensively investigated including different linkages between the piperidine and aromatic rings, numerous heterocyclic alternatives, and a variety of the substitution patterns on the aromatic ring. The potency was optimized from sub-micro molar to double digital nano molar in the enzymatic assay. In addition, compound **20t** showed micro molar cellular potency in a few p53-related cell lines and offered

excellent CYP450 profile. The further optimization of cellular potency and PK profiles of this chemical series will be reported in due course.

Acknowledgments

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References and notes

1. Vousden, K. H.; Ryan, K. M. *Nat. Rev. Cancer* **2009**, *9*, 691. and the references therein.
2. Levine, A. J.; Oren, M. *Nat. Rev. Cancer* **2009**, *9*, 749. and the references therein.
3. Hollstein, M.; Sidransky, D.; Vogelstein, B.; Harris, C. C. *Science* **1991**, *253*, 49.
4. Vogelstein, B.; Lane, D.; Levine, A. J. *Nature* **2000**, *408*, 307.
5. Momand, J.; Zambetti, G. P.; Olson, D. C.; George, D.; Levine, A. J. *Cell* **1992**, *69*, 1237.
6. Oliner, J. D. *Nature* **1993**, *362*, 857.
7. Barak, Y.; Juven, T.; Haffner, R.; Oren, M. *EMBO J.* **1993**, *12*, 461.
8. Haupt, Y.; Maya, R.; Kazaz, A.; Oren, M. *Nature* **1997**, *387*, 296.
9. Honda, R.; Tanaka, H.; Yasuda, H. *FEBS Lett.* **1997**, *420*, 25.
10. Kubbutat, M. H.; Jones, S. N.; Vousden, K. H. *Nature* **1997**, *387*, 299.
11. Menendez, D.; Inga, A.; Resnick, M. A. *Nat. Rev. Cancer* **2009**, *9*, 724. the references therein.
12. Chen, F.; Wang, W.; El-Deiry, W. S. *Biochem. Pharmacol.* **2010**, *80*, 724.
13. Hu, C. Q.; Hu, Y. Z. *Curr. Med. Chem.* **2008**, *15*, 1720.
14. Patel, S.; Player, M. R. *Expert Opin. Investig. Drugs* **2008**, *17*, 1865.
15. Beretta, G. L.; Gatti, L.; Benedetti, V.; Perego, P.; Zunino, F. *Mini-Rev. Med. Chem.* **2008**, *8*, 856.
16. Weber, L. *Expert Opin. Ther. Pat.* **2010**, *20*, 179.
17. (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.
18. Zhang, R.; Mayhood, T.; Lipari, P.; Wang, Y.; Durkin, J.; Syto, R.; Gesell, J.; McNemar, C.; Windsor, W. *Anal. Biochem.* **2004**, *331*, 138.
19. Wang, Y.; Zhang, R.; Ma, Y.; Lahue, B. R.; Shipps, G. W. U.S. Pat. 2,008,004,286, 2008.
20. Ma, Y.; Lahue, B. R.; Shipps, G. W.; Wang, Y.; Bogen, S. L.; Voss, M. E.; Nair, L. G.; Tian, Y.; Doll, R. J.; Guo, Z.; Strickland, C. O.; Zhang, R.; McCoy, M. A.; Pan, W.; Siegel, E. M.; Gibeau, C. R. U.S. Pat. 2,008,004,287, 2008.
21. Ma, Y.; Lahue, B. R.; Gibeau, C. R.; Shipps, G. W.; Bogen, S. L.; Wang, Y.; Guo, Z.; Guzi, T. J. *ACS Med. Chem. Lett.* **2014**, *5*, 572.
22. Ma, Y.; Lahue, B. R.; Shipps, G. W.; Brookes, J.; Wang, Y. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 1026.