ACS Medicinal Chemistry Letters

pubs.acs.org/acsmedchemlett Letter

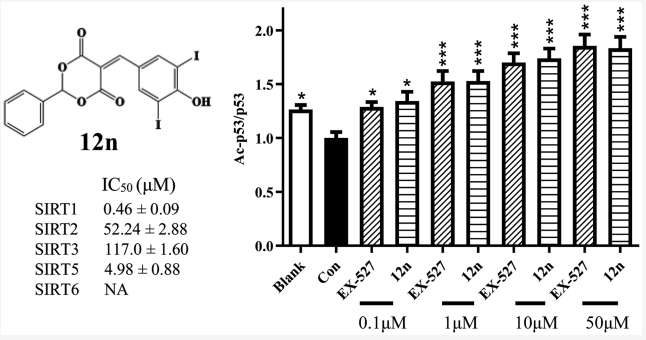
Discovery of 5-Benzylidene-2-phenyl-1,3-dioxane-4,6-diones as Highly Potent and Selective SIRT1 Inhibitors

Chunpu Li,[⊥] Sha-Sha Hu,[⊥] Lisheng Yang,[⊥] Min Wang, Jian-Dong Long, Bing Wang, Haozhen Han, Haoran Zhu, Sen Zhao, Jing-Gen Liu,* Dongxiang Liu,* and Hong Liu*

Cite This: *ACS Med. Chem. Lett.* 2021, 12, 397−403 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: SIRT1, a member of the sirtuin family, catalyzes the deacetylation of proteins with the transformation of NAD^+ into nicotinamide and 2′-*O*-acetyl-ADP-ribose. Selective SIRT1/2 inhibitors have potential application in the chemotherapy of colorectal carcinoma, prostate cancer, and myelogenous leukemia. Here we identified novel SIRT1 inhibitors with the scaffold of 5-benzylidene-2-phenyl-1,3-dioxane-4,6-dione. The most potent inhibitor **12n** displayed an IC_{50} of 460 nM and a selectivity for SIRT1 over SIRT2, SIRT3, and SIRT5 of 113.5-, 254.3-, and 10.83-fold, respectively. It did not affect the activity of SIRT6. To elucidate the inhibitory mechanism, we determined the inhibition type of the inhibitor by enzyme kinetic analysis, showing that the inhibitor was competitive to the acetyl peptide and noncompetitive to NAD^+. Further, the interaction of the inhibitor in SIRT1 was studied by using molecular docking, which was validated by the structure−activity relationship analysis of the inhibitors and the site-directed mutagenesis of SIRT1. Consistent with the *in vitro* assays, the inhibitors increased the acetylation level of p53 in a concentration-dependent manner in cells.



KEYWORDS: *SIRT1, inhibitor, structure−activity relationship, interaction, deacetylase*

The acetylation state of proteins regulates diverse biological processes and is related with age-related diseases.[1] Sirtuins are a family of NAD^+-dependent deacetylases that catalyze the deacetylation of proteins with the transformation of NAD^+ into nicotinamide and 2′-*O*-acetyl-ADP-ribose.[2] Human sirtuins include seven family members, SIRT1−7. They have different subcellular localizations and functions. SIRT1, mostly present in the nucleus, is associated with a variety of metabolic pathways and involved in diseases including metabolic syndrome and insulin resistance.[3,4] Activation of SIRT1 was found to mediate the progesterone resistance, which is believed to contribute to the infertility and progression of endometriosis.[5] Besides, SIRT1 suppresses intestinal tumorigenesis and colon cancer growth through deacetylation of *β*-catenin[6] but functions as a tumor promoter in colorectal carcinoma, prostate cancer, and myelogenous leukemia.[7−11] Selective SIRT1/2 inhibitors have potential application in the chemotherapy of these cancers.[12−16]

To date, several SIRT1 inhibitors have been discovered. For instance, Cambinol (**1**), a SIRT1/2 inhibitor, exhibits antitumor activity in the Daudi Burkitt lymphoma xenograft model (Figure 1).[17] EX-527 (Selisistat, **2**), a selective SIRT1 inhibitor, was entered into clinical trials for Huntington's disease and endometrial receptivity.[18,19] In a double-blind, randomized phase II clinical trial involving Huntington's disease patients, EX-527 was found to be safe and well-tolerated.[20] However, no

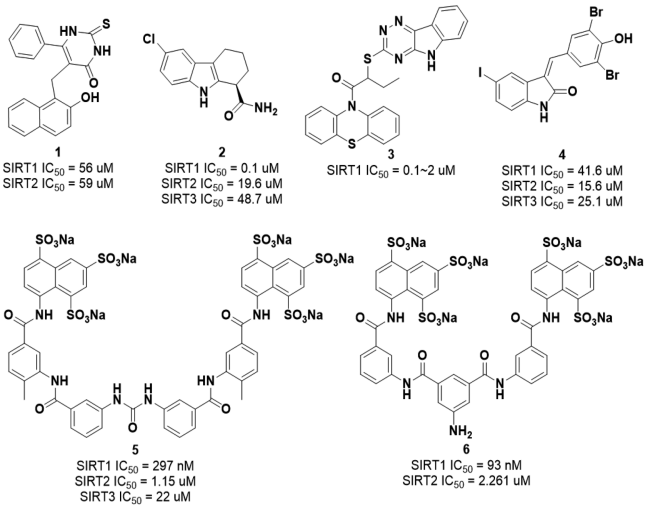
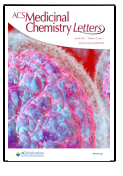


Figure 1. Representative SIRT1 inhibitors.

