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Discovery of novel dihydroimidazothiazole derivatives as p53–MDM2 protein–protein interaction inhibitors: Synthesis, biological evaluation and structure–activity relationships

Masaki Miyazaki [a,*], Haruko Kawato [a], Hiroyuki Naito [a], Masahiro Ikeda [a], Masaya Miyazaki [b], Mayumi Kitagawa [b], Takahiko Seki [b], Setsuko Fukutake [b], Masashi Aonuma [b], Tsunehiko Soga [a]

[a] Lead Discovery & Optimization Research Laboratories II, R&D Division, Daiichi Sankyo Co., Ltd, 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan
[b] Oncology Research Laboratories, R&D Division, Daiichi Sankyo Co., Ltd, 1-16-13 Kitakasai, Edogawa-ku, Tokyo 134-8630, Japan

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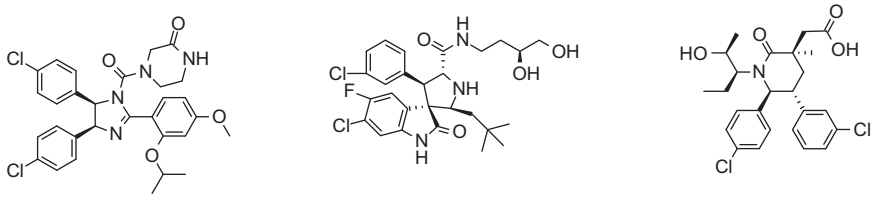
ABSTRACT

Starting with Nutlins as an initial lead, we designed and generated bicyclic scaffolds aiming to place *cis*-bischlorophenyl moiety at the equivalent location where the hydrophobic interaction with MDM2 could be expected. As a result, we discovered novel MDM2 inhibitors possessing a dihydroimidazothiazole scaffold. Further exploration of the side chains on the dihydroimidazothiazole scaffold aided by molecular modeling resulted in compounds exhibiting almost comparable in vitro potency to Nutlin-3a.

The p53 tumor suppressor protein plays an important role in the growth suppression and apoptosis to cancer cells.[1] About 50% of human cancers have wild-type p53,[2] whose functions are regulated by an overexpression or an amplification of human murine double minute 2 (MDM2) gene.[3,4] MDM2 combines with the N-terminal transcriptional activation domain of p53, and promotes export of p53 from nucleus to cytoplasm, thereby promoting proteasomal degradation of p53 via ubiquitination through its E3 ligase activity.[5–7] Thus, activation of the p53 function by the inhibition of the protein–protein interaction of p53–MDM2 is regarded as an effective approach in cancer therapy. In fact, there have been many reports regarding the relevance between MDM2 inhibition and growth inhibition of cancer cells.[8–10]

In the last ten years, various small molecules (Fig. 1) which inhibit p53–MDM2 interactions have been reported.[11–16] The p53–MDM2 binding inhibitory activity (IC_{50}) of these compounds in the reports are roughly 0.1–10 μM on a cell free assay, among which potent inhibitor reported first was Nutlin-3a (**1**) whose IC_{50} was 0.09 μM. AM-8553 (**3**), which was just reported recently, had improved potency considerably by lead optimization researches from their HTS hit compounds.[17] We also focused our attention on these compounds and investigated further in order to obtain a novel and more potent molecule as a p53–MDM2 interaction inhibitor.

We report herein the synthesis of novel p53–MDM2 interaction inhibitors having a dihydroimidazothiazole scaffold, together with their p53–MDM2 binding inhibitory activities.



1 (Nutlin-3a) **2** (MI-219) **3** (AM-8553)

Figure 1. Previously reported small molecules as p53–MDM2 inhibitors.

* Corresponding author. Tel.: +81 3 3492 3131; fax: +81 3 5740 3640.
E-mail address: miyazaki.masaki.p3@daiichisankyo.co.jp (M. Miyazaki).

