Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

Discovery of 4-((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-1′-ethyl-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamido)bicyclo[2.2.2]octane-1-carboxylic Acid (AA-115/APG-115): A Potent and Orally Active Murine Double Minute 2 (MDM2) Inhibitor in Clinical Development

Angelo Aguilar,[†] Jianfeng Lu,[†] Liu Liu,[†] Ding Du,[†] Denzil Bernard,[†] Donna McEachern,[†] Sally Przybranowski,[†] Xiaoqin Li,[‡] Ruijuan Luo,[‡] Bo Wen,[‡] Duxin Sun,[‡] Hengbang Wang,[§,#] Jianfeng Wen,[§,#] Guangfeng Wang,[§,#] Yifan Zhai,[§,#] Ming Guo,[§,#] Dajun Yang,[§,#,±] and Shaomeng Wang*[,†]

[†]University of Michigan Comprehensive Cancer Center, and Departments of Internal Medicine, Pharmacology, and Medicinal Chemistry, University of Michigan, 1600 Huron Parkway, Ann Arbor, Michigan 48109, United States

[‡]Department of Pharmaceutical Sciences, College of Pharmacy, University of Michigan, Ann Arbor, Michigan 48109, United States

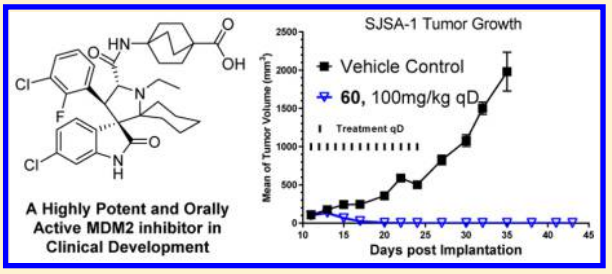
[§]Jiangsu Ascentage Biomed Development Inc., China Medical City, Taizhou, Jiangsu 225300, China

[#]Suzhou Ascentage Pharma Inc., Suzhou, Jiangsu 215123, China

[±]Department of Experimental Research, Sun Yat-Sen University Cancer Center, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, 651 Dongfeng Road East, Guangzhou, China

Supporting Information

ABSTRACT: We previously reported the design of spirooxindoles with two identical substituents at the carbon-2 of the pyrrolidine core as potent MDM2 inhibitors. In this paper we describe an extensive structure−activity relationship study of this class of MDM2 inhibitors, which led to the discovery of **60** (AA-115/APG-115). Compound **60** has a very high affinity to MDM2 (K_i < 1 nM), potent cellular activity, and an excellent oral pharmacokinetic profile. Compound **60** is capable of achieving complete and long-lasting tumor regression in vivo and is currently in phase I clinical trials for cancer treatment.



INTRODUCTION

The tumor suppressor function of p53 is compromised in essentially all human cancers. In about half of human cancers, *TP53*, the gene encoding p53 protein, is mutated or deleted, and this inactivates the tumor suppressor function of p53.[1] In the remaining 50%, p53 retains its wild-type status but its function is effectively inhibited by the murine double minute 2 (MDM2) protein through a direct protein−protein interaction.[2−8] Through direct binding, the MDM2 protein blocks the transactivation domain of p53, transports p53 from the nucleus to the cytoplasm, and ubiquitinates p53 for proteasomal degradation.[6] Small-molecule inhibitors designed to block the MDM2−p53 interaction (hereafter called MDM2 inhibitors) can liberate the tumor suppressor function of p53[9−13] and may have a promising therapeutic potential for cancer treatment. Intense research efforts have resulted in advancement of several potent, selective, non-peptide MDM2 inhibitors (**1**−**5**),[14−20] shown in Figure 1, into clinical development.

Our laboratory has designed and optimized spirooxindoles as a new class of MDM2 inhibitors and has advanced compound **2** (MI-77301) into clinical development.[18] One shortcoming of **2** and its earlier analogues that we have found is that they slowly isomerize in solution.[21,22] To overcome this stability issue, we recently reported the design of new spirooxindoles containing two identical substituents at C-2 of the pyrrolidine ring, which can undergo a rapid and irreversible conversion to a single diastereoisomer.[21,22] In the present study, we report an extensive structure−activity relationship (SAR) study for this class of MDM2 inhibitors. This study has led to the discovery of **60** (AA-115/APG-115), a highly potent, chemically stable and efficacious MDM2 inhibitor, which has entered clinical development for cancer treatment (ClinicalTrials.gov identifier for **60**: NCT02935907).

Received: November 17, 2016
Published: March 24, 2017

DOI: 10.1021/acs.jmedchem.6b01665
J. Med. Chem. 2017, 60, 2819−2839

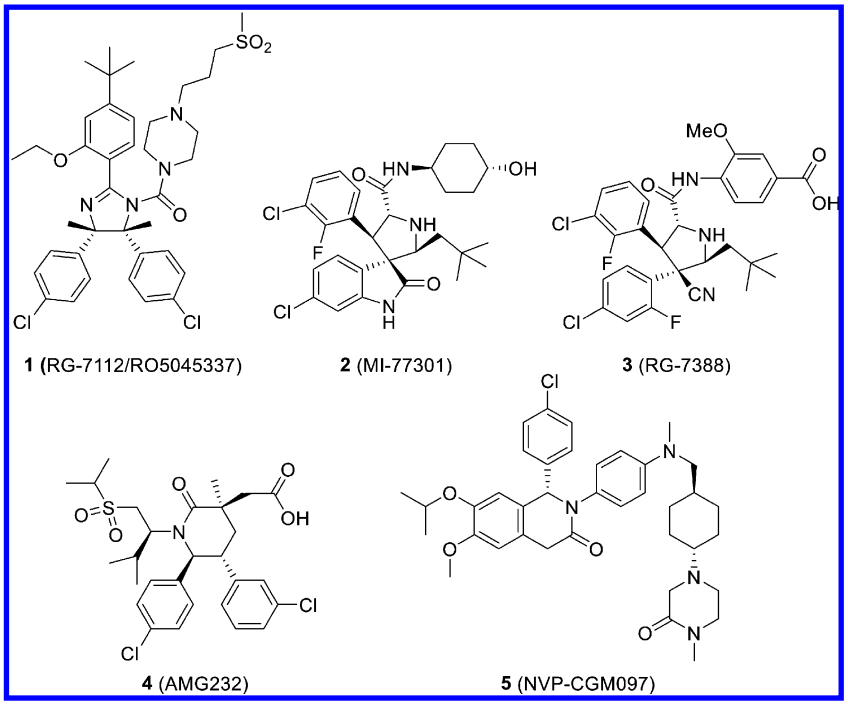


Figure 1. Chemical structures of representative MDM2 inhibitors in clinical trials.

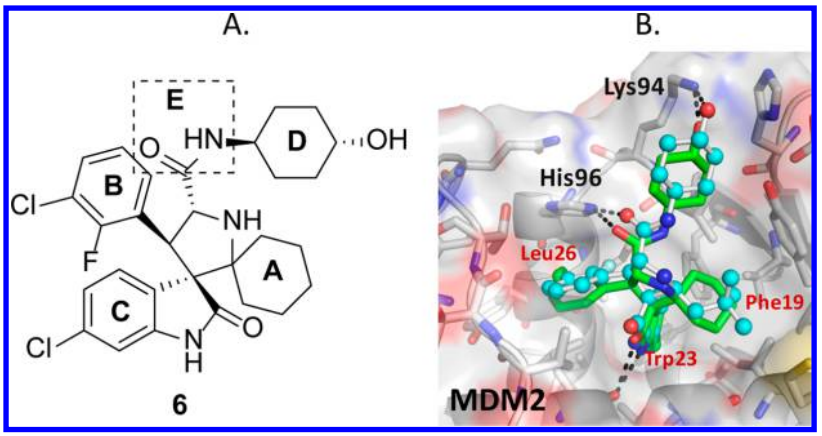


Figure 2. (A) Chemical structure of compound **6** and five regions (A–E) where chemical modifications were made. (B) Cocrystal structure of **2** (light blue spheres, PDB code 5TRF) and model of the binding mode of **6** (green sticks) in complex with MDM2.

RESULTS AND DISCUSSION

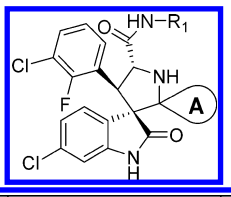
Our exploration started with lead molecule **6**[21] (Figure 2A) that binds to MDM2 with K_i = 2.9 nM, inhibits the growth of the SJSA-1 cancer cell line with IC_{50} = 190 nM, and achieves strong tumor growth inhibition but not complete tumor regression in an SJSA-1 osteosarcoma xenograft model in mice.[21] In a search for superior compounds, we have performed extensive modifications of **6**.

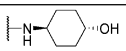
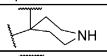
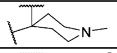
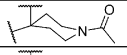
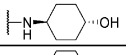
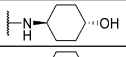
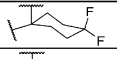
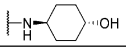
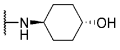
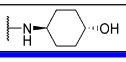
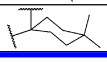
To guide our chemical modifications, we modeled the structure of compound **6** in a complex with MDM2 based upon the cocrystal structure of compound **2** complexed with human MDM2 (Figure 2B).[18] Our model shows that the oxindole-phenyl, 3-chloro-2-fluorophenyl, and cyclohexyl groups of **6** project into the Trp23, Leu26, and Phe19 p53 binding pockets of the MDM2 protein and the 4-hydroxyl of the *N*-cyclohexylcarboxamide group forms a hydrogen bond with Lys94 of MDM2 (Figure 2B). Guided by this model, we performed extensive modifications on five regions of the molecule (labeled as sites A–E, Figure 2A).

We began by replacing the 4-hydroxycyclohexyl group at site D in compound **6** with a methyl group, giving compound **7**. Since compounds **6** and **7** have similar binding affinities to MDM2, we have used either compound **6** or **7** as the template for modifications of site A (Table 1). Replacement of the cyclohexyl group at site A with 4-piperidinyl group led to compound **8**, which has no appreciable binding up to 2 μM to MDM2. Methylation or acetylation of the amine group in **8** resulted in compound **9** or **10**, respectively, and these also have very weak affinities for MDM2. Replacement of the cyclohexyl group at site A in **6** with 4-tetrahydro-2*H*-pyran generated compound **11**, which has a moderate binding affinity to MDM2 (K_i = 75.9 nM). Introduction of two fluorine substituents at the 4-position of the cyclohexyl group in compound **6** resulted in compound **12**, which has a good affinity to MDM2 (K_i = 9.8 nM) but is 4 times less potent than **6**. However, introduction of two methyl groups at the same 4-position in **6** yielded compound **15**, which has a K_i value of <1 nM to MDM2 and is several times more potent than **6**. Replacement of the

DOI: 10.1021/acs.jmedchem.6b01665
J. Med. Chem. 2017, 60, 2819–2839

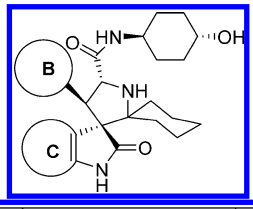
Table 1. Structure–Activity Relationship of Pyrrolidine-2-spirocyclohexyl Substituent[c]

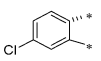
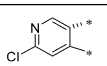
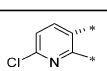
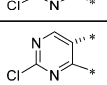
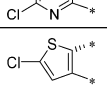


Compound ID	-N-R_1	A	Binding Affinity	
			IC_{50} [nM]	K_i [nM]
6[b]			17.2 ± 4.6	2.9 ± 0.8
7			21.2 ± 5.0	4.3 ± 1.3
8			>2000[a]	
9			>2000[a]	
10			2110[a]	525
11			282[a]	75.9
12			41[a]	9.8
13[b]			149 ± 32	19 ± 4
14			14 ± 1.3	2.4 ± 0.4
15			6.5 ± 0.9	<1

[a]Value of one experiment. [b]Reference 21. [c]Values are average of at least two tests.

Table 2. SAR of the B and C Aryl Rings[a]



Compound ID	B	C	Binding Affinity	
			IC_{50} [nM]	K_i [nM]
16			260 ± 29	77 ± 16
17			52.6 ± 3	13.9 ± 0.8
18			51.4 ± 0.9	13.2 ± 0.3
19			56.8 ± 4.9	13.7 ± 2.2
20			31.2 ± 3.7	6.5 ± 1.6

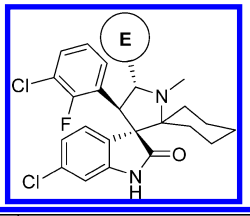
[a]Values are average of at least two tests.

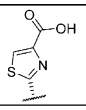
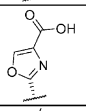
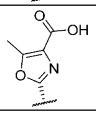
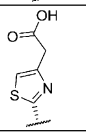
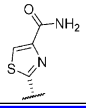
cyclohexyl group with a cyclobutyl group generated compound **13**,[21] which is 6 times less potent than **6**. Installation of methyl groups at the 3-position of the cyclobutyl ring led to compound **14**, which is equally potent as compound **6** and 7 times more potent than compound **13**. Hence, our SAR data for site A clearly show that a hydrophobic group is highly desirable for achieving a strong binding affinity to MDM2, and cyclohexyl, 4,4′-dimethylcyclohexyl, and 3,3′-cyclobutyl groups are the most preferred.

Next, we explored modifications of the 3-chloro-2-fluorophenyl and the oxindolephenyl substituents (sites B and C in Figure 2A, respectively). To improve solubility, we inserted one or more nitrogen atoms into the aryl rings, producing compounds **16**, **17**, **18**, and **19** (Table 2). Insertion of a nitrogen into the 3-chloro-2-fluorophenyl substituent (ring B) gave **16**, which has a K_i of 77 nM, 26 times weaker than **6**. Interestingly, although the Trp23 pocket is hydrophobic in nature, compounds **17**, **18**, and **19** containing a pyridinyl or a pyrimidinyl oxindole group (ring C) are all quite potent with K_i = 13–14 nM. Our data, however, are consistent with a previous study, which showed that a compound containing a chloropyridyl oxindole group is a potent MDM2 inhibitor.[23]

DOI: 10.1021/acs.jmedchem.6b01665
J. Med. Chem. 2017, 60, 2819–2839

Table 3. Carboxamide (E) Bioisostere Replacement[c]



Compound ID	E	Binding Affinity		SJSA-1
		IC_{50} [nM]	K_i [nM]	IC_{50} [μM]
21		12.8 ± 1.5	~1	>10[a]
22		27.5 ± 4.7	2.7 ± 0.6	>10[a]
23		603 ± 177	81.2 ± 24.3	12.1[a]
24		161[a]	21[a]	5.0[a]
25		38.6 ± 22.3	4.2 ± 3.0	3.0[a]
26		30.9 ± 1.7	3.1 ± 0.2	6.0[a]
27		12.5 ± 1.1	~1.0	2.69[a]
28		156[a]	20[a]	NT[b]

[a]Value of only one experimnent. [b]NT = not tested. [c]Values are average of at least two experiments.

This previous study also showed that replacing the oxindole with a thienopyrrolone led to potent MDM2 inhibitors.[23] We therefore synthesized compound **20** containing a thienopyrrolone, which has a K_i of 6.5 nM and is 2-fold less potent than **6**. We found that the thiophene group in **20** can be oxidized during the oxidative removal of the chiral auxiliary, preventing further investigation of this compound.