Received: October 18, 2020
Accepted: February 22, 2021
Published: March 1, 2021



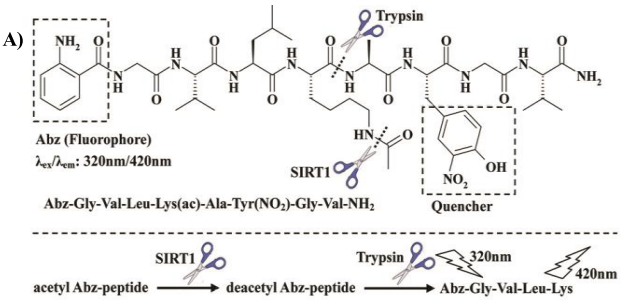
https://dx.doi.org/10.1021/acsmedchemlett.0c00559
ACS Med. Chem. Lett. 2021, 12, 397−403

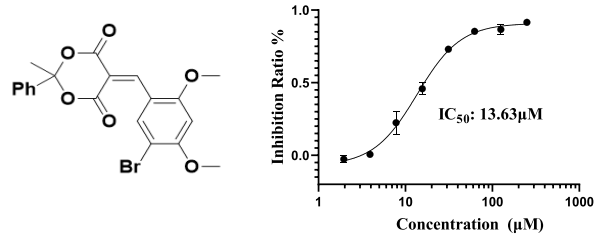
clinical benefit was observed after 2 weeks' treatment.[21] Recently, a SAFER trial for EX-527 was started in the clinical setting of *in vitro* fertilization and embryo transfer (clinical trial number NCT04184323).[22] Besides, Lu *et al.* disclosed a small molecule, Inauhzin (**3**), that promotes the p53-dependent apoptosis of cancer cells by inhibiting SIRT1.[23] Some benzylidene compounds such as 3-benzylideneindolin-2-one (GW 5074, **4**) have also been described as sirtuin inhibitors.[24−26] A screen for sirtuin activators identified suramin (**5**) as a SIRT1 inhibitor.[27,28] Further, Jung *et al.* developed a suramin analogue, NF675 (**6**), with an improved inhibitory potency.[29] Even so, the road of SIRT1 inhibitors to the clinical applications remains challenging. Structurally diverse and highly active SIRT1 inhibitors are still in urgent need, which highlights the significance of developing a novel chemotype of selective SIRT1 inhibitors.

To identify novel SIRT1 inhibitors, a fluorescent assay that detects the deacetylation of an Abz-labeled acetyl peptide by SIRT1 was employed to screen our in-house compound library (Figure 2A).[30] We found that compound **7** (Figure 2B) inhibited the activity of SIRT1 with an IC_{50} of 13.63 μM (Figure 2C). To confirm the inhibitory effect, we purchased 14 derivatives of the compound from SPECS company (https://www.specs.net) and determined their IC_{50}. The assay showed that 13 derivatives possessed the inhibitory effect with IC_{50} lower than 20 μM (Figure 2D). Among them, compound **8m** is the most active with an IC_{50} of 1.9 μM. In order to develop more potent SIRT1 inhibitors, we synthesized the derivatives of the compound and studied their structure−activity relationship (SAR).

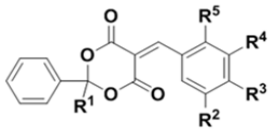
The synthesis of compounds **11a−11j** and **12a−12s** is outlined in Scheme 1. Benzaldehyde (**9**) reacts with malonic acid to obtain the 2-phenyl-1,3-dioxane-4,6-dione (**10**) in moderate yield. The condensation of 2-phenyl-1,3-dioxane-4,6-dione (**10**) and various substituted benzaldehydes gives the target compounds **11a−11j** and **12a−12s**.

We evaluated the inhibitory effect of the synthesized derivatives. EX-527, a selective SIRT1 inhibitor whose IC_{50} was originally determined with the substrate of RHKKac-AMC,[18] was used as the positive control. Herein, we used the peptide of Abz-GVLKacAY$_{NO2}$GY-NH_2 as the substrate of the enzyme assay. As summarized in Table 1, compound **11a** (R^1 = F) exhibited a potent effect against SIRT1 in low micromolar concentration (IC_{50}: 3.76 μM). Replacement of fluorine with bromide (**11b**) maintains the activity. Both compounds displayed better activities than compound **8k**, indicating that small substituent at R^1 may enhance the interaction of the compound in SIRT1. Similar result may be obtained by comparing the activities of compounds **11c** and **11i**. Meanwhile, derivatives with various substituents (such as methyl, halogens, alkoxyl) at the *ortho* position (i.e., R^2) of hydroxy (**11c−11h**) showed the inhibitory potency in the low micromolar range. Interestingly, the chlorine-substituted analogue **11e** exhibits a better activity than fluorine-substituted analogue **11d**. The bromide-substituted analogue **11f** exhibited an improved potency compared with **11e** with an IC_{50} of 1.40 μM. In addition, the atom linked to the *ortho* position has a marked impact on the activity. Halogens and oxygen seem more favorable than carbon. Beyond that, introducing an alkyl at R^2 may improve the activity (comparing compound **11b** to **11j**, compound **11g** to **11h**). This suggests that the space in SIRT1 to accommodate R^1 and R^2 should be limited. Substitution at these positions with appropriate chemical groups would enhance the interaction of the compound with SIRT1.