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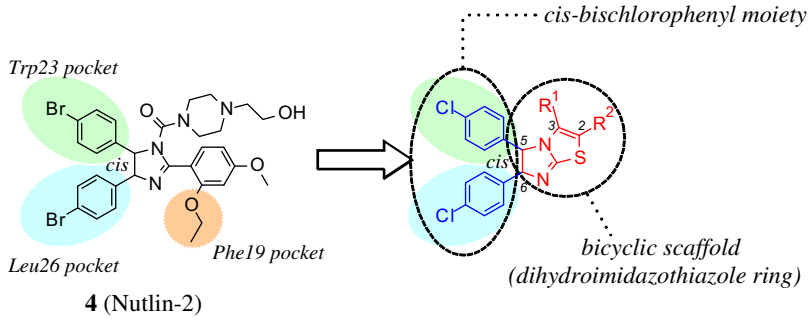
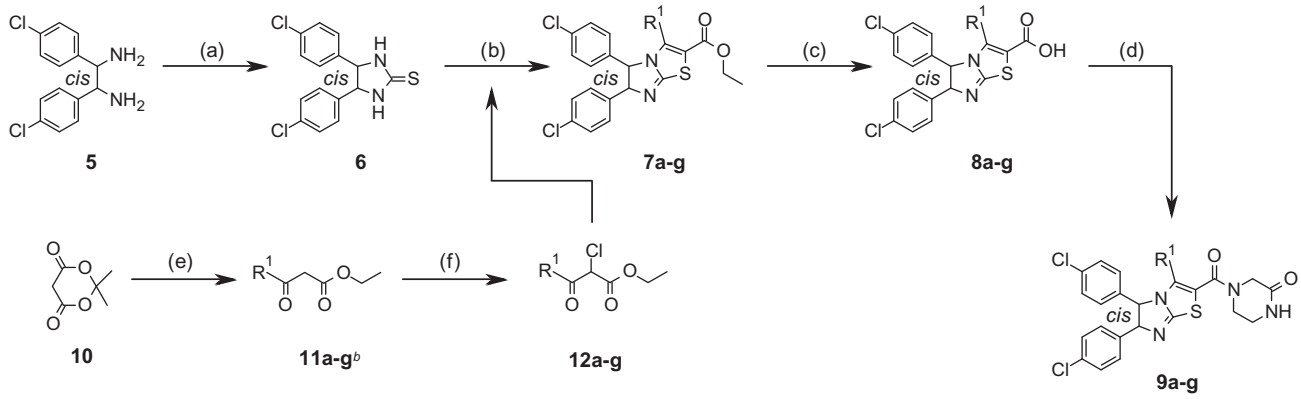


Figure 2. Designs of novel scaffold having a bicyclic skeleton.



compd[a]	R^1
11a	methyl
b	ethyl
c	*i*-propyl
d	*n*-propyl
e	*c*-propyl
f	*t*-butyl
g	$MeOCH_2$-

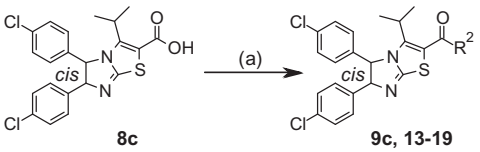
Scheme 1. Synthesis of C-3 substituted dihydroimidazothiazoles **9a**–**g**. [a]Reagents and Conditions: (a) CS_2, EtOH, reflux, 70%; (b) Compound **12a**–**g**, EtOH, reflux, 42–85%; (c) NaOHaq., EtOH, reflux,; (d) 2-oxopiperazine, EDC/HCl, Et_3N, CH_2Cl_2, 30–75% (2 steps); (e) (i) R^1-COCl, Pyridine, CH_2Cl_2, (ii) EtOH, reflux; (f) SO_2Cl_2, CH_2Cl_2, 56–58% from **10**. [b]**11e** and **11g** were just synthesized from **10**. The others were utilized commercially available.