Our modeling of **6** and the cocrystal structure of **2** (Figure 2B) showed that the carbonyl of the amide (site E) forms a hydrogen bond with His96 of the MDM2 protein but the amino group does not interact with the MDM2 protein. We evaluated a series of five-membered heteroaromatic rings as the amide bioisoteres (Table 3). Among these replacements, we found that heteroaromatic rings with carboxylic acid substituents, e.g., **21**, **22**, **26**, and **27** have the best binding affinities with K_i values between 1 and 3 nM. Unfortunately, these compounds show weak cell growth inhibitory activity (IC_{50} > 2 μM, Table 3) in the SJSA-1 cancer cells, probably because the negatively charged acid group in these compounds decreases their cell permeability. We therefore converted the acid group in compound **21** to an amide but found that the resulting compound **28** is 20 times less potent than **21**.

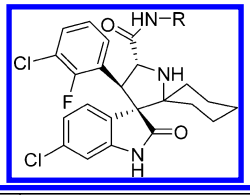
Our modeling of **6** and the cocrystal structure of **2** also showed that the 4-hydroxyl on the cyclohexylcarboxamide substituent of **6** forms a hydrogen bond with Lys94 of the MDM2 protein (Figure 2B). We synthesized a series of compounds containing either a terminal carboxylic acid or a sulfone to investigate the effect on binding (compounds **29**–**38**) (Table 4). These modifications resulted in a series of compounds with high affinities. In particular, compounds **31**, **32**, and **33** bind to MDM2 with K_i < 1 nM and are more than 3 times more potent than **6**.

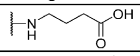
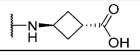
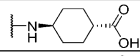
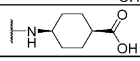
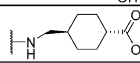
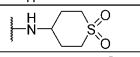
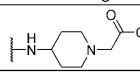
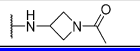
We tested the cell growth inhibitory activity of compounds **29**–**38** in the SJSA-1 cell line, and the data are summarized in Table 4. Among this series of compounds, **32**, **33**, and **38** have the best antiproliferative activity with IC_{50} values of 0.22, 0.15, and 0.24 μM, respectively.

We further evaluated compound **33** for its pharmacodynamic (PD) effect in the SJSA-1 xenograft tissue. Our PD data showed that a single, oral administration of **33** at 100 mg/kg is very effective in inducing upregulation of MDM2, p53, and p21 proteins at both 3 and 6 h time-points in the SJSA-1 tumor tissue, indicating strong activation of p53 (Figure 3A). Upon the basis of its promising PD data, we evaluated compound **33** for its antitumor efficacy in the SJSA-1 xenograft model (Figure 3B,C). While compound **33** can effectively retard tumor growth, it failed to achieve tumor regression. During the course of our research, we also observed that compound **33** can

DOI: 10.1021/acs.jmedchem.6b01665
J. Med. Chem. 2017, 60, 2819–2839

Table 4. SAR of Carboxamide Substituent (D)[d]



Compound ID	-NH-R	Binding Affinity		SJSA-1
		IC_{50} [nM]	K_i [nM]	IC_{50} [μM]
29[a]		200 ± 47	39 ± 8.7	>10
30		65 ± 14.1	12.8 ± 1.0	8.9[c]
31		9.4 ± 2.8	<1	1.4[c]
32		7.0 ± 1.8	<1	0.22 ± 0.03
33		4.4 ± 0.9	<1	0.15 ± 0.02
34		20.4 ± 1.6	4.0 ± 0.8	1.74[c]
35		17.3 ± 1.0	2.2 ± 0.8	1.90[c]
36		20.7 ± 4.4	4.5 ± 1.4	0.54[c]
37		19.0 ± 3.7	3.4 ± 0.3	NT[b]
38		16.9 ± 3.8	3.4 ± 0.4	0.24 ± 0.06

[a]Reference 21. [b]NT = not tested. [c]Value of one time testing. [d]Values are average of at least two tests.

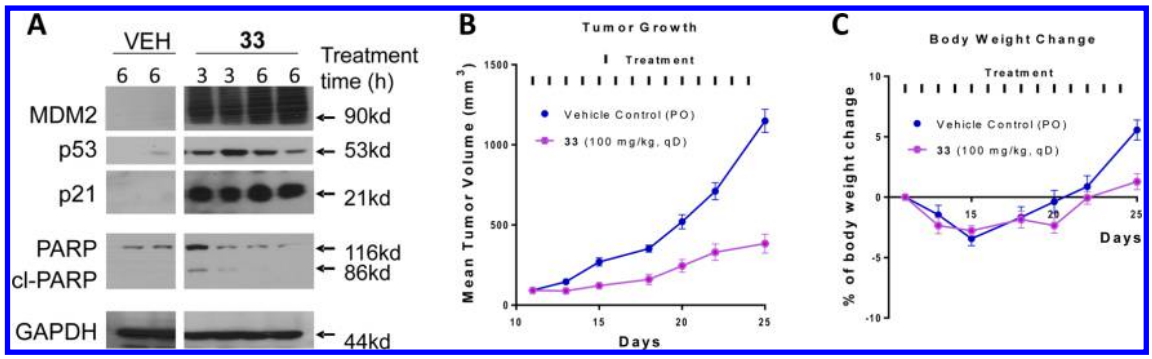


Figure 3. (A) Pharmacodynamics (PD) effect of **33** in SJSA-1 tumors in mice. Mice were given a single, oral dose of the vehicle, or **33** at 100 mg/kg and sacrificed at indicated time points. West blotting was performed on harvested tumor tissue to probe MDM2, p53, p21, PAPR, cleaved PARP (cl-PAPR) proteins. (B) Antitumor activity of **33** in the SJSA-1 osteosarcoma tumor xenograft model in mice. Compound **33** was administered via oral gavage at 100 mg/kg daily for 14 days. (C) Body weight change of mice during administration of **33**.

decompose in solution, particularly in cell culture media (panels A and B in Scheme 1). To analyze the potential structural features affecting the stability of **33**, we determined the stability of compound **31** with a flexible butanoic acid group, compound **6** with a 4-hydroxylcyclohexyl group, and **33**. Compound **6** had negligible (<1%) decomposition (panel C in Scheme 1). On the other hand, compound **31** showed 18% decomposition after 2 days in cell culture media, which is greater than that observed for compound **33** (panel D in Scheme 1). On the basis of these results, we proposed that the carboxylic group assists in the decomposition of **33**.

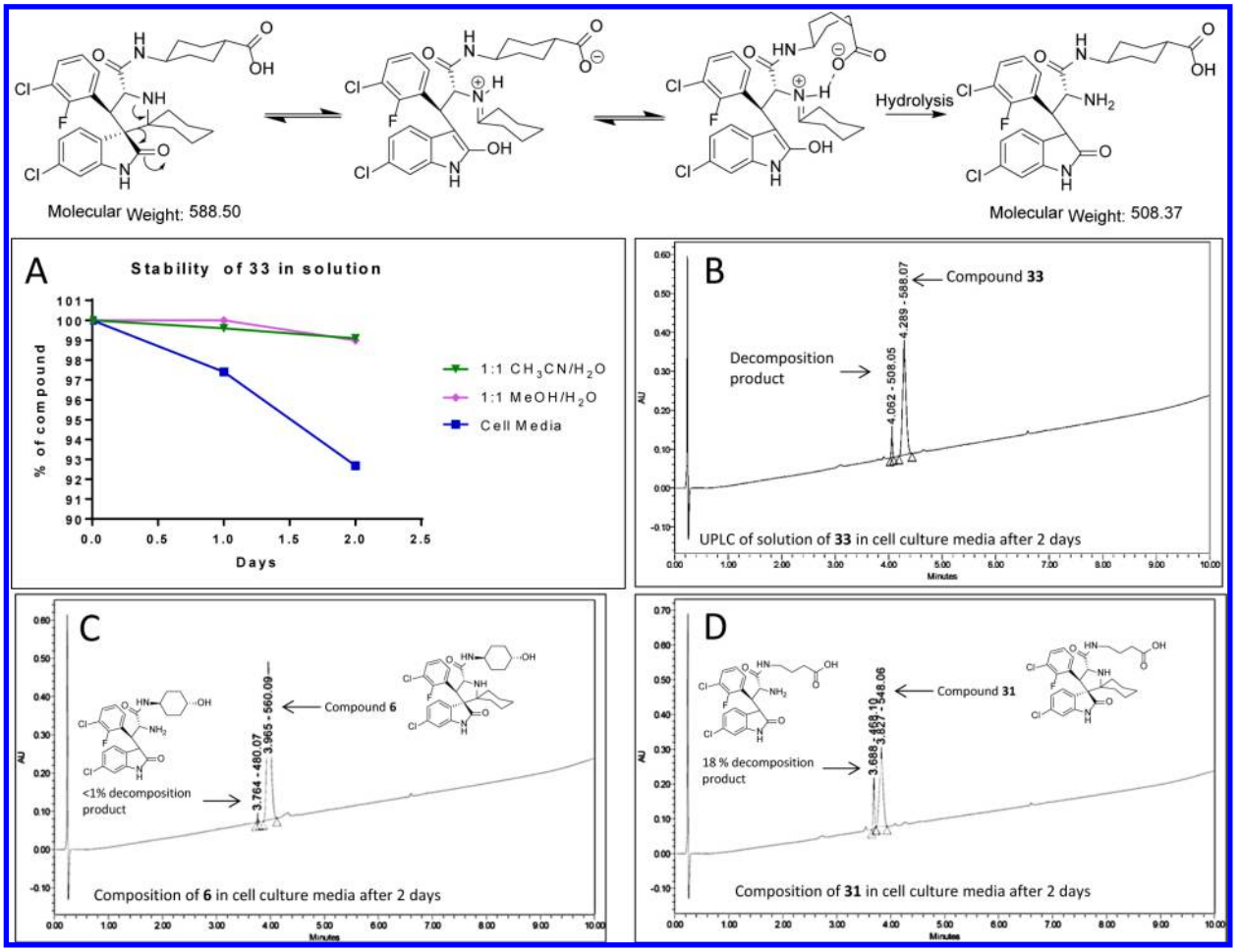
We next performed further chemical modifications of **33** with the goal to improve its chemical stability, cellular potency, and in vivo efficacy.

We investigated if replacement of the cyclohexyl group with an even more rigid aryl group can prevent the decomposition. A series of compounds containing an *N*-arylcarboxamide substituent were synthesized and tested (Table 5). These compounds all display very high binding affinities to MDM2, superior to that of **6** and equivalent to that of **33**. The cell growth inhibition of SJSA-1 cells by these compounds was determined (Table 5). Compounds **39** (MI-1061)[21] and **44** displayed the best cellular activity among the compounds in this series and are 3 times more potent than compound **33** in inhibition of cell growth in the SJSA-1 cell line. Compound **39** was found in our previous study[21] to be very stable in solution. Furthermore, **39** was capable of achieving partial tumor regression in the SJSA-1 xenograft model as shown in our previous report.[21]

We next combined all the favorable modifications on sites A and D and designed and synthesized compound **54** (Scheme 2). Compound **54** has a very high binding affinity to MDM2

DOI: 10.1021/acs.jmedchem.6b01665
J. Med. Chem. 2017, 60, 2819−2839

Scheme 1. Proposed Mechanism of Decomposition for 33[a]



[a](A) Stability of compound **33** in various solutions. Compound **33** was incubated in CH_3CN/H_2O, $MeOH/H_2O$, or cell culture media, and the % composition of **33** in the solutions was determined daily for 2 days by UPLC (ultraperformance liquid chromatography) and plotted. (B, C, D) UPLC/MS spectrum of **33**, **6**, and **31**, respectively, after incubation for 2 days in cell culture media. Peaks are labeled with retention time followed by molecular weight.

(IC_{50} = 2.4 nM, K_i < 1 nM, Scheme 2) and potently inhibits cell growth of SJSA-1 cells with IC_{50} = 13 nM (Scheme 2).

We assessed the antitumor activity of compound **54** in the SJSA-1 xenograft model in mice, with compound **2** included as the control (Figure 4). Compound **54** effectively inhibits tumor growth in a dose-dependent manner compared to the vehicle control. While compound **54** at 50 mg/kg daily dosing effectively retards tumor growth, at 100 mg/kg it achieves a maximum of 87% tumor regression during the treatment. However, while compound **2** at 100 mg/kg achieves complete tumor regression, compound **54** fails to do so.

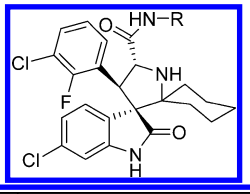
Pharmacokinetic (PK) studies in rats (Table 6) showed that with oral administration, compounds **39** and **54** achieve considerably lower C_{max} and AUC values than compound **2**, suggesting that further improvement of the oral PK for compounds **39** and **54** is needed in order to achieve stronger in vivo antitumor activity. Since **39** and **54** achieve comparable tumor regression in the SJSA-1 xenograft model and **39** is less hydrophobic than **54**, we have selected compound **39** for further optimization of its PK properties and in vivo efficacy.

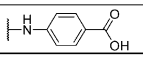
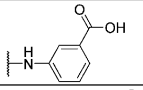
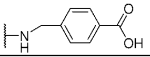
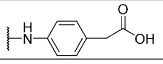
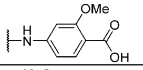
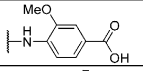
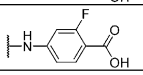
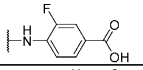
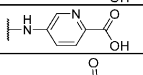
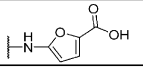
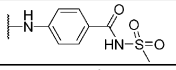
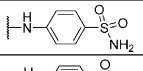
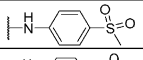
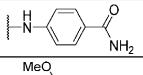
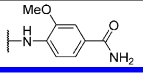
In our attempt to further improve the oral PK properties of compound **39** while retaining its high binding affinity to MDM2 and cellular potency, we investigated the replacement of the benzoic acid with nonclassical benzoic acid mimetics such as a bicyclo[1.1.1]pentane-1-carboxylic acid[24] or a bicyclo[2.2.2]octane-1-carboxylic acid. These efforts led to the design and synthesis of compounds **55** and **56** (Scheme 3). Compound **55** binds to MDM2 with a high affinity (IC_{50} = 6.4 nM, K_i < 1 nM) but is 5 times less potent than compound **39** in inhibition of cell growth in the SJSA-1 cell line. In comparison, compound **56** has a high binding affinity to MDM2 (IC_{50} = 3.7 nM, K_i < 1 nM) and is as potent as compound **39** in inhibition of cell growth in the SJSA-1 cell line (Scheme 3 and Table 7).