D)



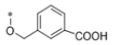
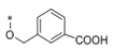
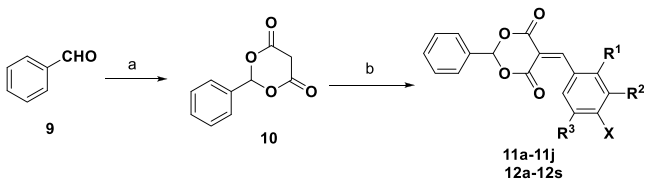
Compound	R^1	R^2	R^3	R^4	R^5	SIRT1 IC_{50} (μM)
8a	H	H	Cl	H	H	18.81±0.42
8b	H	OMe		H	H	8.14±0.97
8c	H	H		H	H	10.04±0.57
8d	Me	H	OH	OEt	H	7.92±1.79
8e	Me	H		OMe	H	12.88±2.09
8f	Me	OMe	H	H	OMe	16.75±0.71
8g	H	H	OH	OEt	H	5.49±0.95
8h	Me	H	H	H	OMe	14.38±1.86
8i	H	OiPr	H	H	H	14.01±2.01
8j	Me	H	OH	H	H	7.06±0.10
8k	H	H	OH	H	H	6.33±0.21
8l	H	H	OMe	OMe	OMe	15.79±1.36
8m	H	OMe	OH	Cl	H	1.90±0.53
8n	Me	H	H	H	H	NA

Figure 2. (A) Fluorescent assay that detects the deacetylation of an Abz-labeled acetyl peptide by SIRT1. (B) Chemical structure of compound **7**. (C) Inhibition curve of compound **7**. (D) Chemical structures and inhibitory activities of 14 derivatives from SPECS.

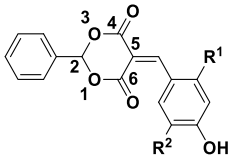
Scheme 1. Synthesis of Compounds 11a−11j and 12a−12s[a]

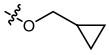


[a]Reagents and conditions: (a) malonic acid, Ac_2O, H_2SO_4 (conc), rt; (b) the appropriate benzaldehyde, NaOAc, DMSO, rt.

https://dx.doi.org/10.1021/acsmedchemlett.0c00559
ACS Med. Chem. Lett. 2021, 12, 397−403

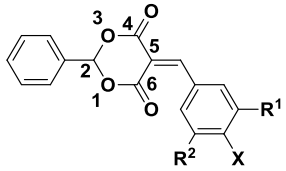
Table 1. Chemical Structures and Inhibitory Activities of Derivatives 11a−11j



Compound	R^1	R^2	IC_{50} (μM)
11a	F	H	3.76 ± 0.16
11b	Br	H	3.56 ± 0.12
11c	H	Me	5.41 ± 0.12
11d	H	F	4.65 ± 0.05
11e	H	Cl	3.84 ± 0.15
11f	H	Br	1.40 ± 0.43
11g	H		2.48 ± 0.02
11h	H	OBn	2.52 ± 0.18
11i	Me	Me	4.40 ± 0.23
11j	Br	OMe	3.52 ± 0.19
EX- 527			0.99 ± 0.10

In light of the above SAR analysis, 19 new compounds were designed and synthesized to improve the potency of **11f** (Table 2). Compounds **12a−12c** contain the same alkyl substituents at R^1 and R^2 on the phenyl group, but the lengths of the substituents are different (methyl, ethyl, and *tert*-butyl). Clearly, the enzymatic assay showed that the inhibitory activities of compounds with more steric hindrance were decreased (**12c** vs **12a**). Compound **12d** was much less potent than **12a** likely due to the electro-donating property of the methoxy group. In contrast, introduction of electro-withdrawing groups (such as ester, nitro, bromide) at R^1 led to enhanced inhibitory activities (2.4−8.0-fold) compared to compound **12d**. Notably, compounds **12i** and **12j** potently inhibited SIRT1 with IC_{50} values of 1.31 and 1.14 μM, respectively. In addition, diiodo-substituted analogue **12n** exhibited improved potency with IC_{50} of 0.46 μM, whereas dichloro-substituted and dibromo-substituted analogues **12l** and **12m** showed slightly weaker potency. The IC_{50} of the compounds displayed a decreasing trend of F > Cl > Br > I. Besides, compounds **12o−12q** containing halogens and benzyloxy/methoxy at the phenol group showed the IC_{50} of 0.68, 0.61, and 0.89 μM, respectively, suggesting that introduction of halogens at the *ortho* position of pheno is essential for the activity. Moreover, replacement of hydroxy by amino group reduced the potency dramatically (compounds **12r** and **12s**), indicating that the hydroxy is important for the interaction of the compounds with SIRT1.

Table 2. Chemical Structures and Inhibitory Activities of Derivatives 12a−12s



Compound	R^1	R^2	X	IC_{50} (μM)
12a	Me	Me	OH	3.07 ± 0.17
12b	Et	Et	OH	3.10 ± 0.15
12c	*t*-Bu	*t*-Bu	OH	18.96 ± 0.10
12d	OMe	OMe	OH	11.90 ± 2.62
12e	Bn	OMe	OH	2.47 ± 0.10
12f	COOBn	OMe	OH	2.66 ± 0.09
12g	NO_2	OEt	OH	3.69 ± 0.10
12h	NO_2	OMe	OH	3.49 ± 0.11
12i	Br	OMe	OH	1.31 ± 0.26
12j	Br	OEt	OH	1.14 ± 0.08
12k	F	F	OH	4.54 ± 0.03
12l	Cl	Cl	OH	1.20 ± 0.09
12m	Br	Br	OH	0.70 ± 0.03
12n	I	I	OH	0.46 ± 0.09
12o	OBn	Br	OH	0.68 ± 0.07
12p	OBn	I	OH	0.61 ± 0.06
12q	OMe	I	OH	0.89 ± 0.01
12r	Br	Br	NH_2	7.63 ± 0.11
12s	I	I	NH_2	10.21 ± 0.17

As each sirtuin has its distinct roles in the physiological process, selectivity among the sirtuins should be considered when developing SIRT1 inhibitors. Thus, we determined the inhibitory potencies of our compounds against SIRT2, SIRT3, SIRT5, and SIRT6. As shown in Table 3, **12i, 12l, 12n**, and **12q** exhibited significant selectivity for SIRT1 over SIRT2, SIRT3, SIRT5, and SIRT6. The most potent inhibitor **12n** displayed a SIRT1 selectivity over SIRT2, SIRT3, or SIRT5 of 113.5-, 254.3-, or 10.83-fold, respectively. All compounds did not affect the activity of SIRT6. In comparison, EX-527 displayed a SIRT1 selectivity over SIRT2 of only 8.78-fold and over SIRT3 of 29.47-fold, indicating that compound **12n** is a very potent SIRT1 inhibitor with high selectivity.