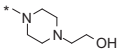
The interaction between p53 and MDM2 depends on van der Waals' forces, which is mainly accomplished by the intervention of three hydrophobic residues of p53, that is side chains of Phe19, Trp23, and Leu26.[18–20] Therefore, proficient filling of the hydrophobic pockets is very important to furnish potent inhibitors. Specifically, the substituents should be carefully placed on a scaffold so as to let them fit the pockets efficiently. Compounds displayed in Figure 1 are considered to apply the concept of the drug design. The co-crystal structure of MDM2 and Nutlin-2 (**4**) supports the analysis, in which two 4-bromophenyl groups and ethoxyphenyl moiety on Nutlin-2 corresponded to the three hydrophobic residues of p53.[11]

In the beginning of our research to obtain a novel scaffold for p53–MDM2 interaction inhibitors, we analyzed the mode of interaction between MDM2 and bis-(4-chlorophenyl) groups of Nutlin-3a (**1**). And we tried to place the *cis*-bischlorophenyl structures in proper positions by synthesizing and evaluating various bicyclic scaffolds having the moieties. We discovered that dihydroimidazothiazole derivatives hold the inhibitory activity (IC_{50} <1 μM). The lead structure is exemplified in Figure 2.

Then, our medicinal effort was moved to the optimization of the substituent at the C-3 position. Syntheses of the C-3 variants are depicted in Scheme 1. *cis*-1,2-Bis(4-chlorophenyl)ethane-1,2-diamine (**5**) was synthesized in accordance with the literature method.[21] Cyclization with CS_2,[22] followed by thiazole formation with α-chloro-β-ketoesters (**12a**–**g**),[23] provided dihydroimidazothiazole derivatives (**7a**–**g**). Compounds **11a**–**c** and **11f** are commercially available, while **11e** and **11g** were prepared via conventional methods using Meldrum's acid.[24] Hydrolysis then gave the corresponding carboxylic acids (**8a**–**g**). EDC mediated amidation with oxopiperazine[25] furnished the amide derivatives (**9a**–**g**) for screening.

As shown in Scheme 2, variants for the amide portion in place of 2-oxopiperazine were also synthesized (**13**–**19**).

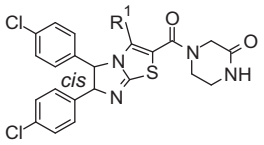


		R^2		
neutral	9c		15	
	13		16	
	14			
basic	17		19	
	18			

Scheme 2. Synthesis of C-2 substituted dihydroimidazothiazoles **13**–**19**. [a]Reagents and Conditions: (a) amine, EDC/HCl, HOBt, Et_3N, DMF or CH_2Cl_2, 30–64%.

We evaluated **9a**–**g** to investigate the influence of C-3 moiety on the activity, wherein the capacity of interacting pockets could be assessed. IC_{50} values of these compounds were measured by an ELISA in which GST-tagged MDM2 binds to p53 immobilized to the surface of a 96-well plate. As a result, compound **9c** bearing *i*-propyl moiety only showed high potency (IC_{50} = 0.26 μM), which is almost comparable to the one of Nutlin-3 (racemate, IC_{50} = 0.18 μM). Other compounds possessing smaller substituents than *i*-propyl groups such as methyl (**9a**) and ethyl (**9b**) showed weak activity. In addition, substituents such as *n*-propyl (**9d**), *c*-propyl (**9e**), methoxymethyl (**9g**) and pivaloyl (**9f**) groups also reduced the activities (IC_{50}s = 1.8–9.5 μM) (Table 1).