A PK study in rats showed that **56** has a higher plasma exposure than compound **2** based upon both the C_{max} (8234 vs 4547 μg/L) and AUC values (73603 vs 17230 h·μg/L). However, our PD experiment showed that oral administration of compound **56** at 100 mg/kg induces only modest p53 activation and no cleavage of PARP and caspase-3 in the SJSA-1 xenograft tumor tissue in mice (Figure 6A). Consistent with the PD data, compound **56** at 100 mg/kg administered daily for 14 days demonstrates only a very moderate tumor growth inhibition in the SJSA-1 xenograft model in mice (Figure 5A). Hence, the antitumor activity of **56** is much inferior to that achieved by **2**, **39**, and **54**. Because of its excellent in vitro cellular potency and excellent plasma exposure, the modest p53

DOI: 10.1021/acs.jmedchem.6b01665
J. Med. Chem. 2017, 60, 2819−2839

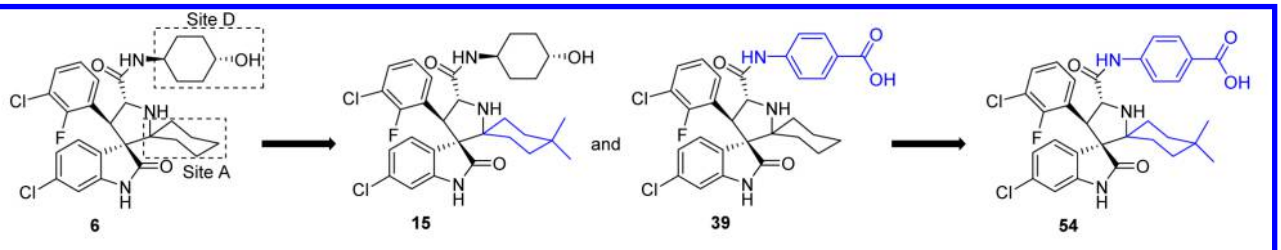
Table 5. SAR of (Aryl acid)carboxamides[d]



Compound ID	-N-R	Binding Affinity		SJSA-1
		IC_{50} [nM]	K_i [nM]	IC_{50} [μM]
39[c]		4.4 ± 1.1	<1	0.10 ± 0.03
40		8.8 ± 2.1	<1	0.76 ± 0.14
41		9.9 ± 1.6	<1	NT[b]
42		17 ± 1.3	2.5 ± 0.6	0.76 ± 0.29
43		7.5	<1	0.12 ± 0.02
44		4.7 ± 1.5	<1	0.061 ± 0.008
45		7.2 ± 3.6	<1	0.19 ± 0.06
46		12.7 ± 5.1	1.2 ± 1.0	0.23 ± 0.06
47		9.5 ± 1.1	1.0 ± 0.3	0.24 ± 0.07
48		18	2.8	6.2
49		8.1 ± 0.7	<1	5.8[a]
50		256	63[a]	11.2[a]
51		128	29[a]	0.14[a]
52		12[a]	1.9[a]	0.12 ± 0.02
53		14[a]	2.2[a]	0.12 ± 0.02

[a]Value of one time testing. [b]NT = not tested. [c]Reference 21. [d]Values are average of at least three tests.

Scheme 2. Design of Compound 54 by Combining the Optimal Substituents of Sites A and D



		Compounds			
		6	**15**	**39**[a]	**54**
Binding affinity to MDM2	IC_{50} [nM]	17.2 ± 4.6	6.5 ± 0.9	4.4 ± 1.1	2.4 ± 0.8
	K_i [nM]	2.9 ± 0.8	<1	<1	< 1
Cell growth inhibition in SJSA-1 cells	IC_{50} [nM]	190 ± 40	48 ± 1	100 ± 30	13 ± 3

[a]Reference 21.

activation and antitumor activity achieved by compound **56** suggests that this compound has a poor penetration into the xenograft tumor tissue. Accordingly, we investigated strategies with which to improve the tissue penetration while retaining

DOI: 10.1021/acs.jmedchem.6b01665
J. Med. Chem. 2017, 60, 2819−2839

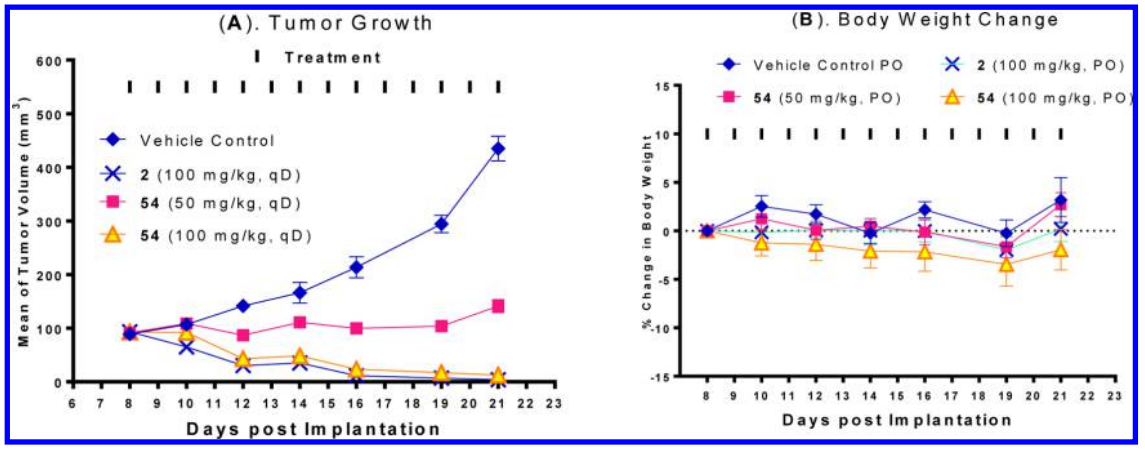
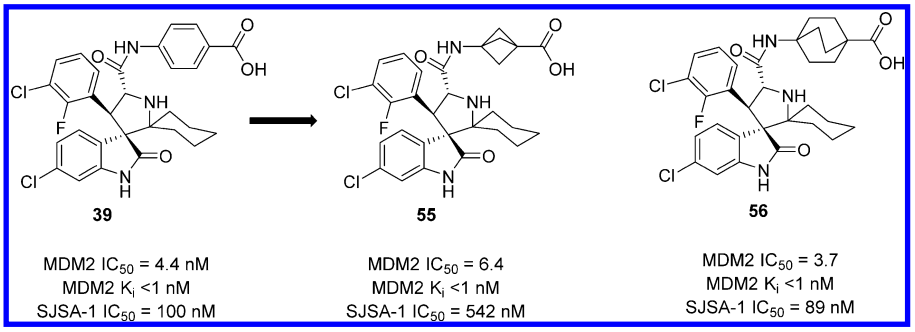


Figure 4. (A) Antitumor activity of compound **54**, in comparison to compound **2** in the SJSA-1 osteosarcoma tumor xenograft model in mice. Compound **54** was administered via oral gavage at 50 or 100 mg/kg daily for 14 days and compound **2** was administered via oral gavage at 100 mg/kg daily for 14 days. (B) Changes in body weight of mice during animal dosing.

Table 6. Pharmacokinetics Table for Comparison of 39, 54, 56, 59, 60, and 2 in Rats

	compound					
	39	**54**	**56**	**59**	**60**	**2**
po dose (mg/kg)	25	25	25	25	25	25
po C_{max} (ng/mL)	1553	968	8234	4391	5453	4547
po AUC (h·ng/mL)	6799	10188	73603	35205	39083	17230
po $T_{1/2}$ (h)	4.0	4.5	4.3	3.9	4.6	1.6
iv dose (mg/kg)	10	10	10	10	10	5
iv AUC (h·ng/mL)	8633	14767	82241	28825	38265	4630
iv CL (L h^{-1} kg^{-1})	1.16	0.690	0.119	0.352	0.257	1.03
iv V_{ss}(L/kg)	2.14	2.45	0.734	1.10	1.25	2.98
F (%)	31.5	27.6	35.0	48.6	40.3	74.9
antitumor efficacy	86% regression	87% regression	no regression	100% regression	100% regression	100% regression

Scheme 3. Replacement of the Benzoic Acid in Compound 39 with Nonclassical Benzoic Acid Bioisosteres



high binding affinity to MDM2, potent cellular activity, and good oral bioavailability of compound **56**.

We investigated three strategies to improve the tissue penetration: (1) decreasing the lipophilicity of the molecule; (2) reducing the acidity of carboxylic acids; and (3) increasing the basicity of the nitrogen atom in the pyrrolidine core.[25]

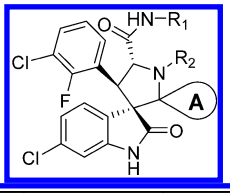
Fluorine substitution on aliphatic groups is known to decrease lipophilicity.[26−28] We therefore synthesized compound **57** with a 4,4-difluorocyclohexyl group at carbon-2 of the pyrrolidine core (Table 7). For the second strategy, we replaced the carboxylic acid group with an acyl sulfonamide bioisostere, which resulted in **58** (Table 7). For the third strategy, we installed a methyl or ethyl group on the nitrogen of the pyrrolidine core, which led to **59** and **60**, respectively (Table 7).

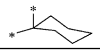
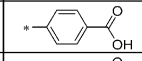
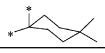
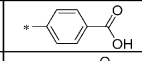
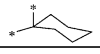
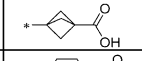
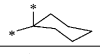
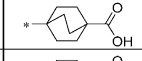
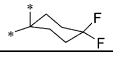
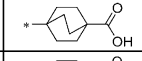
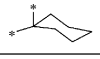
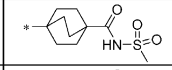
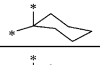
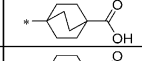
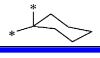
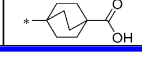
Compound **57** binds to MDM2 with a high affinity but is less potent than **56** and is 2 times less potent than **56** in inhibition of cell growth in the SJSA-1 cell line (Table 7). Compound **58** is 2 times less potent than **56** in binding to MDM2 and is 4 times less potent than **56** in inhibition of cell growth in the SJSA-1 cell line (Table 7). The PD study showed that oral administration of **57** or **58** at 100 mg/kg only has a modest effect on activation of p53 and induction of cleavage of PARP and caspase-3 (Figure 6B).

In comparison, compounds **59** and **60** bind to MDM2 with a high binding affinity (K_i < 1 nM, Table 7). Both compounds potently inhibit cell growth in the SJSA-1 cell line with IC_{50} values of 70 and 60 nM, respectively, and are thus as potent as compound **56** (Table 7). PD experiments showed that a single, oral dose of **59** and **60** at 100 mg/kg achieves strong p53

DOI: 10.1021/acs.jmedchem.6b01665
J. Med. Chem. 2017, 60, 2819−2839

Table 7. SAR of Benzoic Acid Nonclassical Bioisosteres and Alkylation of the Pyrrolidine Nitrogen[c]



Compound	A	R_1	R_2	MDM2 IC_{50}, (nM)	MDM2 K_i, (nM)	SJSA-1 IC_{50}, (μM)
39[b]			H	4.4 ± 1.1	<1	0.10 ± 0.03
54			H	2.4 ± 0.8	<1	0.013 ± 0.003
55			H	6.4 ± 1.3	<1	0.54 ± 0.16
56			H	3.7 ± 0.5	<1	0.089 ± 0.033
57			H	11.5 ± 3.7	1.3 ± 0.6	0.17[a]
58			H	6.3 ± 2.3	<1	0.37[a]
59			Me	4.5 ± 1.0	<1	0.070 ± 0.021
60			Et	3.8 ± 1.1	<1	0.060 ± 0.022

[a]Value from one experiment. [b]Reference 21. [c]Values are average of at least two independent experiments.

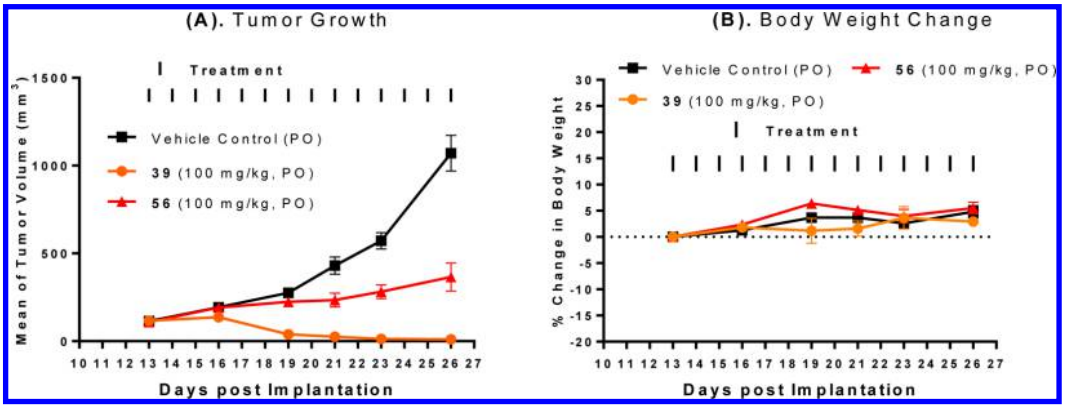


Figure 5. (A) Antitumor activity of compounds **39** and **56** in the SJSA-1 osteosarcoma xenograft model in mice. (B) Body weight change of mice during administration period.

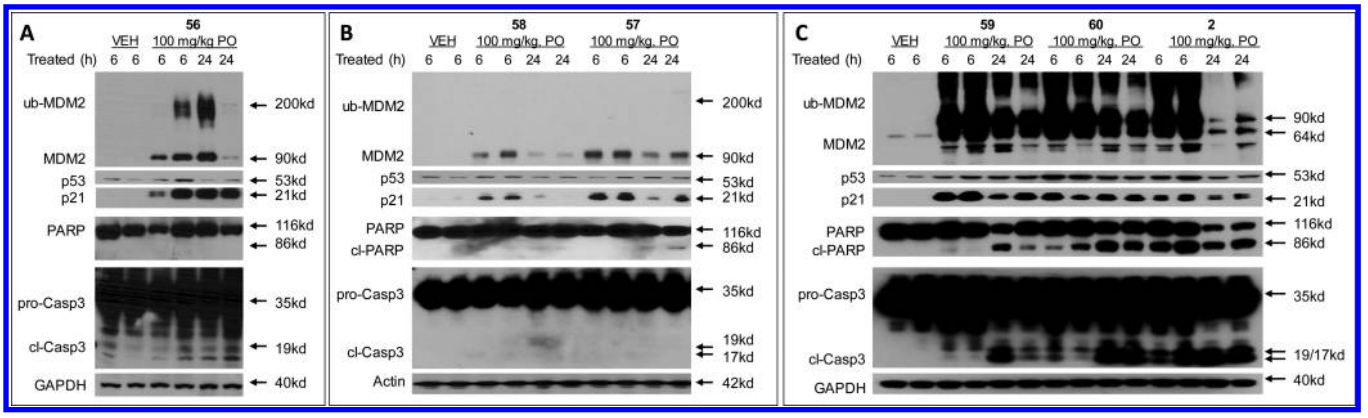


Figure 6. Pharmacodynamic (PD) analysis of the effect of MDM2 inhibitors in SJSA-1 tumors. Mice bearing SJSA-1 tumors were dosed with a single oral dose of **56**, **57**, **58**, **59**, **60** and **2** at 100 mg/kg and the tumors were harvested at 6 and 24 hours for western blot analysis. (A, B) PD of **56**, **57** and **58** show modest activation of p53 as seen with the low levels of p53, MDM2, and p21; (C) Compounds **59**, **60** and **2** show robust activation of p53 as seen with increased levels of p53, MDM2, ubiquitinated MDM2 (ub-MMD2) and p21, and induce strong apoptosis as seen with increased levels of cleaved PARP (cl-PAPR) and cleaved caspase-3 (cl-Casp3).