As known, the deacetylation catalyzed by SIRT1 is a bisubstrates reaction (i.e., the acetyl lysine and NAD^+). The acetyl lysine binds to the D-site whereas NAD^+ occupies the A-, B-, and C-sites in SIRT1.[31] To unveil the inhibitory mechanism, we determined the inhibition type of compound **12i** and examined how the inhibitor affects the binding of acetyl substrate and NAD^+ in SIRT1.

From the Lineweaver−Burk plot of the acetyl substrate at various concentrations of **12i** (Figure 3B), we may see that the lines intersect in the third quadrant, indicating that the initial velocity V_0 and the apparent Michaelis−Menten constant K_m of the acetyl substrate decrease with the increasing concentration of **12i**. Thus, compound **12i** is a mix-type competitive inhibitor for the acetyl lysine. It may occupy the D-site, which is the binding site of the acetyl lysine in SIRT1. Meanwhile, the Lineweaver−Burk plot of NAD^+ shows that the lines intersect at the horizontal axis (Figure 3D), indicating that the initial velocity V_0 decreases but the apparent Michaelis−Menten constant K_m of NAD^+ remains unchanged with the increasing concentration of **12i**. Therefore, compound **12i** did not affect the interaction of NAD^+ in SIRT1. It is a noncompetitive inhibitor for NAD^+ and should not occupy the binding sites of NAD^+.

To elucidate the inhibitory mechanism, we simulated the interaction of **12i** in SIRT1 (PDB ID: 4I5I) by using swissdock (http://www.swissdock.ch).[32] SIRT1 contains a catalytic core constituted by a Rossmann-fold domain and a Zn^{2+}-binding domain. The acetyl lysine binds to the cleft enclosed by two domains (i.e., D-site). As shown in Figure 4, compound **12i** occupies the binding site of acetyl lysine. A hydrophobic

https://dx.doi.org/10.1021/acsmedchemlett.0c00559
ACS Med. Chem. Lett. 2021, 12, 397−403

Table 3. Inhibitory Activities of Our Compounds and EX-527 against SIRT1, SIRT2, SIRT3, SIRT5, and SIRT6 (IC_{50}: μM)[a]

	SIRT1	SIRT2	SIRT3	SIRT5	SIRT6
12i	1.31 ± 0.26	54.04 ± 6.3	136.70 ± 14.30	19.84 ± 1.24	NA
12l	1.20 ± 0.09	56.44 ± 0.44	171.80 ± 13.60	37.42 ± 1.47	NA
12n	0.46 ± 0.09	52.24 ± 2.88	117.0 ± 1.60	4.98 ± 0.88	NA
12q	0.89 ± 0.01	49.45 ± 5.95	135.80 ± 14.70	22.45 ± 0.78	NA
EX-527	0.99 ± 0.10	8.69 ± 0.08	29.18 ± 1.60	NA	NA

[a]NA means "not active".

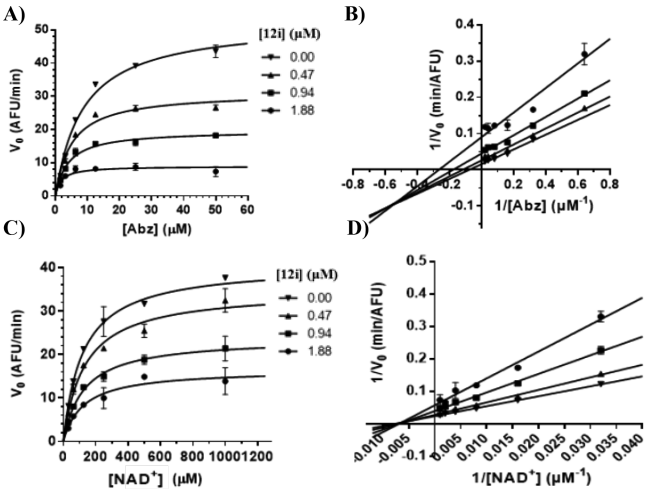


Figure 3. Enzyme kinetic study of compound **12i**. (A) Michaelis–Menten plot of the Abz-labeled acetyl peptide at various concentrations of **12i** and 1 mM of NAD^+. (B) Lineweaver–Burk plot of the Abz-labeled acetyl peptide at various concentrations of **12i**. (C) Michaelis–Menten plot of NAD^+ at various concentrations of **12i** and 30 μM of the Abz-labeled acetyl peptide. (D) Lineweaver–Burk plot of NAD^+ at various concentrations of **12i**.

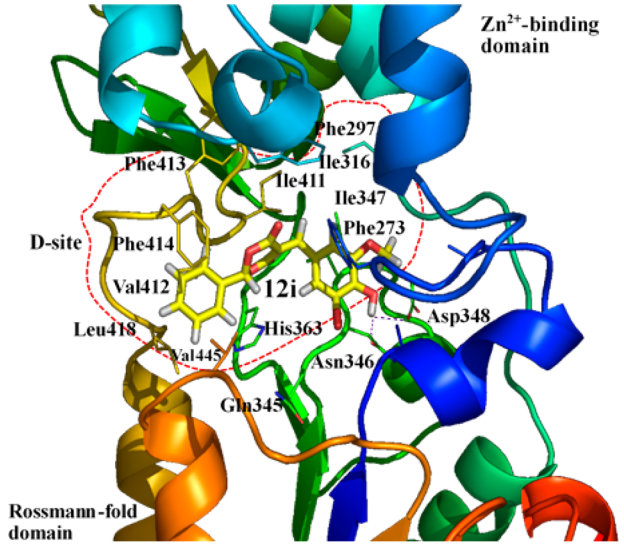


Figure 4. Interaction of compound **12i** in SIRT1 (dash line: H-bond).