Table 1
Cell free p53–MDM2 inhibitory activity of analogues **9a**–**g**

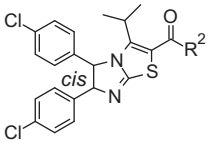


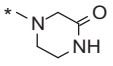
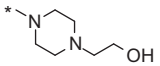
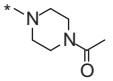
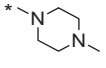
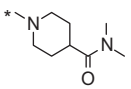
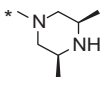
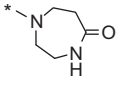
compd[a]	R^1	IC_{50} (μM)
9a	Methyl	2.7
9b	Ethyl	1.8
9c	*i*-Propyl	0.26
9d	*n*-Propyl	3.1
9e	*c*-Propyl	9.5
9f	*t*-Butyl	3.0
9g	$MeOCH_2$-	7.1
Nutlin-3		0.18[b]

[a] All compounds were racemate.
[b] *In house* data.

Next, the result of the SAR for C-2 position (amide site), in which C-3 substituent was fixed to the *i*-propyl group, is depicted in Table 2. We introduced neutral or basic 6-membered rings into C-2, referring to Nutlin's side chain, for the purpose of evaluating the efficiency of the newly generated scaffold. In this connection, the optimization of the C-2 position will be reported elsewhere in due course. As for neutral substituents, compound **15** having a morpholino group showed potency almost equivalent to **9c** (IC_{50} = 0.34 μM). *N*-acetylpiperazine (**13**) or homopiperazine (**16**) variants gave less potency at around 1 μM on IC_{50}, whilst the one for *N,N*-dimethylcarbamoylpiperidine (**14**) diminished (IC_{50} = 4.4 μM). Compounds **17** and **18,** which have basic substituents, were less active (IC_{50} = 1.8 and 1.2 μM). On the other hand, compound **19** possessing 2,5-dimethylpiperazine, which is a rather

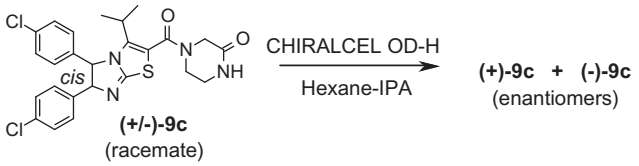
Table 2
Cell free p53–MDM2 inhibitory activity of analogues **13**–**19**



compd[a]	R^2	IC_{50} (μM)	compd[a]	R^2	IC_{50} (μM)
9c		0.26	**17**		1.8
13		0.84	**18**		1.2
14		4.4	**19**		0.14
15		0.34			
16		1.1	Nutlin-2		0.14[b]

[a] All compounds were racemate.
[b] *Lit.* data (see Ref. 11).

Table 3
Chiral separation of compound **9c**



compd	IC_{50} (μM)
(+/−)-9c	0.26
(+)-9c	0.16
(−)-9c	11.9
Nutlin-3a	0.09[a]
Nutlin-3b	13.6[a]

[a] *Lit.* data (see Ref. 11).

weaker basic moiety, showed the most potent activity (IC_{50} = 0.14 μM).

These dihydroimidazothiazole derivatives upon which we are reporting thus far were racemates, and each enantiomer could be separated by using HPLC with a chiral column. With regard to Nutlin, each enantiomer was obtained by optical resolution of the racemate, and only one of these showed potency.[11] Thus we also separated compound **9c** [equal to **(+/−)-9c**] using chiral HPLC (CHIRALCEL® OD-H, eluant: hexane-IPA), and acquired each enantiomer ((+)-**9c** and (−)-**9c**)[26] (Table 3). Compound (+)-**9c** possessed potent activity, in contrast, the IC_{50} of the other isomer (−)-**9c** was found to be 1/85 times weaker. This result suggested the same tendency as Nutlin's SAR[11]; hence the mode of placing the two halophenyl groups with the dihydroimidazothiazole scaffold was considered to be almost equivalent to the Nutlin's imidazole as expected, although the absolute configurations of (+)-**9c** and (−)-**9c** are yet to be determined. Co-crystal structure analysis of the dihydroimidazothiazoles and MDM2 has not been conducted yet, however, the mode of the interaction of our lead would be interpreted as follows: From our drug design which referred to the structural configuration of Nutlins and the result of optical resolution, it is considered that Nutlin-3a and our active lead **(+)-9c** make an interaction to MDM2 protein in the same manner. As shown in Figure 3, **(+)-9c** illustrated with an estimated configuration, was posed by superposition to the co-crystal structure of MDM2/Nutlin-2 (PDB code: 1RV1) using docking calculation. In this model, two chlorophenyl groups and the *i*-propyl group of **(+)-9c** are fitted with the three MDM2 pockets efficiently.