DOI: 10.1021/acs.jmedchem.6b01665
J. Med. Chem. 2017, 60, 2819−2839

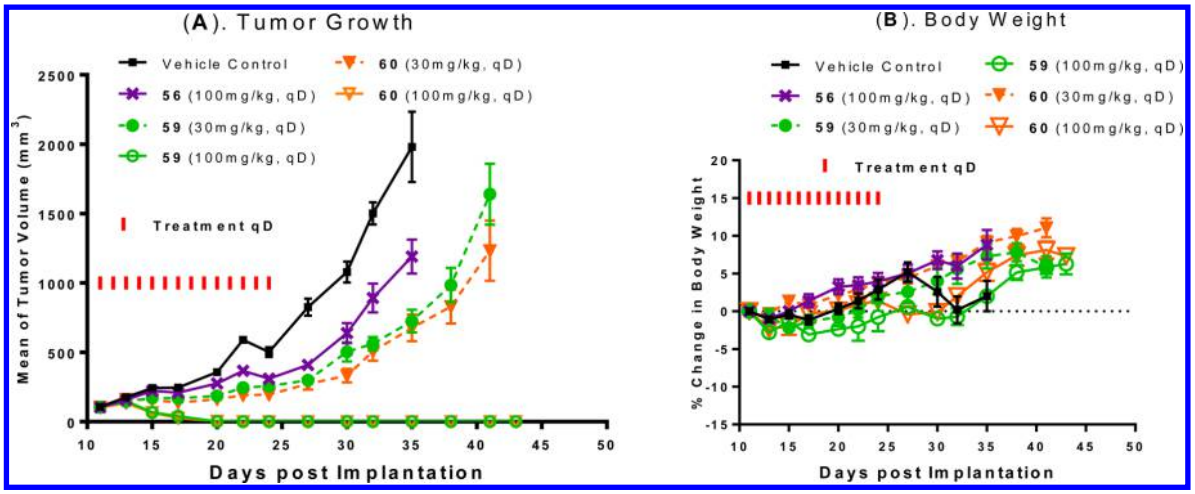


Figure 7. (A) Antitumor activity of compound **59**, **60** in comparison to **56** in the SJSA-1 osteosarcoma xenograft model in mice. Compound **59** and **60** were administered at 30 mg/kg or 100 mg/kg daily for 14 days and **56** at 100 mg/kg daily for 14 days. (B) Animal weight changes of mice during the experiment.

Table 8. Cell Growth Inhibition Activity of 56, 59, and 60 in Different Human Cancer Cell Lines[a]

			cell growth inhibition (IC_{50})		
cell line	tumor type	p53 status	**56**	**59**	**60**
SJSA-1	osteosarcoma	wild-type	89 ± 33 (nM)	70 ± 21(nM)	60 ± 22 (nM)
Saos2	osteosarcoma	null	26.7 ± 5.1 (μM)	25 ± 6 (μM)	22.7 ± 4.7 (μM)
RS4;11	leukemia	wild-type	62 ± 26 (nM)	56 ± 18 (nM)	38 ± 5 (nM)
LNCaP	prostate cancer	wild-type	36 ± 19 (nM)	30 ± 15 (nM)	18 ± 13 (nM)
PC3	prostate cancer	null	12.3 ± 2.5 (μM)	24 ± 5 (μM)	22 ± 7.2 (μM)
HCT116	colon cancer	wild-type	137 ± 31 (nM)	117 ± 33 (nM)	104 ± 36 (nM)
HCT116 p53−/−	colon cancer	knockout	14 ± 2 (μM)	18 ± 8 (μM)	8 ± 1 (μM)

[a]IC_{50} values are average of at least three independent experiments.

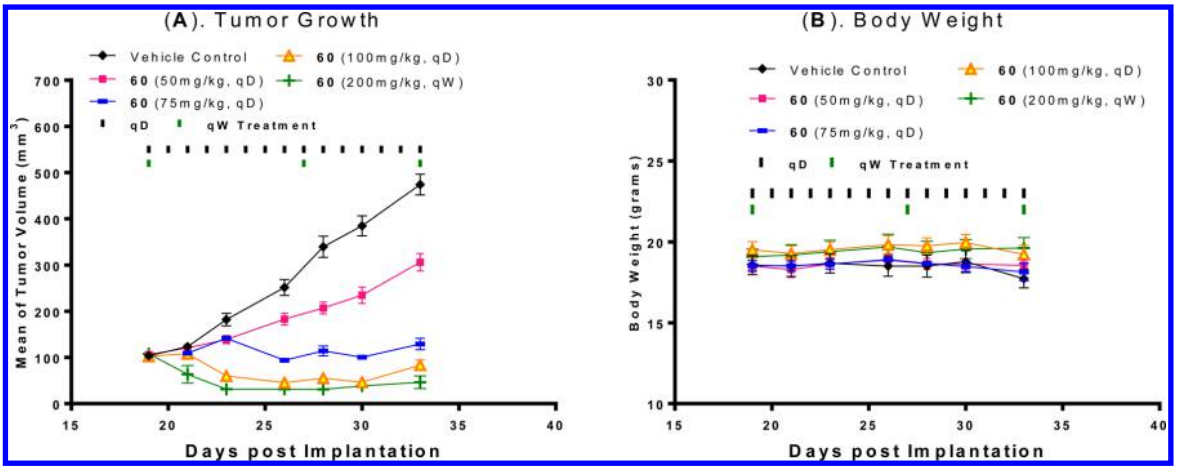


Figure 8. (A) Antitumor activity of compound **60** in the RS4; 11 acute lymphoblastic leukemia xenograft model in mice. Compound **60** was administered at 50 mg/kg, 75 mg/kg, or 100 mg/kg daily for 14 days and 200 mg/kg weekly for 3 weeks. (B) Animal body weight of mice during administration.

activation, with the effect persisting for 24 h in the SJSA-1 xenograft tumor tissue (Figure 6C). Compounds **59** and **60** also achieve strong apoptosis induction in the SJSA-1 xenograft tumor, based upon cleavage of PARP and caspase-3 (Figure 6C).

We next tested **59** and **60** for the antitumor activity in the SJSA-1 xenograft model (Figure 7). Compound **59** or **60** orally administered at 30 mg/kg daily for 14 days effectively inhibits tumor growth. Compound **59** or **60** orally administered at 100 mg/kg daily for 14 days effectively achieves complete and long-lasting tumor regression. In comparison, compound **56** at 100 mg/kg inhibits tumor growth only moderately in the same experiment (Figure 7A). Compounds **56**, **59**, and **60** cause no or minimal weight loss during the entire experiment (Figure 7B).

Our PK studies in rats with oral administration showed that **59** and **60** achieve a C_{max} value similar to that of **2** but have a 2 times higher AUC than compound **2** (Table 6).

DOI: 10.1021/acs.jmedchem.6b01665
J. Med. Chem. 2017, 60, 2819−2839

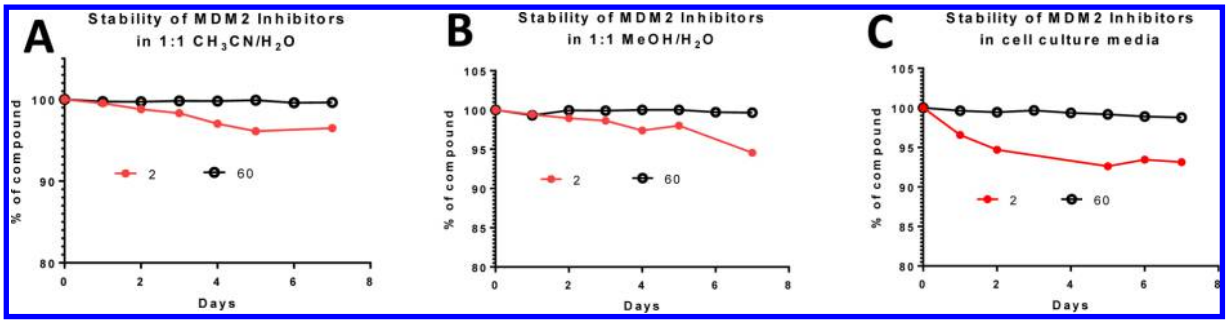
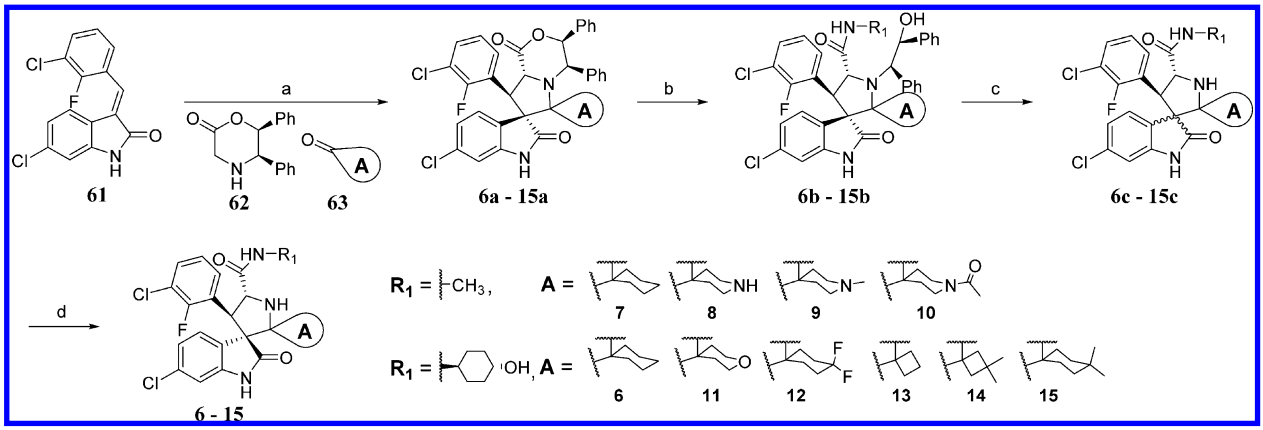


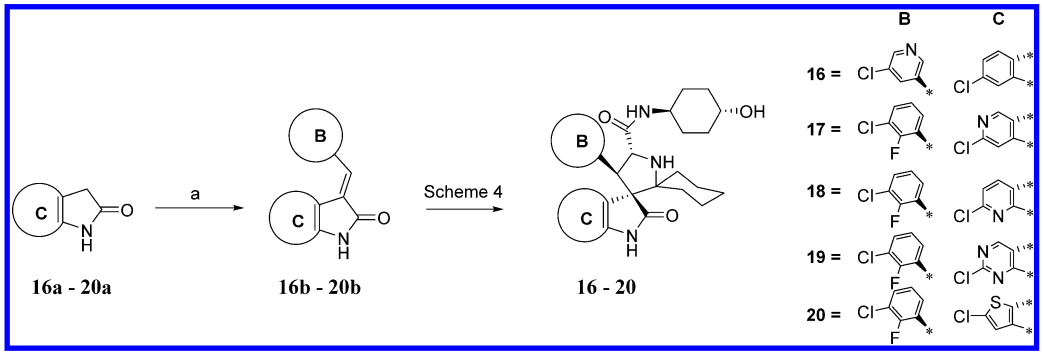
Figure 9. Stability comparison of compounds **2** and **60** in (A) 1:1 CH_3CN to H_2O; (B) 1:1 MeOH to H_2O; (C) cell culture media.

Scheme 4. Synthesis of Compounds 6−15[a]



[a]Reagents and conditions: (a) toluene, reflux, 2 h; (b) R_1NH_2, THF, reflux overnight; (c) CH_3CN/H_2O (1:1), CAN, 15 min; (d) CH_3CN/H_2O, overnight.

Scheme 5. Synthesis of Compounds 16−20[a]



[a]Reagents and conditions: (a) MeOH, piperidine, reflux.

We evaluated compounds **56**, **59**, and **60** for their activity and selectivity in a number of human cancer cell lines of different tumor types (Table 8). Consistent with their mechanism of action, these three compounds potently inhibit cell growth in cancer cell lines with wild-type p53 and display outstanding selectivity in cancer cell lines with deleted p53. Compound **60** is the most potent compound, achieving IC_{50} values of 38, 18, and 104 nM in the RS4;11 acute leukemia, LNCaP prostate cancer, and HCT116 colon cancer cell lines, respectively.

We next evaluated compound **60** for its antitumor activity in the RS4;11 acute leukemia xenograft model in mice. Compound **60** effectively inhibits tumor growth in a dose-dependent manner and achieves partial tumor regression at 100 mg/kg, daily, oral administration for 14 days, or at 200 mg/kg weekly dosing for 3 weeks (Figure 8). Compound **60** is also well tolerated at all doses and schedules in this experiment.

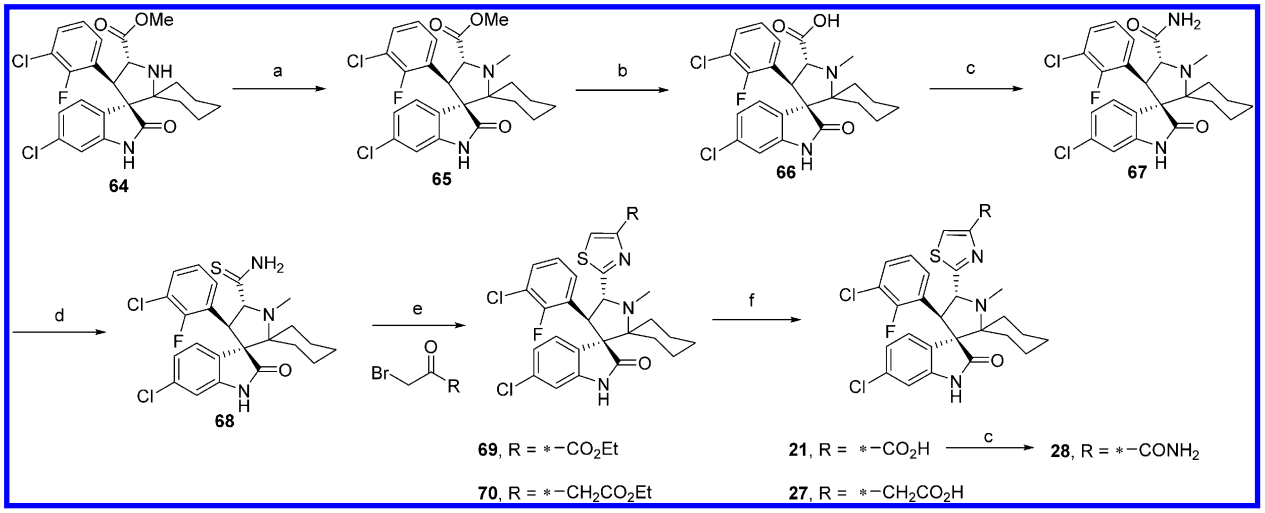
We evaluated the chemical stability of compound **60** in three different solutions and found that this compound is very stable in solutions for a period of 7 days (Figure 9). In comparison, compound **2** slowly isomerizes in solutions and retains only 93−96% purity after 7 days (Figure 9).