interaction was formed between the phenyl at the C2 position of **12i** and the residues Phe413, Phe414, and Val445 of SIRT1. The benzylidene at the C5 position of the inhibitor is in the vicinity of Ile347 and forms an edge-to-face $\pi-\pi$ interaction with Phe273. Substitution with bromine atom may cause electron deficiency of the benzene ring of benzylidene and enhance the $\pi-\pi$ interaction between compound **12i** and Phe273, which may explain the positive correlation between halogen substitution at the benzylidene and the inhibitory potency of inhibitors. In addition, the hydroxy at the benzene ring of benzylidene forms hydrogen-bonds with the side-chains of Asn346 and Asp348. Substitution of the hydroxy with amino dramatically reduced the inhibitory activity (Table 2).

To validate the binding mode of **12i** in SIRT1, the residues Phe273, Asn346, Ile347, Asp348, and Phe414 of SIRT1 were selected for single-site mutation. The dissociation equilibrium constant K_i of compound **12i** with SIRT1, $SIRT1^{F273L}$, $SIRT1^{N346A}$, $SIRT1^{I347A}$, $SIRT1^{D348A}$, or $SIRT1^{F414A}$ mutant was determined by the secondary plots of the kinetics data of **12i** (Figure 5). We may see that the K_i of **12i** for wild type SIRT1 is

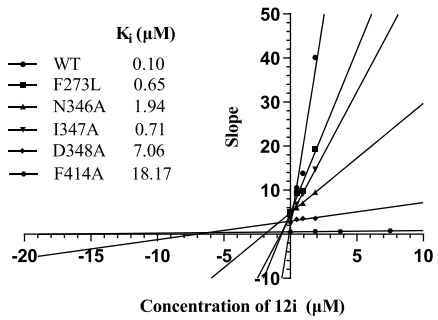


Figure 5. Secondary plots for determining K_i of **12i** for wild-type SIRT1 and its mutants. The slopes of the Lineweaver–Burk plot of NAD^+ for wild-type SIRT1 (Figure 3D) and the mutants (Figure S3) were plotted versus the concentration of **12i**. The values of K_i were calculated from the x intercepts of the lines.

0.10 μM whereas the K_i values for $SIRT1^{F273L}$, $SIRT1^{N346A}$, $SIRT1^{I347A}$, $SIRT1^{D348A}$, and $SIRT1^{F414A}$ mutants are 0.65, 1.94, 0.71, 7.06, and 18.17 μM, respectively. These demonstrated that Phe273, Asn346, Ile347, Asp348, and Phe414 did interact with compound **12i**, among which Phe414 had the greatest contribution to the interaction with the compound.

All-trans retinoic acid (ATRA) had been shown to induce the differentiation of SH-SY5Y cells through activation of SIRT1.[33] As p53 is a substrate of SIRT1, modulation of SIRT1 activity may change the acetylation state of p53.[34] To examine whether our inhibitors are active in cells, we treated SH-SY5Y cells with ATRA and the inhibitors and detected the expression of acetyl p53 by Western blot. As shown in Figure 6A, treatment of SH-SY5Y cells with ATRA alone (lane: control) decreased the expression level of acetyl p53 (compared to the lane of blank), while incubation of the cells with ATRA and 10 μM of compounds **12i**, **12q**, **8m**, **12l**, or **12n** for 72 h significantly increased the acetyl-p53 level. To further confirm the result, we examined the inhibitory activity of **12n** through a titration assay with the concentrations of 0.1, 1, 10, and 50 μM. The result showed that **12n** increased the ratio of acetyl-p53 to p53 in a

https://dx.doi.org/10.1021/acsmedchemlett.0c00559
ACS Med. Chem. Lett. 2021, 12, 397–403