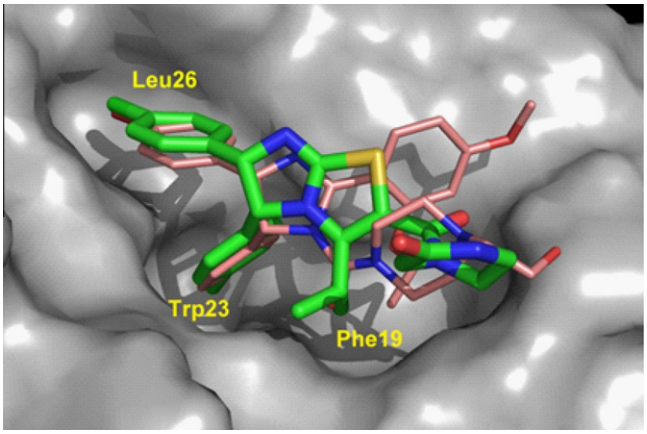


Figure 3. Predicted binding model of **(+)-9c** (estimated configuration) in green superposed on the MDM2/Nutlin-2 co-crystal structure (PDB code: 1RV1) by docking calculation. The three substituents of dihydroimidazothiazole scaffold are fitted to hydrophobic pockets. The *i*-propyl group has the same role for Phe19 of p53, which is placed on ethoxy moiety of Nutlin-2 in light red.

With regard to the substituent at the C-2 position, it seems that there is a slight difference between our lead and Nutlin's. Compound **9c** with 2-oxopiperazine as Nutlin-3 kept potent activity, whilst compound **17** having hydroxyethylpiperazine like Nutlin-2 (**4**) reduced the activity, which was inconsistent with Nutlin's SAR. For dihydroimidazothiazoles, incorporation of basic moiety at the C-2 position resulted in reduction of activity exemplified by compounds **17** and **18**, while improvement of activity was observed by introducing a weak basic moiety demonstrated by compound **19**. Thus, there seems to be more space and opportunity for derivatization at the C-2 position, and for further improvement of potency and physicochemical properties as well.

In conclusion, we discovered novel inhibitors of the p53–MDM2 interaction possessing a dihydroimidazothiazole scaffold. Especially 2-oxopiperazine (**9c**) and 2,5-dimethylpiperazine (**19**) variants possessed high activity, which was almost equivalent to Nutlin's. Moreover, since p53–MDM2 inhibitory activity was drastically influenced by altering substituents at the C-2 or C-3 positions, further optimization aiming to discover more potent inhibitors is considered feasible. Investigation and further optimization are now underway, and the results will be reported in due course.

Acknowledgment

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.08.086.

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25. Experimental details of the general procedure for the final products are provided in Supplementary data.
26. *Chiral separation*: DAICEL CHIRALCEL® OD-H, 4.6 × 250 mm, hexane: IPA = 65:35 (v/v), Flow rate: 1.0 ml/min, rt; (+)-9c: 14.5 min, $[\alpha]_D$ = +80° (*c* = 0.1, $CHCl_3$, 24 °C); (−)-9c: 21.6 min, $[\alpha]_D$ = −62° (*c* = 0.1, $CHCl_3$, 24 °C).