CHEMISTRY

To explore achiral substituents in carbon-2 of the pyrrolidine core, the compounds in Table 1 were synthesized using the method[21] outlined in Scheme 4. The azomethine ylide generated in situ by reaction of the chiral amine (**62**) with the cyclic ketone (**63**) was allowed to react with **61** in a 1,3-dipolar cycloaddition reaction, generating the chiral spiroox-

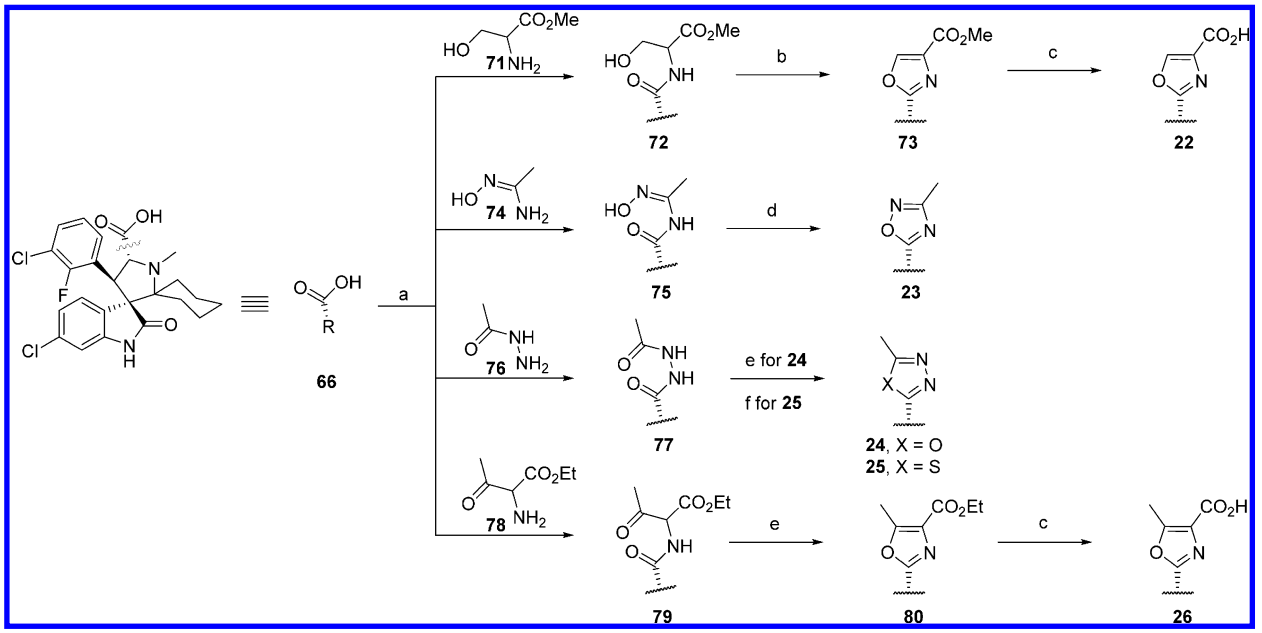
DOI: 10.1021/acs.jmedchem.6b01665
J. Med. Chem. 2017, 60, 2819−2839

Scheme 6. Synthesis of Compounds 21, 27, and 28[a]



[a]Reagents and conditions: (a) DCM/AcOH (1:1), paraformaldehyde, $NaBH(OAc)_3$; (b) THF/MeOH/H_2O, LiOH·H_2O; (c) DCE, CDI, DIEA, 50 °C 30 min, then NH_4OH; (d) DCM, Lawesson's reagent, reflux overnight; (e) (1) THF, $BrCH_2COR$, Et_3N, reflux overnight, (2) DME, TFAA, pyridine, −20 °C to rt, 30 min; (f) THF/MeOH/H_2O (1:1:1), NaOH, rt, prep-HPLC.

Scheme 7. Synthesis of Compounds 22−26[a]



[a]Reagents and conditions: (a) DCE, CDI, DIEA, DMAP, 50 °C for 30 min, then **71**, **74**, **76**, **78**, and reflux; (b) (1) THF, Deoxo-Fluor, −20 °C for 30 min, (2) $BrCCl_3$, DBU at rt overnight; (c) THF/H_2O (2:1), LiOH·H_2O, rt for 30 min, then preparative HPLC; (d) pyridine, 100 °C for 4 h; (e) DCM, PPh_3, I_2, Et_3N, rt; (f) DCM, Lawesson's reagent, reflux overnight.

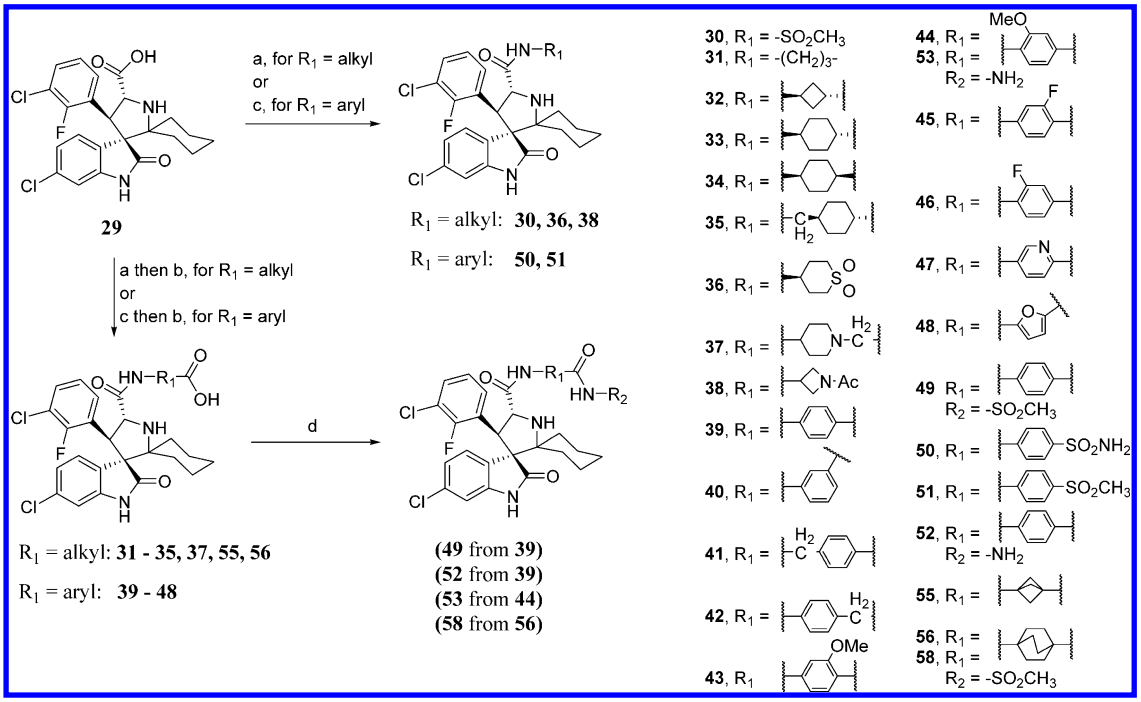
indoles (**6a−15a**). Reaction of this morpholine (**6a−15a**) with an aliphatic amine led to the open-ring compound (**6b−15b**). Upon oxidation with ceric ammonium nitrate (CAN), **6c−15c** were formed as a mixture of two diastereoisomers. "Aging" in acetonitrile/water resulted in formation of the single diastereoisomers **6−15**.

Scheme 5 shows the method by which the B and C aryl rings were replaced. First the intermediates **16b−20b** were synthesized by condensation of the pyrrolidones (**16a−20a**) with an arylaldehyde. Subsequently, the compounds (**16−20**) in Table 2 were obtained by using the intermediates (**16b−20b**), which are equivalent to **61**, in the synthetic method outlined in Scheme 4.

Compounds containing various five-membered heteroaromatic rings as bioisostere replacements for the carboxamide group E were synthesized as outlined in Schemes 6 and 7. The *N*-methylpyrrolidine (**65**) was generated by reductive amination of **64** with paraformaldehyde and $NaBH(OAc)_3$. Hydrolysis of the methyl ester (**65**) was followed by conversion of the acid (**66**) to the corresponding carboxamide (**67**). Refluxing of **67** with Lawesson's reagent produced the thioamide (**68**) which was reacted with an α-bromoketone in a Hantzsch thiazole synthesis reaction, generating the thiazoles **69** and **70**.[29] Basic hydrolysis of the esters produced the acid analogues **21** and **27**. Compound **21** was further elaborated by amide coupling, generating the carboxamide (**28**).

DOI: 10.1021/acs.jmedchem.6b01665
J. Med. Chem. 2017, 60, 2819−2839

Scheme 8. Synthesis of Compounds in Tables 4 and 5[a]



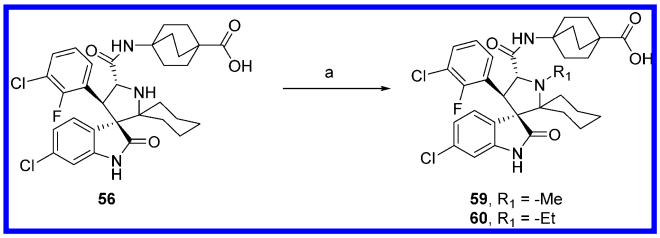
[a]Reagents and conditions: (a) DCM, HATU, DIEA, alkylamine; (b) THF/MeOH/H_2O (1:1:1), LiOH·H_2O for alkyl esters or NaOH for aryl esters; (c) DCM, Ph_2POCl, DIEA 30 min, then arylamine and DMAP; (d) DCE, CDI, DIEA, DMAP, 50 °C for 30 min, then R_2NH_2 and reflux.

Several five-membered heteroaromatic rings were formed from the carboxylic acid (**66**). The carboxylic acid (**66**) was activated with CDI (1,1′-carbonyldiimidazole), and this was followed by addition of commercially available compounds **71**, **74**, **76**, **78**, resulting in the formation of intermediates **72**, **75**, **77**, **79**, respectively. Cyclization to the respective five-membered heteroaromatic compounds (**73**,[30] **23**,[31] **24**,[32] **25**, **80**) was accomplished under the conditions outlined in Scheme 7. The carboxylic acid compounds **22** and **26** were produced by hydrolysis of the esters in compounds **73** and **80**, respectively.

The compounds in Tables 4 and 5 were obtained by the synthesis outlined in Scheme 8. Activation of the carboxylic acid (**29**) by HATU (1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate) followed by addition of an alkylamine gave the *N*-alkylcarboxamide intermediates **30**, **36**, **38**, **31**−**35**, **37**, **55**, and **56**. When *N*-arylamines were used, HATU failed to accomplish the coupling, but the coupling was successful when the acid was activated with Ph_2POCl [17] and produced the *N*-arylcarboxamides **39**−**48**, **50**, and **51**. Basic hydrolysis of the esters produced the carboxylic acid compounds listed in Tables 4 and 5. Further elaboration of the acids (**56** to give **58**; **39** to give **49** and **52**; and **44** to give **53**) was accomplished by CDI activation of the carboxylic acid followed by reaction with the respective amine.

Reductive amination of **56** with paraformaldehyde or acetaldehyde produced **59** and **60** as shown in Scheme 9.

Scheme 9. Synthesis of Compound 59 and 60[a]



[a]Reagents and conditions: (a) DCM/AcOH (1:1), paraformaldehyde or acetaldehyde, $NaBH(OAc)_3$, rt overnight.

CONCLUSION

In this study, we have performed an extensive SAR study on spirooxindoles containing two substituents at the carbon-2 of the pyrrolidine core as MDM2 inhibitors. Extensive modifications in five different regions of the molecule have led to discovery of a number of potent and highly efficacious MDM2 inhibitors. In particular, compound **60** has a very high binding affinity to MDM2 (K_i < 1 nM), potently activates wild-type p53, inhibits cell growth with low nanomolar IC_{50} values in human cancer cell lines carrying wild-type p53, and demonstrates an outstanding cellular selectivity over human cancer cell lines with deleted p53. Compound **60** is very stable in solutions, has excellent oral pharmacokinetics, and effectively activates p53 in the SJSA-1 xenograft tumor tissue in mice following a single oral administration. Significantly, **60** achieves complete and long-lasting regression of the SJSA-1 xenograft tumors in mice and demonstrates strong antitumor activity in the RS4;11 acute leukemia model at well tolerated dose schedules. Compound **60** has been advanced into phase I clinical development for the treatment of human cancer.

EXPERIMENTAL SECTION

General Information. Unless otherwise stated, all commercial reagents were used as supplied without further purification and all reactions were performed under a nitrogen atmosphere in dry solvents under anhydrous conditions. NMR spectra were obtained on either a Bruker 300 UltraShield spectrometer at a ^1H frequency of 300 MHz and ^{13}C frequency of 75 MHz or Bruker 400 Ascend spectrometer at a

DOI: 10.1021/acs.jmedchem.6b01665
J. Med. Chem. 2017, 60, 2819−2839

^{1}H frequency of 400 MHz and ^{13}C frequency of 100 MHz. Chemical shifts (δ) are reported in parts per million (ppm) relative to an internal standard. The final products were purified on a preparative HPLC (Waters 2545, quaternary gradient module) with a SunFire Prep C18 OBD 5 μm, 50 mm × 100 mm reverse phase column. The mobile phase was a gradient of solvent A (H_2O with 0.1% TFA) and solvent B (MeOH with 0.1% TFA or MeCN with 0.1% TFA) at a flow rate of 40 mL/min and 1%/2 min increase of solvent B or a flow rate of 60 mL/min and 1%/min increase of solvent B. All final compounds have purity of ≥95% as determined by Waters ACQUITY UPLC using reverse phase column (SunFire, C18-5 μm, 4.6 mm × 150 mm) and a solvent gradient of A (H_2O with 0.1% of TFA) and solvent D (MeOH with 0.1% of TFA) or A (H_2O with 0.1% of TFA) and solvent B (CH_3CN with 0.1% of TFA). ESI mass spectrum analysis was performed on a Thermo-Scientific LCQ Fleet mass spectrometer. No PAINS liability was found in any of the compounds presented as determined by analysis in the online filter SmartsFilter (http://pasilla.health.unm.edu/tomcat/biocomp/smartsfilter).

Compounds **6**, **13**, **29**, **39** and intermediate **64** were reported previously.[21] Compounds **16b**–**20b** were synthesized as reported previously.[23]

Compounds **7**–**20** were synthesized according to our reported method[21] which is described below for the synthesis of compound **6**.

(3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-*N*-((1*r*,4*R*)-4-hydroxycyclohexyl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamide (6).[21] In a round-bottom flask, (*E*)-6-chloro-3-(3-chloro-2-fluorobenzylidene)indolin-2-one (500 mg, 1.62 mmol), (5*R*,6*S*)-5,6-diphenyl-2-morpholinone (492 mg, 1.94 mmol), and cyclohexanone (4.86 mmol) were suspended in toluene (10 mL) and heated at reflux for 2 h at 140 °C. Then the reaction was allowed to cool to room temperature and the solvent was removed by rotoevaporation. The crude product was purified by column chromatography (the compound was eluted with 100% DCM) to give 665 mg (64% yield) of **6a** as a pale yellow solid. *trans*-4-Aminocyclohexanol (475 mg, 4.13 mmol) was added to a solution of **6a** (530 mg, 0.826 mmol) in THF (20 mL) and heated at reflux overnight. Then the reaction was cooled to room temperature and the solvent was removed by rotoevaporation. The crude **6b** was purified by column chromatography to produce 224 mg of **6b**. Cerium ammonium nitrate (324 mg, 0.592 mmol) was added to a solution of the resulting **6b** (224 mg, 0.296 mmol) in MeCN (6 mL) and stirred for 5 min at room temperature, then H_2O (6 mL) was added. After stirring the reaction for an additional 10 min, it was quenched with saturated sodium bicarbonate, brine was added, and the solution was extracted with EtOAc. The EtOAc solution was dried over sodium sulfate, filtered through Celite and the solvent was removed by rotoevaporation to produce crude **6c** as a mixture of diastereisomers. The crude material was dissolved in a 3:1 mixture of MeOH/H_2O that was acidified with TFA and aged. After 2 days this solution was purified by preparative HPLC. The combined fractions of the pure compound were concentrated and then redissolved in a minimum amount of MeCN, H_2O was added, and the solution was frozen and lyophilized to produce the TFA salt of **6** (116 mg, 70% yield) as a white powder. Chemical data were reported previously.[21]

(3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-*N*-methyl-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamide (7). 7 was obtained using the synthetic method described for the preparation of **6**. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 8.29 (br s, 1H), 7.63 (t, 1H, J = 7.2 Hz), 7.51 (dd, 1H, J = 2.4, 8.2 Hz), 7.39 (t, 1H, J = 7.6 Hz), 7.21–7.07 (m, 2H), 6.77 (d, 1H, J = 1.5 Hz), 5.13 (d, 1H, J = 10.9 Hz), 4.83 (d, 1H, J = 11.0 Hz), 2.83 (d, 1H, J = 8.3 Hz), 2.73 (s, 3H), 2.17 (d, 1H, J = 15.3 Hz), 2.04–1.68 (m, 5H), 1.52 (q, 1H, J = 14.6 Hz), 1.31–1.09 (m, 2H); ESI-MS m/z 476.25 $(M + 1)^+$.