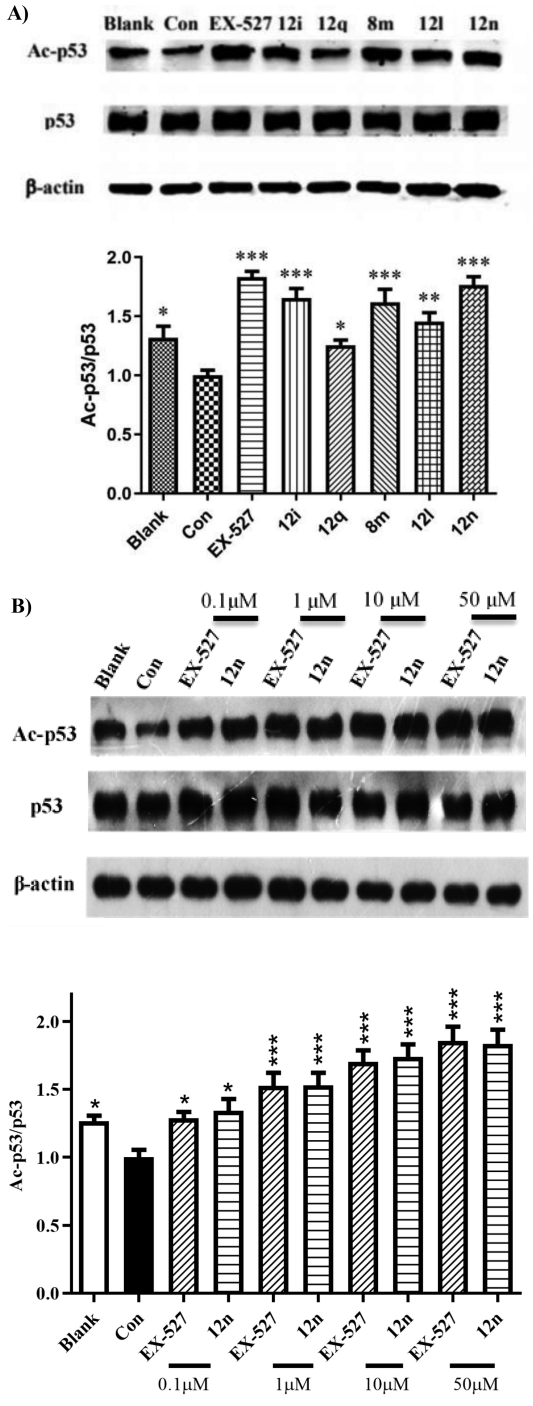


Figure 6. Effects of inhibitors on acetylation of p53. (A) The control group was treated with 10 μM of ATRA while the blank was untreated. The other groups were cotreated with 10 μM of ATRA and inhibitor (EX-527, **12i**, **12q**, **8m**, **12l** or **12n**). (B) **12n** showed a dose-dependent effect on the acetylation level of p53. Error bars represent mean ± SEM ($N = 5$). *$p < 0.05$, ***$p < 0.001$, compared with the control group. One-way ANOVA with Newman-Keuls post hoc test.

dose-dependent manner (Figure 6B). It displayed a comparable activity as EX-527 in cells.

In summary, here we identified novel SIRT1 inhibitors with the scaffold of 5-benzylidene-2-phenyl-1,3-dioxane-4,6-dione. The derivatives of the inhibitor were synthesized and evaluated for their SIRT1 inhibitory activity. Based on the SAR of the derivatives, we developed a highly potent and selective SIRT1 inhibitor, **12n**. The enzyme kinetic analysis showed that the inhibitor was competitive to the acetyl peptide and non-competitive to NAD^+. We also simulated the interaction of the inhibitor in SIRT1 by molecular docking, which was supported by the SAR analysis of the derivatives and the SIRT1 mutagenesis studies. In line with the *in vitro* assays, the inhibitor significantly increased the acetylation of p53 in cells.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.0c00559.

Experimental details, characterization of all compounds, and the Lineweaver−Burke plots of NAD^+ for SIRT1 and its mutants (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Jing-Gen Liu − *Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China*; Email: jgliu@simm.ac.cn

Dongxiang Liu − *Department of Pharmacology III, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China; University of Chinese Academy of Sciences, Beijing 100049, China*; orcid.org/0000-0002-7639-5497; Email: dxl@mail.shcnc.ac.cn

Hong Liu − *State Key Laboratory of Drug Research and Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China; University of Chinese Academy of Sciences, Beijing 100049, China; School of Pharmaceutical Science and Technology, Hangzhou Institute for Advanced Study, UCAS, Hangzhou 310024, China*; orcid.org/0000-0003-3685-6268; Phone: +86-21-50806600; Email: hliu@simm.ac.cn; Fax: +86-21-50807088

Authors

Chunpu Li − *State Key Laboratory of Drug Research and Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China; School of Pharmaceutical Science and Technology, Hangzhou Institute for Advanced Study, UCAS, Hangzhou 310024, China*

Sha-Sha Hu − *Department of Pharmacology III, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China; University of Chinese Academy of Sciences, Beijing 100049, China*

Lisheng Yang − *State Key Laboratory of Drug Research and Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China*

Min Wang − *Department of Pharmacology III, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China*

Jian-Dong Long − *Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China*

Bing Wang − *Department of Pharmacology III, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China; University of Chinese Academy of Sciences, Beijing 100049, China*

https://dx.doi.org/10.1021/acsmedchemlett.0c00559
ACS Med. Chem. Lett. 2021, 12, 397−403

Haozhen Han − *Department of Pharmacology III, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China; University of Chinese Academy of Sciences, Beijing 100049, China*

Haoran Zhu − *State Key Laboratory of Drug Research and Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China; University of Chinese Academy of Sciences, Beijing 100049, China*

Sen Zhao − *State Key Laboratory of Drug Research and Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsmedchemlett.0c00559

Author Contributions

[⊥]C.L., S.-S.H., and L.Y. contributed equally. The manuscript was written through contributions of all authors.

Funding

The work was supported by National Natural Science Fund of China (Grants 21672233, 81973239, 81620108027, and 21632008), the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA12020375 and XDA12050411), SA-SIBS Scholarship Program, and the Youth Innovation Promotion Association CAS.

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

SIRT1, silent mating type information regulation 2 homologue-1; NAD^+, nicotinamide adenine dinucleotide; Ac_2O, acetic anhydride; SAR, structure−activity relationship; ATRA, all-trans retinoic acid

■ REFERENCES

(1) Li, T. M.; Coan, J. P.; Krajewski, K.; Zhang, L. C.; Elias, J. E.; Strahl, B. D.; Gozani, O.; Chua, K. F. Binding to medium and long chain fatty acyls is a common property of heat and arm repeat modules. *Sci. Rep.* **2019**, *9*, 14226.

(2) Imai, S.; Armstrong, C. M.; Kaeberlein, M.; Guarente, L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* **2000**, *403*, 795−800.

(3) Cohen, H. Y.; Miller, C.; Bitterman, K. J.; Wall, N. R.; Hekking, B.; Kessler, B.; Howitz, K. T.; Gorospe, M.; de Cabo, R.; Sinclair, D. A. Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. *Science* **2004**, *305*, 390−392.

(4) Mitchell, S. J.; Martin-Montalvo, A.; Mercken, E. M.; Palacios, H. H.; Ward, T. M.; Abulwerdi, G.; Minor, R. K.; Vlasuk, G. P.; Ellis, J. L.; Sinclair, D. A.; Dawson, J.; Allison, D. B.; Zhang, Y.; Becker, K. G.; Bernier, M.; de Cabo, R. The SIRT1 activator SRT1720 extends lifespan and improves health of mice fed a standard diet. *Cell Rep.* **2014**, *6*, 836−843.

(5) Yoo, J. Y.; Kim, T. H.; Fazleabas, A. T.; Palomino, W. A.; Ahn, S. H.; Tayade, C.; Schammel, D. P.; Young, S. L.; Jeong, J.-W.; Lessey, B. A. KRAS activation and over-expression of SIRT1/BCL6 contributes to the pathogenesis of endometriosis and progesterone resistance. *Sci. Rep.* **2017**, *7*, 6765.

(6) Firestein, R.; Blander, G.; Michan, S.; Oberdoerffer, P.; Ogino, S.; Campbell, J.; Bhimavarapu, A.; Luikenhuis, S.; de Cabo, R.; Fuchs, C.; Hahn, W. C.; Guarente, L. P.; Sinclair, D. A. The SIRT1 deacetylase suppresses intestinal tumorigenesis and colon cancer growth. *PLoS One* **2008**, *3*, No. e2020.

(7) Bradbury, C. A.; Khanim, F. L.; Hayden, R.; Bunce, C. M.; White, D. A.; Drayson, M. T.; Craddock, C.; Turner, B. M. Histone deacetylases in acute myeloid leukaemia show a distinctive pattern of expression that changes selectively in response to deacetylase inhibitors. *Leukemia* **2005**, *19*, 1751−1759.

(8) Huffman, D. M.; Grizzle, W. E.; Bamman, M. M.; Kim, J.-s.; Eltoum, I. A.; Elgavish, A.; Nagy, T. R. SIRT1 is significantly elevated in mouse and human prostate cancer. *Cancer Res.* **2007**, *67*, 6612−6618.

(9) Li, L.; Wang, L.; Li, L.; Wang, Z.; Ho, Y.; McDonald, T.; Holyoake, T. L.; Chen, W.; Bhatia, R. Activation of p53 by SIRT1 inhibition enhances elimination of CML leukemia stem cells in combination with imatinib. *Cancer Cell* **2012**, *21*, 266−281.

(10) Stünkel, W.; Peh, B. K.; Tan, Y. C.; Nayagam, V. M.; Wang, X.; Salto-Tellez, M.; Ni, B.; Entzeroth, M.; Wood, J. Function of the SIRT1 protein deacetylase in cancer. *Biotechnol. J.* **2007**, *2*, 1360−1368.

(11) Yamakuchi, M.; Ferlito, M.; Lowenstein, C. J. MiR-34a repression of SIRT1 regulates apoptosis. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 13421−13426.

(12) Huhtiniemi, T.; Salo, H. S.; Suuronen, T.; Poso, A.; Salminen, A.; Leppänen, J.; Jarho, E.; Lahtela-Kakkonen, M. Structure-based design of pseudopeptidic inhibitors for SIRT1 and SIRT2. *J. Med. Chem.* **2011**, *54* (13), 6456−6468.

(13) Kumar, A.; Chauhan, S. How much successful are the medicinal chemists in modulation of SIRT1: A critical review. *Eur. J. Med. Chem.* **2016**, *119*, 45−69.

(14) Monaldi, D.; Rotili, D.; Lancelot, J.; Marek, M.; Wossner, N.; Lucidi, A.; Tomaselli, D.; Ramos-Morales, E.; Romier, C.; Pierce, R. J.; Mai, A.; Jung, M. Structure-Reactivity relationships on substrates and inhibitors of the lysine deacylase sirtuin 2 from schistosoma mansoni (SmSirt2). *J. Med. Chem.* **2019**, *62*, 8733−8759.

(15) Peck, B.; Chen, C. Y.; Ho, K. K.; Di Fruscia, P.; Myatt, S. S.; Coombes, R. C.; Fuchter, M. J.; Hsiao, C. D.; Lam, E. W. SIRT inhibitors induce cell death and p53 acetylation through targeting both SIRT1 and SIRT2. *Mol. Cancer Ther.* **2010**, *9*, 844−855.

(16) Rotili, D.; Tarantino, D.; Nebbioso, A.; Paolini, C.; Huidobro, C.; Lara, E.; Mellini, P.; Lenoci, A.; Pezzi, R.; Botta, G.; Lahtela-Kakkonen, M.; Poso, A.; Steinkühler, C.; Gallinari, P.; De Maria, R.; Fraga, M.; Esteller, M.; Altucci, L.; Mai, A. Discovery of salermide-related sirtuin inhibitors: binding mode studies and antiproliferative effects in cancer cells. *J. Med. Chem.* **2012**, *55* (24), 10937−10947.

(17) Heltweg, B.; Gatbonton, T.; Schuler, A. D.; Posakony, J.; Li, H.; Goehle, S.; Kollipara, R.; DePinho, R. A.; Gu, Y.; Simon, J. A.; Bedalov, A. Antitumor activity of a small-molecule inhibitor of human silent information regulator 2 enzymes. *Cancer Res.* **2006**, *66*, 4368−4377.

(18) Napper, A. D.; Hixon, J.; McDonagh, T.; Keavey, K.; Pons, J.-F.; Barker, J.; Yau, W. T.; Amouzegh, P.; Flegg, A.; Hamelin, E.; Thom-As, R. J.; Kates, M.; Jones, S.; Navia, M. A.; Saunders, J. O.; DiSte-Fano, P. S.; Curtis, R. Discovery of indoles as potent and selective inhibitors of the deacetylase SIRT1. *J. Med. Chem.* **2005**, *48*, 8045−8054.

(19) Zhao, X.; Allison, D.; Condon, B.; Zhang, F.; Gheyi, T.; Zhang, A.; Ashok, S.; Russell, M.; MacEwan, I.; Qian, Y.; Jamison, J. A.; Luz, J. G. The 2.5 Å crystal structure of the SIRT1 catalytic domain bound to nicotinamide adenine dinucleotide (NAD^+) and an indole (EX527 analogue) reveals a novel mechanism of histone deacetylase inhibition. *J. Med. Chem.* **2013**, *56*, 963−969.