(3*R*,4′*S*,5′*R*)-6-Chloro-4′-(3-chloro-2-fluorophenyl)-*N*-methyl-2-oxodispiro[indoline-3,3′-pyrrolidine-2′,4″-piperidine]-5′-carboxamide (8). Starting with *tert*-butyl 4-oxopiperidine-1-carboxylate in place of cyclohexanone, compound **8-Boc** (compound **8** with *N*-Boc piperidine) was prepared according to the synthetic method described for the preparation of **6**. Compound **8-Boc** was dissolved in trifluoroacetic acid and stirred. After 30 min the trifluoroacetic acid was removed and the crude was purified by HPLC to give **8** (TFA salt). ^{1}H NMR (300 MHz, CD_3OD) δ ppm 8.24 (br s, 1H), 7.64 (t, 1H, J = 7.2 Hz), 7.49 (dd, 1H, J = 2.2, 8.1 Hz), 7.27 (t, 1H, J = 7.3 Hz), 7.13–7.03 (m, 2H), 6.68 (s, 1H), 4.79 (d, 1H, J = 9.6 Hz), 4.64 (d, 1H, J = 9.6 Hz), 3.70 (t, 1H, J = 13.1 Hz), 3.44–3.18 (m, 3H), 2.77 (d, 3H, J = 4.3 Hz), 2.39 (d, 1H, J = 14.5 Hz), 2.10–1.88 (m, 2H), 1.50–1.26 (m, 1H). ESI-MS m/z 477.17 $(M + 1)^+$.

(3*R*,4′*S*,5′*R*)-6-Chloro-4′-(3-chloro-2-fluorophenyl)-*N*,1″-dimethyl-2-oxodispiro[indoline-3,3′-pyrrolidine-2′,4″-piperidine]-5′-carboxamide (9). Starting with 1-methylpiperidin-4-one in place of cyclohexanone, compound **9** was prepared according to the synthetic method described for the preparation of **6**. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 8.25 (br s, 1H), 7.62 (t, 1H, J = 7.3 Hz), 7.47 (dd, 1H, J = 2.1, 8.2 Hz), 7.25 (t, 1H, J = 7.5 Hz), 7.12–7.01 (m, 2H), 6.77 (d, 1H, J = 1.6 Hz), 4.76 (d, 1H, J = 9.5 Hz), 4.62 (d, 1H, J = 9.5 Hz), 3.75 (t, 1H, J = 12.5 Hz), 3.52–3.40 (m, 2H), 3.24–3.12 (m, 1H), 2.86 (s, 3H), 2.76 (d, 3H, J = 4.0 Hz), 2.40 (d, 1H, J = 14.4 Hz), 2.12–1.86 (m, 2H), 1.54–1.34 (m, 1H); ESI-MS m/z 491.08 $(M + 1)^+$.

(3*R*,4′*S*,5′*R*)-1″-Acetyl-6-chloro-4′-(3-chloro-2-fluorophenyl)-*N*-methyl-2-oxodispiro[indoline-3,3′-pyrrolidine-2′,4″-piperidine]-5′-carboxamide (10). Starting with 1-acetylpiperidin-4-one in place of cyclohexanone, compound **10** was prepared according to the synthetic method described for the preparation of **6**. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 7.63 (t, 1H, J = 7.8 Hz), 7.47 (dd, 1H, J = 2.6, 8.2 Hz), 7.31 (t, 1H, J = 8.3 Hz), 7.15–7.04 (m, 2H), 6.75 (d, 1H, J = 1.7 Hz), 4.77 (d, 1H, J = 10.1 Hz), 4.61 (d, 0.5H, J = 16.4 Hz, rotamer), 4.43 (d, 0.5H, J = 11.9 Hz, rotamer), 4.00 (d, 0.5H, J = 13.1 Hz, rotamer), 3.85 (d, 0.5H, J = 12.6 Hz, rotamer), 3.81–3.68 (m, 1H), 2.76 (s, 3H), 2.56–2.40 (m, 1H), 2.16–1.76 (m, 5H), 1.45–1.11 (m, 2H); ESI-MS m/z 519.17 $(M + 1)^+$.

(3*R*,4′*S*,5′*R*)-6-Chloro-4′-(3-chloro-2-fluorophenyl)-*N*-((1*r*,4*R*)-4-hydroxycyclohexyl)-2-oxo-2″,3″,5″,6″-tetrahydrodispiro[indoline-3,3′-pyrrolidine-2′,4″-pyran]-5′-carboxamide (11). Starting with tetrahydro-4*H*-pyran-4-one in place of cyclohexanone, compound **11** was prepared according to the synthetic method described for the preparation of **6**. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 8.19 (d, J = 7.9 Hz 1H), 7.63 (ddd, 1H, J = 1.5, 6.5, 7.9 Hz), 7.51 (dd, 1H, J = 2.3, 8.2 Hz), 7.37 (t, 1H, J = 8.3 Hz), 7.19–7.07 (m, 2H), 6.80 (d, 1H, J = 1.9 Hz), 5.02 (d, 1H, J = 10.8 Hz), 4.74 (d, 1H, J = 10.8 Hz), 4.11–3.93 (m, 2H), 3.87 (dd, 1H, J = 3.9, 12.4 Hz), 3.69–3.55 (m, 2H), 3.50–3.38 (m, 1H), 2.62 (d, 1H, J = 13.2 Hz), 2.26–2.12 (m, 1H), 2.04–1.73 (m, 4H), 1.70–1.17 (m, 5H), 1.08 (ddd, 1H, J = 3.5, 12.7, 24.0 Hz); ESI-MS m/z 562.67 $(M + 1)^+$.

(3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-4,4-difluoro-*N*-((1*r*,4*R*)-4-hydroxycyclohexyl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamide (12). Starting with 4,4-difluorocyclohexan-1-one in place of cyclohexanone, compound **12** was prepared according to the synthetic method described for the preparation of **6**. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 8.12 (d, 1H, J = 8.1 Hz), 7.62 (t, 1H, J = 7.2 Hz), 7.49 (dd, 1H, J = 2.3, 8.2 Hz), 7.33 (t, 1H, J = 8.3 Hz), 7.16–7.05 (m, 2H), 6.78 (d, 1H, J = 1.9 Hz), 4.77 (d, 1H, J = 10.3 Hz), 3.70–3.41 (m, 2H), 2.74–1.64 (m, 11H), 1.48–1.21 (m, 4H), 1.18–1.02 (m, 1H); ESI-MS m/z 596.75 $(M + 1)^+$.

(3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-*N*-((1*r*,4*R*)-4-hydroxycyclohexyl)-3,3-dimethyl-2″-oxodispiro[cyclobutane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamide (14). Starting with 3,3-dimethylcyclobutan-1-one in place of cyclohexanone, compound **14** was prepared according to the synthetic method described for the preparation of **6**. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.61 (dd, 1H, J = 1.9, 8.2 Hz), 7.52 (ddd, 1H, J = 1.5, 6.4, 7.9 Hz), 7.39 (ddd, 1H, J = 1.5, 7.3, 8.6 Hz), 7.18–7–11 (m, 2H), 6.89 (d, 1H, J = 1.9 Hz), 4.92 (d, 1H, J = 10.9 Hz), 4.46 (d, 1H, J = 10.9 Hz), 3.68–3.58 (m, 1H), 3.50–3.39 (m, 1H), 2.78 (dd, 2H, J = 14.5, 39.1 Hz), 2.37 (d, 1H, J = 14.2 Hz), 1.95–1.76 (m, 3H), 1.69–1.59 (m, 1H), 1.38–1.17 (m, 7H), 0.98 (ddd, 1H, J = 3.6, 12.9, J = 24.3 Hz), 0.54 (s, 3H); ESI-MS m/z 560.25 $(M + H)^+$.

DOI: 10.1021/acs.jmedchem.6b01665
J. Med. Chem. 2017, 60, 2819–2839

(3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-*N*-((1*r*,4*R*)-4-hydroxycyclohexyl)-4,4-dimethyl-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamide (15). Starting with 4,4-dimethylcyclohexan-1-one in place of cyclohexanone, compound **15** was prepared according to the synthetic method described for the preparation of **6**. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.62 (t, 1H, J = 7.9 Hz), 7.48 (dd, 1H, J = 1.4, 7.8 Hz), 7.35−7.25 (m, 1H), 7.15−7.04 (m, 2H), 6.78 (d, 1H, J = 1.7 Hz), 4.73 (d, 1H, J = 9.9 Hz), 3.67−3.57 (m, 1H), 3.52−3.43 (m, 1H), 2.08−1.64 (m, 8H), 1.58−1.42 (m, 2H), 1.41−1.20 (m, 6H), 0.98 (s, 3H), 0.73 (s, 3H); ESI-MS m/z 588.25 $(M + H)^+$.

(3′*R*,4′*R*,5′*R*)-6″-Chloro-4′-(5-chloropyridin-3-yl)-*N*-((1*r*,4*R*)-4-hydroxycyclohexyl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamide (16). Starting with (*E*)-6-chloro-3-((5-chloropyridin-3-yl)methylene)indolin-2-one (**16b**) in place of (*E*)-6-chloro-3-(3-chloro-2-fluorobenzylidene)indolin-2-one (**61**), compound **16** was prepared according to the synthetic method described for the preparation of **6**. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 8.45 (d, 1H, J = 2.1 Hz), 8.24 (d, 1H, J = 1.7 Hz), 7.89 (s, 1H), 7.60 (d, 1H, J = 8.2 Hz), 7.14 (dd, 1H, J = 1.8, 8.2 Hz), 6.78 (d, 1H, J = 1.8 Hz), 5.10 (d, 1H, J = 10.9 Hz), 4.47 (d, 1H, J = 10.9 Hz), 3.73−3.57 (m, 1H), 3.50−3.36 (m, 1H), 2.83 (d, 1H, J = 12.5 Hz), 2.17 (d, 1H, J = 14.3 Hz), 2.03−1.70 (m, 8H), 1.70−1.13 (m, 7H), 1.08−0.88 (m, 1H); ESI-MS m/z 543.75 $(M + H)^+$.

(3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-*N*-((1*r*,4*R*)-4-hydroxycyclohexyl)-2″-oxo-1″,2″-dihydrodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-pyrrolo[3,2-*c*]pyridine]-5′-carboxamide (17). Starting with (*E*)-6-chloro-3-(3-chloro-2-fluorobenzylidene)-1,3-dihydro-2*H*-pyrrolo[3,2-*c*]pyridin-2-one (**17b**) in place of (*E*)-6-chloro-3-(3-chloro-2-fluorobenzylidene)indolin-2-one (**61**), compound **17** was prepared according to the synthetic method described for the preparation of **6**. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.32 (s, 1H), 7.58 (t, 1H, J = 6.7 Hz), 7.36−7.27 (m, 1H), 7.11 (t, 1H, J = 8.6 Hz), 6.81 (s, 1H) 3.67−3.57 (m, 1H), 3.55−3.45 (m, 1H), 2.01−1.51 (m, 10H), 1.42−1.00 (m, 8H); ESI-MS m/z 561.25 $(M + H)^+$.

(3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-*N*-((1*r*,4*R*)-4-hydroxycyclohexyl)-2″-oxo-1″,2″-dihydrodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-pyrrolo[2,3-*b*]pyridine]-5′-carboxamide (18). Starting with (*E*)-6-chloro-3-(3-chloro-2-fluorobenzylidene)-1,3-dihydro-2*H*-pyrrolo[2,3-*b*]pyridin-2-one (**18b**) in place of (*E*)-6-chloro-3-(3-chloro-2-fluorobenzylidene)indolin-2-one (**61**), compound **18** was prepared according to the synthetic method described for the preparation of **6**. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 7.89 (dd, 1H, J = 2.14, 7.84 Hz), 7.61 (t, 1H, J = 7.43 Hz), 7.40 (t, 1H, J = 7.14 Hz), 7.17 (t, 1H, J = 8.40 Hz), 7.12 (d, 1H, J = 7.96 Hz), 5.07−4.94 (m, 1H), 4.78 (d, 1H, J = 10.91 Hz), 3.71−3.57 (m, 1H), 3.51−3.39 (m, 1H), 2.89−2.66 (m, 1H), 2.17−2.02 (m, 1H), 1.99−1.43 (m, 10H), 1.43−1.11 (m, 5H), 1.09−0.93 (m, 1H); ESI-MS m/z 561.58 $(M + H)^+$.

(3′*R*,4′*S*,5′*R*)-2″-Chloro-4′-(3-chloro-2-fluorophenyl)-*N*-((1*r*,4*R*)-4-hydroxycyclohexyl)-6″-oxo-6″,7″-dihydrodispiro[cyclohexane-1,2′-pyrrolidine-3′,5″-pyrrolo[2,3-*d*]pyrimidine]-5′-carboxamide (19). Starting with (*E*)-2-chloro-5-(3-chloro-2-fluorobenzylidene)-5,7-dihydro-6*H*-pyrrolo[2,3-*d*]pyrimidin-6-one (**19b**) in place of (*E*)-6-chloro-3-(3-chloro-2-fluorobenzylidene)-indolin-2-one (**61**), compound **19** was prepared according to the synthetic method described for the preparation of **6**. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.55 (d, 1H, J = 2.2 Hz), 7.55 (t, 1H, J = 7.8 Hz), 7.39 (t, 1H, J = 7.0 Hz), 7.16 (t, 1H, J = 7.9 Hz), 3.67−3.56 (m, 1H), 3.53−3.43 (m, 1H), 1.99−1.48 (m, 10H), 1.42−1.04 (m, 8H); ESI-MS m/z 562.33 $(M + H)^+$.

(3′*R*,4′*R*,5′*R*)-2″-Chloro-4′-(3-chloro-2-fluorophenyl)-*N*-((1*r*,4*R*)-4-hydroxycyclohexyl)-5″-oxo-4″,5″-dihydrodispiro[cyclohexane-1,2′-pyrrolidine-3′,6″-thieno[3,2-*b*]pyrrole]-5′-carboxamide (20). Starting with (*Z*)-2-chloro-6-(3-chloro-2-fluorobenzylidene)-4,6-dihydro-5*H*-thieno[3,2-*b*]pyrrol-5-one (**20b**) in place of (*E*)-6-chloro-3-(3-chloro-2-fluorobenzylidene)indolin-2-one (**61**), compound **20** was prepared according to the synthetic method described for the preparation of **6**. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.40 (td, 2H, J = 6.8, 15.1 Hz), 7.15 (t, 1H, J = 8.0 Hz), 6.71 (s, 1H), 4.58 (d, 1H, J = 10.3 Hz), 4.45 (d, 1H, J = 10.4 Hz), 3.68−3.57 (m, 1H), 3.53−3.43 (m, 1H), 2.16 (d, 1H, J = 11.4 Hz), 2.00−1.79 (m, 4H), 1.77−1.46 (m, 7H), 1.40−1.07 (m, 6H); ESI-MS m/z 566.25 $(M + H)^+$.