(20) Sussmuth, S. D.; Haider, S.; Landwehrmeyer, G. B.; Farmer, R.; Frost, C.; Tripepi, G.; Andersen, C. A.; Di Bacco, M.; Lamanna, C.; Diodato, E.; Massai, L.; Diamanti, D.; Mori, E.; Magnoni, L.; Drey-Haupt, J.; Schiefele, K.; Craufurd, D.; Saft, C.; Rudzinska, M.; Ry-Glewicz, D.; Orth, M.; Brzozy, S.; Baran, A.; Pollio, G.; Andre, R.; Tabrizi, S. J.; Darpo, B.; Westerberg, G.; Consortium, P. An exploratory double-blind, randomized clinical trial with selisistat, a SirT1 inhibitor, in patients with Huntington's disease. *Br. J. Clin. Pharmacol.* **2015**, *79*, 465−476.

(21) Broussy, S.; Laaroussi, H.; Vidal, M. Biochemical mechanism and biological effects of the inhibition of silent information regulator 1 (SIRT1) by EX-527 (SEN0014196 or selisistat). *J. Enzyme Inhib. Med. Chem.* **2020**, *35*, 1124−1136.

(22) SIRT-1 Antagonism for Endometrial Receptivity (SAFER). 2019. Retrieved from https://clinicaltrials.gov/ct2/show/NCT04184323 (Identification No. NCT04184323).
(23) Zhang, Q.; Zeng, S. X.; Zhang, Y.; Zhang, Y.; Ding, D.; Ye, Q.; Meroueh, S. O.; Lu, H. A small molecule Inauhzin inhibits SIRT1 activity and suppresses tumour growth through activation of p53. *EMBO Mol. Med.* **2012**, *4*, 298−312.
(24) Trapp, J.; Jochum, A.; Meier, R.; Saunders, L.; Marshall, B.; Kunick, C.; Verdin, E.; Goekjian, P.; Sippl, W.; Jung, M. Adenosine mimetics as inhibitors of NAD^+-dependent histone deacetylases, from kinase to sirtuin inhibition. *J. Med. Chem.* **2006**, *49*, 7307−7316.
(25) Huber, K.; Schemies, J.; Uciechowska, U.; Wagner, J. M.; Rumpf, T.; Lewrick, F.; Süss, R.; Sippl, W.; Jung, M.; Bracher, F. Novel 3-arylideneindolin-2-ones as inhibitors of NAD^+ -dependent histone deacetylases (sirtuins). *J. Med. Chem.* **2010**, *53*, 1383−1386.
(26) Uciechowska, U.; Schemies, J.; Neugebauer, R. C.; Huda, E. M.; Schmitt, M. L.; Meier, R.; Verdin, E.; Jung, M.; Sippl, W. Thiobarbiturates as sirtuin inhibitors: virtual screening, free-energy calculations, and biological testing. *ChemMedChem* **2008**, *3*, 1965−1976.
(27) Howitz, K. T.; Bitterman, K. J.; Cohen, H. Y.; Lamming, D. W.; Lavu, S.; Wood, J. G.; Zipkin, R. E.; Chung, P.; Kisielewski, A.; Zhang, L. L.; Scherer, B.; Sinclair, D. A. Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. *Nature* **2003**, *425*, 191−196.
(28) Schuet, A.; Min, J.; Antoshenko, T.; Wang, C. L.; Hasani, A. A.; Dong, A.; Loppnau, P.; Vedadi, M.; Bochkarev, A.; Sternglanz, R.; Plotnikov, A. N. Structural Basis of Inhibition of the Human NAD^+-Dependent Deacetylase SIRT5 by Suramin. *Structure* **2007**, *15*, 377−389.
(29) Trapp, J.; Meier, R.; Hongwiset, D.; Kassack, M. U.; Sippl, W.; Jung, M. Structure-activity studies on suramin analogues as inhibi-tors of NAD^+-dependent histone deacetylases (sirtuins). *ChemMedChem* **2007**, *2*, 1419−1431.
(30) Roessler, C.; Tüting, C.; Meleshin, M.; Steegborn, C.; Schutkowski, M. A novel continuous assay for the deacylase sirtuin 5 and other deacetylases. *J. Med. Chem.* **2015**, *58*, 7217−7223.
(31) Davenport, A. M.; Huber, F. M.; Hoelz, A. Structural and functional analysis of human SIRT1. *J. Mol. Biol.* **2014**, *426* (3), 526−541.
(32) Grosdidier, A.; Zoete, V.; Michielin, O. Fast docking using the CHARMM force field with EADock DSS. *J. Comput. Chem.* **2011**, *32*, 2149−2159.
(33) Kim, M. J.; Ahn, K.; Park, S. H.; Kang, H. J.; Jang, B. G.; Oh, S. J.; Oh, S. M.; Jeong, Y. J.; Heo, J. I.; Suh, J. G.; Lim, S. S.; Ko, Y. J.; Huh, S. O.; Kim, S. C.; Park, J. B.; Kim, J.; Kim, J. I.; Jo, S. A.; Lee, J. Y. SIRT1 regulates tyrosine hydroxylase expression and differentiation of neuroblastoma cells via FOXO3a. *FEBS Lett.* **2009**, *583*, 1183−1188.
(34) Peck, B.; Chen, C. Y.; Ho, K. K.; Di Fruscia, P.; Myatt, S. S.; Coombes, R. C.; Fuchter, M. J.; Hsiao, C. D.; Lam, E. W. F. SIRT inhibitors induce cell death and p53 acetylation through targeting both SIRT1 and SIRT2. *Mol. Cancer Ther.* **2010**, *9*, 844−855.

https://dx.doi.org/10.1021/acsmedchemlett.0c00559
ACS Med. Chem. Lett. 2021, 12, 397−403