2-((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-1′-methyl-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indolin]-5′-yl)thiazole-4-carboxylic Acid (21). Ethyl 3-bromo-2-oxopropanoate (48 mg, 0.244 mmol) and triethylamine (0.033 mL, 0.244 mmol), were added to a solution of **68** (30 mg, 0.061 mmol) in THF (3 mL) and refluxed overnight. Then the reaction was cooled, and the solvent was removed. The crude product was redissolved in DME (3 mL), and to this solution, at −20 °C, was added trifluoracetic anhydride (0.025 mL, 0.183 mmol) and pyridine (0.025 mL, 0.305 mmol), and then the reaction was allowed to warm to rt. After 30 min, saturated sodium bicarbonate solution was added and the reaction was extracted with EtOAc. The EtOAc solution was dried over sodium sulfate, filtered, and purified by column chromatography to produce 18 mg of compound **69**. Lithium hydroxide monohydrate (50 mg, 1.19 mmol) was added to a solution of **69** (18 mg, 0.031 mmol) in THF/MeOH/H_2O (1:1:1, 3 mL). After 2 h the reaction was quenched with ammonium chloride, and brine was added and extracted with EtOAc. The EtOAc solution was dried over sodium sulfate, filtered, concentrated and the crude was purified by preparative HPLC to produce compound **21** (TFA salt). ^{1}H NMR (300 MHz, CD_3OD) δ ppm 8.34 (s, 1H), 7.70 (t, 1H, J = 6.8 Hz), 7.53 (d, 1H, J = 7.1 Hz), 7.27 (t, 1H, J = 7.5 Hz), 7.13−7.03 (m, 2H), 6.73 (d, 1H, J = 1.8 Hz), 5.78−5.59 (m, 1H), 4.81−4.68 (m, 1H), 3.10 (s, 3H), 2.51−2.38 (m, 1H), 2.25 (d, 1H, J = 15.1 Hz), 2.13−1.94 (m, 1H), 1.87−1.10 (m, 7H); ESI-MS m/z 560.33 $(M + H)^+$.

General Procedure A: Amide Coupling to Compound 66. CDI (3 equiv), DIEA (5 equiv), DMAP (1 equiv) were added to a suspension of compound **66** (1 equiv) in 1,2-dichloroethane and heated at 50 °C. After 30 min, the respective amine (5 equiv) was added and the reaction was refluxed overnight. Then the solvent was removed and the crude product was purified to produce the carboxamide.

2-((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-1′-methyl-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indolin]-5′-yl)oxazole-4-carboxylic Acid (22). Compound **72** was produced according to general procedure A. At −20 °C, Deoxo-Fluor (50 μL) was added to a solution of **72** (51 mg) in THF (3 mL). After 30 min bromotrichloromethane (500 μL) and DBU (400 μL) were added and the reaction was allowed to warm to room temperature. After 5 h the reaction was quenched with saturated sodium bicarbonate (stirred for 5 min) and then extracted with EtOAc. The EtOAc solution was dried over sodium sulfate, filtered, and purified by column chromatography to produce 34 mg of compound **73**. Lithium hydroxide monohydrate (60 mg, 1.43 mmol) was added to a solution of **73** (34 mg, 0.061 mmol) in THF/H_2O (1:1, 4 mL). After 2 h the reaction was quenched with TFA, concentrated and the crude was redissolved in 3:1 MeOH/H_2O and purified by HPLC to produce **22** (TFA salt) as a white powder. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.50 (s, 1H), 7.62−7.53 (m, 2H), 7.24 (t, 1H, J = 7.3 Hz), 7.07 (dd, 1H, J = 1.7, 8.2 Hz), 7.03 (t, 1H, J = 8.1 Hz), 6.73 (d, 1H, J = 1.8 Hz), 5.34 (d, 1H, J = 10.3 Hz), 5.02 (d, 1H, J = 11.6 Hz), 2.97 (s, 3H), 2.33 (d, 1H, J = 13.1 Hz), 2.15 (d, 1H, J = 14.1 Hz), 1.95−1.85 (m, 1H), 1.74−1.44 (m, 5H), 1.38−1.26 (m, 1H), 1.19−1.05 (m, 1H); ESI-MS m/z 544.42 $(M + H)^+$.

(3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-1′-methyl-5′-(3-methyl-1,2,4-oxadiazol-5-yl)dispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indolin]-2″-one (23). Starting with 50 mg of compound **66**, compound **75** was obtained using general procedure A. Crude **75** was dissolved in pyridine (5 mL) and heated to 100 °C. After 5 h the solution was cooled and the solvent was removed by rotoevaporation and then the crude product was purified by HPLC to produce **23** (TFA salt) as a white powder. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.60 (ddd, 1H, J = 1.5, 6.4, 7.9 Hz), 7.50 (dd, 1H, J = 3.0, 8.2 Hz), 7.25 (ddd, 1H, J = 1.6, 7.2, J = 8.6 Hz), 7.07−7.01 (m, 2H), 6.72 (d, 1H, J = 1.9 Hz), 5.30 (d, 1H, J = 11.0 Hz), 4.98 (d, 1H, J = 11.1 Hz), 2.99 (s, 3H), 2.32 (s, 3H), 2.24 (d, 1H, J = 13.2 Hz),

DOI: 10.1021/acs.jmedchem.6b01665
J. Med. Chem. 2017, 60, 2819−2839

2.19−2.10 (m, 1H), 1.86−1.47 (m, 6H), 1.21 (ddd, 1H, J = 5.0, 12.6 Hz, 13.9 Hz), 1.15−1.02 (m, 1H); ESI-MS m/z 514.92 $(M + H)^+$.

(3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-1′-methyl-5′-(5-methyl-1,3,4-oxadiazol-2-yl)dispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indolin]-2″-one (24). Starting with 77 mg of compound **66**, 63 mg of **77** was obtained using general procedure A. A solution of **77** (30 mg, 0.056 mmol) in DCM (3 mL) was slowly added to a solution of PPh_3 (30 mg, 0.112 mmol), Et_3N (31 μL), and iodine (28 mg, 0.112 mmol) in DCM (1 mL). After 3 h, H_2O and brine were added and extracted with EtOAc. The EtOAc solution was dried over sodium sulfate, filtered, concentrated and the crude product was purified by HPLC to produce compound **24** (TFA salt) as a white powder. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.60 (t, 1H, J = 7.9 Hz), 7.51 (dd, 1H, J = 2.9, 8.2 Hz), 7.25 (t, 1H, J = 8.3 Hz), 7.08−7.00 (m, 2H), 6.72 (d, 1H, J = 1.9 Hz), 5.29 (d, 1H, J = 11.1 Hz), 4.95 (d, 1H, J = 10.8 Hz), 2.95 (s, 3H), 2.52 (s, 3H), 2.24 (d, 1H, J = 13.8 Hz), 2.15 (dd, 1H, J = 2.3, 14.1 Hz), 1.86−1.02 (m, 8H); ESI-MS m/z 515.25 $(M + H)^+$.

(3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-1′-methyl-5′-(5-methyl-1,3,4-thiadiazol-2-yl)dispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indolin]-2″-one (25). Lawesson's reagent (45 mg, 0.112 mmol) was added to a solution of **77** (30 mg, 0.056 mmol) in DCM and refluxed. After standing overnight the solvent was removed and the crude was purified by HPLC to produce compound **25** (TFA salt) as a white powder. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.69 (t, 1H, J = 7.9 Hz), 7.46 (dd, 1H, J = 2.8, 8.2 Hz), 7.25 (t, 1H, J = 8.4 Hz), 7.08 (dt, 1H, J = 1.1, 8.1 Hz), 7.03 (dd, 1H, J = 2.0, 8.2 Hz), 6.72 (d, 1H, J = 1.9 Hz), 5.50 (d, 1H, J = 10.2 Hz), 4.63 (d, 1H, J = 11.0 Hz), 3.00 (s, 3H), 2.71 (s, 3H), 2.27 (d, 1H, J = 13.0 Hz), 2.23−2.15 (m, 1H), 1.88 (t, 1H, J = 11.6 Hz), 1.83−1.60 (m, 4H), 1.60−1.49 (m, 1H), 1.31−1.07 (m, 2H); ESI-MS m/z 531.17 $(M + H)^+$.

2-((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-1′-methyl-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indolin]-5′-yl)-5-methyloxazole-4-carboxylic Acid (26). Starting with 44 mg of compound **66**, 25 mg of **79** was obtained using general procedure A. Cyclization to generate **80** (13 mg) was accomplished according to the same procedure used to obtain **24**. Lithium hydroxide monohydrate (30 mg, 0.715 mmol) was added to a solution of **80** (13 mg, 0.023 mmol) in THF/H_2O (1:1, 2 mL). After 2 h the reaction was quenched with TFA, concentrated and the crude was redissolved in 3:1 MeOH/H_2O and purified by HPLC to produce **26** (TFA salt) as a white powder. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.60 (t, 1H, J = 7.2 Hz), 7.52 (dd, 1H, J = 2.7, 8.1 Hz), 7.21 (t, 1H, J = 8.3 Hz), 7.07−6.98 (m, 2H), 6.70 (d, 1H, J = 1.9 Hz), 5.07 (d, 1H, J = 10.8 Hz), 4.91 (d, 1H, J = 11.7 Hz), 2.83 (s, 3H), 2.59 (s, 3H), 2.31−2.23 (m, 1H), 2.12−2.04 (m, 1H), 1.85−1.02 (m, 8H); ESI-MS m/z 558.75 $(M + H)^+$.

2-(2-((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-1′-methyl-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indolin]-5′-yl)thiazol-4-yl)acetic Acid (27). Compound **27** was produced using the same synthetic strategy used for **21**. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.65 (t, 1H, J = 6.7 Hz), 7.57 (d, 1H, J = 7.1 Hz), 7.42 (s, 1H), 7.27 (t, 1H, J = 8.2 Hz), 7.13−7.04 (m, 2H), 6.75 (d, 1H, J = 1.9 Hz), 5.92−5.70 (m, 1H), 3.78 (s, 2H), 3.20 (s, 3H), 2.58−2.44 (m, 1H), 2.26 (d, 1H, J = 13.8 Hz), 2.22−2.08 (m, 1H), 1.83−1.43 (m, 5H), 1.38−1.27 (m, 1H), 1.25−1.13 (m, 1H); ESI-MS m/z 574.08 $(M + H)^+$.

2-((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-1′-methyl-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indolin]-5′-yl)thiazole-4-carboxamide (28). Starting with acid **21** in place of **66**, compound **28** was produced using general procedure A. Crude **28** was purified by HPLC to produce **28** as its TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.11 (s, 1H), 7.75 (t, 1H, J = 7.2 Hz), 7.42 (d, 1H, J = 8.1 Hz), 7.26 (t, 1H, J = 8.46 Hz), 7.09 (t, 1H, J = 7.83), 7.01 (dd, 1H, J = 1.7, 8.1 Hz), 6.71 (d, 1H, J = 1.8 Hz), 3.00 (s, 3H), 2.36−2.16 (m, 2H), 2.09−2.00 (m, 1H), 1.91−1.52 (m, 5H), 1.38−1.24 (m, 1H), 1.21−1.06 (m, 1H); ESI-MS m/z 559.42 $(M + H)^+$.

General Procedure B: Amide Coupling of Alkylamines. HATU (2 equiv) and DIEA (4 equiv) were added to a suspension of acid compound **29** (1 equiv) in DCM. After 10 min, alkylamine (4 equiv) was added. After 12 h, the reaction was concentrated and purified by column chromatography to produce alkyl amide compounds.

General Procedure C: Hydrolysis of Alkyl Esters. $LiOH·H_2O$ (3 equiv) was added to a solution of ester (1 equiv) in THF/MeOH/H_2O (1:1:1). After 2 h, TFA was added and the solution was concentrated, redissolved in MeOH/H_2O (3:1), and purified by reverse phase preparative HPLC. The pure fractions were combined and lyophilized to give the carboxylic acid products (TFA salt) as a white powder.

General Procedure D: Amide Coupling of Arylamines. Ph_2POCl (3 equiv) and DIEA (5 equiv) were added to a suspension of compound **29** (1 equiv) in DCM. After 30 min, arylamine (4 equiv) and DMAP (1 equiv) were added. After 12 h, the reaction was concentrated and purified by column chromatography to produce arylamide compounds.

General Procedure E: Hydrolysis of Aryl Esters. NaOH (3 equiv) was added to a solution of ester (1 equiv) in THF/MeOH/H_2O (1:1:1). After 2 h, TFA was added and the reaction was concentrated, redissolved in MeOH/H_2O (3:1), and purified by reverse phase preparative HPLC. The pure fractions were combined and lyophilized to give the carboxylic acid products as the TFA salt as a white powder.

(3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-*N*-(methylsulfonyl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamide (30). Starting with compound **29** in place of **66**, compound **30** was obtained according to general procedure A. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 7.61 (t, 1H, J = 7.5 Hz), 7.53 (dd, 1H, J = 2.1, 8.2 Hz), 7.33 (t, 1H, J = 8.0 Hz), 7.17−7.06 (m, 2H), 6.76 (d, 1H, J = 1.7 Hz), 4.98 (d, 1H, J = 10.4 Hz), 3.09 (s, 3H), 2.60 (d, 1H, J = 13.7 Hz), 2.09 (d, 1H, J = 16.1 Hz), 2.01−1.43 (m, 6H), 1.34−1.06 (m, 2H); ESI-MS m/z 540.08 $(M + 1)^+$.

4-((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamido)butanoic Acid (31). **31** was obtained according to general procedure B followed by general procedure C. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 7.64 (t, 1H, J = 7.1 Hz), 7.51 (dd, 1H, J = 2.2, 8.3 Hz), 7.39 (t, 1H, J = 7.5 Hz), 7.17 (t, 1H, J = 8.1 Hz), 7.11 (dd, 1H, J = 1.6, 8.2 Hz), 6.78 (d, 1H, J = 1.6 Hz), 5.12 (d, 1H, J = 11.0 Hz), 4.80 (d, 1H, J = 11.1 Hz), 3.21−3.04 (m, 1H), 2.81 (d, 1H, J = 7.4 Hz), 2.17 (d, 1H, J = 12.0 Hz), 2.06 (t, 2H, J = 7.4 Hz), 2.01−1.84 (m, 3H), 1.84−1.40 (m, 5H), 1.32−1.12 (m, 2H); ESI-MS m/z 548.42 $(M + 1)^+$.

(1*R*,3*r*)-3-((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamido)cyclobutane-1-carboxylic Acid (32). **32** was obtained by general procedure B followed by general procedure C. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 7.65 (t, 1H, J = 7.0 Hz), 7.49 (dd, 1H, J = 1.7, 8.0 Hz), 7.40 (t, 1H, J = 7.3 Hz), 7.18 (t, 1H, J = 8.0 Hz), 7.11 (d, 1H, J = 8.3 Hz), 6.79 (s, 1H), 5.06 (d, J = 10.8, 1H), 4.79 (d, 1H, J = 10.8 Hz), 4.47 (p, 1H, J = 8.1 Hz), 2.96−2.71 (m, 2H), 2.63−2.38 (m, 2H), 2.29−2.08 (m, 2H), 2.05−1.83 (m, 4H), 1.76 (d, 2H, J = 16.2 Hz), 1.62−1.39 (m, 1H), 1.33−1.11 (m, 2H) ESI-MS m/z 560.50 $(M + 1)^+$.

(1*R*,4*r*)-4-((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamido)cyclohexane-1-carboxylic Acid (33). **33** was obtained by general procedure B followed by general procedure C. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 7.64 (t, 1H, J = 7.1 Hz), 7.49 (d, 1H, J = 8.1 Hz), 7.39 (t, 1H, J = 7.5 Hz), 7.17 (t, 1H, J = 8.1 Hz), 7.11 (d, 1H, J = 8.1 Hz), 6.79 (s, 1H), 5.08 (d, 1H, J = 11.1 Hz), 4.79 (d, 1H, J = 11.1 Hz), 3.72−3.54 (m, 1H), 2.84 (d, 1H, J = 8.4 Hz), 2.25−2.07 (m, 2H), 2.04−1.84 (m, 6H), 1.77 (d, 2H, J = 12.0 Hz), 1.63 (d, 1H, J = 13.0 Hz), 1.56−1.34 (m, 3H), 1.32−1.11 (m, 3H), 0.99−0.82 (m, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ ppm 179.01, 178.21, 167.66, 157.76 (d, J_{C-F} = 248.87 Hz), 145.27, 137.07, 132.35, 129.50, 128.91, 126.48 (d, J_{C-F} = 4.74 Hz), 123.39, 122.45, 122.31, 122.05, 121.87, 111.77, 73.24, 67.76, 62.25, 46.72, 46.68, 43.15, 32.33, 32.09, 32.07, 31.37, 28.79, 28.72, 25.35, 23.11, 21.70; ESI-MS m/z 588.33 $(M + 1)^+$.

DOI: 10.1021/acs.jmedchem.6b01665
J. Med. Chem. 2017, 60, 2819−2839

(1*S*,4*s*)-4-((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamido)cyclohexane-1-carboxylic Acid (34). **34** was obtained according to general procedure B followed by general procedure C. ^{1}H NMR (300 MHz, CD3OD) δ ppm 7.67 (t, 1H, J = 6.61 Hz), 7.49 (dd, 1H, J = 2.0, 8.1 Hz), 7.37 (t, 1H, J = 7.5 Hz), 7.16 (t, 1H, J = 7.9 Hz), 7.10 (dd, 1H, J = 1.7, 8.3 Hz), 6.79 (d, 1H, J = 1.7 Hz), 5.20 (d, 1H, J = 11.3 Hz), 4.76 (d, 1H, J = 11.2 Hz), 3.89−3.75 (m, 1H), 2.84 (d, 1H, J = 9.1 Hz), 2.41−2.29 (m, 1H), 2.20 (d, 1H, J = 15.3 Hz), 2.03−1.12 (m, 16H); ESI-MS m/z 588.50 $(M + H)^+$.

(1*R*,4*r*)-4-(((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamido)methyl)cyclohexane-1-carboxylic Acid (35). **35** was obtained according to general procedure B followed by general procedure C. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 8.36−8.26 (m, 1H), 7.67 (t, 1H, J = 6.8 Hz), 7.52 (d, 1H, J = 8.2 Hz), 7.42 (t, 1H, J = 7.5 Hz), 7.20 (t, 1H, d J = 8.0 Hz), 7.12 (dd, 1H, J = 1.4, 8.2 Hz), 6.78 (d, 1H, J = 1.5 Hz), 5.14 (d, 1H, J = 11.1 Hz), 4.78 (d, 1H, J = 11.3 Hz), 3.48−3.34 (m, 1H), 2.90−2.64 (m, 2H), 2.19 (d, 1H, J = 11.3 Hz), 2.09−1.70 (m, 8H), 1.61−1.11 (m, 8H), 0.79−0.59 (m, 2H); ESI-MS m/z 602.58 $(M + 1)^+$.

(3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-*N*-(1,1-dioxidotetrahydro-2*H*-thiopyran-4-yl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamide (36). **36** was obtained according to general procedure B. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 7.64 (t, 1H, J = 7.1 Hz), 7.49 (dd, 1H, J = 2.1, 8.1 Hz), 7.41 (t, 1H, J = 7.5 Hz), 7.18 (t, 1H, J = 9.9 Hz), 7.11 (dd, 1H, J = 1.6, 8.2 Hz), 6.79 (d, 1H, J = 1.6 Hz), 5.10 (d, 1H, J = 11.1 Hz), 4.80 (d, 1H, J = 11.2 Hz), 4.10−3.94 (m, 1H), 3.27−2.91 (m, 3H), 2.88−2.74 (m, 2H), 2.35−1.64 (m, 10H), 1.52 (q, 1H, J = 13.9 Hz), 1.31−1.11 (m, 2H); ESI-MS m/z 594.50 $(M + 1)^+$.

2-(4-((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamido)piperidin-1-yl)acetic Acid (37). **37** was obtained according to general procedure B followed by general procedure C. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 7.66 (t, 1H, J = 7.1 Hz), 7.48 (dd, 1H, J = 2.1, 8.2 Hz), 7.39 (t, 1H, J = 7.3 Hz), 7.17 (t, 1H, J = 8.0 Hz), 7.10 (dd, 1H, J = 1.6, 8.1 Hz), 6.79 (d, 1H, J = 1.7 Hz), 5.12 (d, 1H, J = 11.0 Hz), 4.80 (d, 1H, J = 10.9 Hz), 4.05−3.89 (m, 2H), 3.79−3.39 (m, 2H), 3.29−3.04 (m, 2H), 2.79 (d, 1H, J = 9.4 Hz), 2.17 (d, 2H, J = 9.4 Hz), 2.03−1.42 (m, 8H), 1.42−1.33 (m, 2H), 1.31−1.10 (m, 2H); ESI-MS m/z 603.67 $(M + 1)^+$.

(3′*R*,4′*S*,5′*R*)-*N*-(1-Acetylazetidin-3-yl)-6″-chloro-4′-(3-chloro-2-fluorophenyl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamide (38). **38** was obtained according to general procedure B. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 7.64 (t, 1H, J = 7.2 Hz), 7.50 (dd, 1H, J = 1.8, 8.2 Hz), 7.40 (t, 1H, J = 7.7 Hz), 7.17 (t, 1H, J = 8.1 Hz), 7.11 (dd, 1H, J = 1.7, 8.2 Hz), 6.79 (s, 1H), 5.10 (d, 1H, J = 10.6 Hz), 4.64−4.36 (m, 2H), 4.29−4.11 (m, 1H), 4.09−3.56 (m, 2H), 2.79 (d, 1H, J = 9.8 Hz), 2.16 (d, 1H, J = 14.9 Hz), 2.01−1.68 (m, 8H), 1.64−1.37 (m, 1H), 1.34−1.11 (m, 2H); ESI-MS m/z 560.08 $(M + 1)^+$.

3-((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamido)benzoic Acid (40). **40** was obtained according to general procedure D followed by general procedure E. ^{1}H NMR (300 MHz, CD3OD) δ ppm 8.14 (s, 1H), 7.84−7.67 (m, 3H), 7.55 (dd, 1H, J = 1.9, 8.2 Hz), 7.48−7.34 (m, 2H), 7.19 (t, 1H, J = 8.0 Hz), 7.12 (dd, 1H, J = 1.6, 8.2 Hz), 6.79 (d, 1H, J = 1.6 Hz), 5.29 (d, 1H, J = 11.0 Hz), 4.97 (d, 1H, J = 10.7 Hz), 2.96−2.84 (m, 1H), 2.18 (d, J = 14.0 Hz 1H), 2.08−1.85 (m, 3H), 1.78 (d, 2H, J = 12.2 Hz), 1.63−1.42 (m, 1H), 1.35−1.13 (m, 2H); ESI-MS m/z 582.58 $(M + H)^+$.

4-(((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamido)methyl)benzoic Acid (41). **41** was obtained according to general procedure B followed by general procedure E. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 7.87 (d, 2H, J = 8.2 Hz), 7.66 (t, 1H, J = 7.0 Hz), 7.50 (dd, 1H, J = 2.3, 8.3 Hz), 7.40 (t, 1H, J = 7.6 Hz), 7.22−7.08 (m, 2H), 7.04 (d, 2H, J = 8.2 Hz), 6.78 (d, 1H, J = 1.6 Hz), 5.20 (d, 1H, J = 11.2 Hz), 4.80 (d, 1H, J = 11.1 Hz), 4.66 (d, 1H, J = 15.3 Hz), 4.20 (d, 1H, J = 15.3 Hz), 2.83 (d, 1H, J = 10.0 Hz), 2.20 (d, 1H, J = 15.8 Hz), 2.04−1.85 (m, 3H), 1.77 (d, 2H, J = 11.9 Hz), 1.52 (q, 1H, J = 13.7 Hz), 1.33−1.10 (m, 2H); ESI-MS m/z 596.33 $(M + 1)^+$.

2-(4-((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamido)phenyl)acetic Acid (42). **42** was obtained according to general procedure D followed by general procedure E. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.70 (t, 1H, J = 7.8 Hz), 7.52 (dd, 1H, J = 2.5, 8.2 Hz), 7.47 (d, 2H, J = 8.6 Hz), 7.35 (t, 1H, J = 8.1 Hz), 7.24 (d, 2H, J = 8.6 Hz), 7.16 (t, 1H, J = 8.0 Hz), 7.10 (dd, 1H, J = 1.9, 8.2 Hz), 6.78 (d, 1H, J = 1.9 Hz), 5.20 (d, 1H, J = 9.8 Hz), 4.93 (d, 1H, J = 10.8 Hz), 3.57 (s, 2H), 2.87−2.69 (m, 1H), 2.13 (d, 1H, J = 13.3 Hz), 1.99−1.70 (m, 5H), 1.64−1.48 (m, 1H), 1.27−1.12 (m, 2H); ESI-MS m/z 596.42 $(M + H)^+$.

4-((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamido)-2-methoxybenzoic Acid (43). **43** was obtained according to general procedure D followed by general procedure E. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 7.83 (d, 1H, J = 8.51 Hz), 7.70 (t, 1H, J = 6.92 Hz), 7.62 (s, 1H), 7.52 (dd, 1H, J = 2.17, 8.08 Hz), 7.35 (t, 1H, J = 7.08 Hz), 7.20−7.02 (m, 3H), 6.78 (d, 1H, J = 1.71 Hz), 5.24−5.09 (m, 1H), 4.94 (d, 1H, J = 10.49 Hz), 3.89 (s, 3H), 2.80−2.58 (m, 1H), 2.09 (d, 1H, J = 14.12 Hz), 2.01−1.47 (m, 6H), 1.26−1.09 (m, 2H); ESI-MS m/z 612.42 $(M + H)^+$.

4-((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamido)-3-methoxybenzoic Acid (44). **44** was obtained according to general procedure D followed by general procedure E. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 8.26 (d, 1H, J = 8.6 Hz), 7.79 (t, 1H, J = 7.1 Hz), 7.66 (dd, 1H, J = 1.4, 8.4 Hz), 7.60 (s, 1H), 7.51 (dd, 1H, J = 2.2, 8.3 Hz), 7.40 (t, 1H, J = 8.2 Hz), 7.21 (t, 1H, J = 8.6 Hz), 7.10 (dd, 1H, J = 1.8, 8.1 Hz), 6.79 (d, 1H, J = 1.7 Hz), 5.49−5.23 (m, 1H), 3.80 (s, 3H), 2.68−2.47 (m, 1H), 2.21−1.52 (m, 7H), 1.35−1.07 (m, 2H); ESI-MS m/z 612.17 $(M + 1)^+$.

4-((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamido)-2-fluorobenzoic Acid (45). **45** was obtained according to general procedure D followed by general procedure E. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 7.90 (t, 1H, J = 8.4 Hz), 7.74−7.61 (m, 2H), 7.55 (dd, 1H, J = 2.5, 8.2 Hz), 7.38 (t, 1H, J = 7.2 Hz), 7.26 (dd, 1H, J = 1.7, 8.6 Hz), 7.19 (t, 1H, J = 8.2 Hz), 7.12 (dd, 1H, J = 1.8, 8.2 Hz), 6.80 (d, 1H, J = 1.7 Hz), 5.31 (d, 1H, J = 10.8 Hz), 4.97 (d, 1H, J = 10.8 Hz), 2.94−2.84 (m, 1H), 2.20 (d, 1H, J = 15.7 Hz), 2.06−1.85 (m, 3H), 1.79 (d, 2H, J = 10.9 Hz), 1.65−1.43 (m, 1H), 1.34−1.11 (m, 2H); ESI-MS m/z 600.42 $(M + H)^+$.

4-((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamido)-3-fluorobenzoic Acid (46). **46** was obtained according to general procedure D followed by general procedure E. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 8.21 (t, 1H, J = 8.0 Hz), 7.91−7.79 (m, 1H), 7.79−7.67 (m, 2H), 7.52 (dd, 1H, J = 2.4, 8.2 Hz), 7.37 (t, 1H, J = 7.3 Hz), 7.25−7.06 (m, 2H), 6.79 (d, 1H, J = 1.7 Hz), 5.41 (d, 1H, J = 9.4 Hz), 2.81−2.67 (m, 1H), 2.14 (d, 1H, J = 14.7 Hz), 2.00−1.84 (m, 3H), 1.84−1.70 (m, 2H), 1.68−1.47 (m, 1H), 1.41−1.09 (m, 2H); ESI-MS m/z 600.83 $(M + H)^+$.

5-((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamido)picolinic Acid (47). **47** was obtained according to general procedure D followed by general procedure E. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 8.80 (s, 1H), 8.27 (d, 1H, J = 8.8 Hz), 8.15 (d, 1H, J = 8.7 Hz), 7.71 (t, 1H, J = 7.2 Hz), 7.55 (d, 1H, J = 8.7 Hz), 7.37 (t, 1H, J = 7.1 Hz), 7.18 (t, 1H, J = 7.9 Hz), 7.12 (d, 1H, J = 8.1 Hz), 6.80 (s, 1H), 5.30 (d, 1H, J = 11.1 Hz), 5.00 (d, 1H, J = 10.8 Hz), 2.90−2.75 (m, 1H), 2.16 (d, 1H, J = 16.9 Hz), 2.06−1.84 (m, 3H), 1.78 (d, 2H, J = 13.2 Hz), 1.66−1.42 (m, 1H), 1.32−1.11 (m, 2H); ESI-MS m/z 583.96 $(M + 1)^+$.

5-((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamido)furan-2-carboxylic Acid (48). **48** was obtained according to general procedure D followed by general procedure E. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 7.66 (t, 1H, J = 7.23 Hz), 7.49

DOI: 10.1021/acs.jmedchem.6b01665
J. Med. Chem. 2017, 60, 2819−2839

(dd, 1H, J = 2.46, 8.24 Hz), 7.31 (t, 1H, J = 7.44 Hz), 7.22 (d, 1H, J = 3.60 Hz), 7.16−7.04 (m, 2H), 6.76 (d, 1H, J = 1.68 Hz), 6.51 (d, 1H, J = 3.53 Hz), 5.06−4.90 (m, 2H), 2.53−2.37 (m, 1H), 2.06−1.50 (m, 7H), 1.25−1.00 (m, 2H); ESI-MS m/z 572.42 $(M + H)^+$.

(3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-*N*-(4-((methylsulfonyl)carbamoyl)phenyl)-2″-oxodispiro-[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamide (49). Starting with compound **39** in place of **66**, compound **49** was obtained according to general procedure A. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 7.89 (d, 2H, J = 8.8 Hz), 7.78−7.66 (m, 3H), 7.52 (dd, 1H, J = 2.5, 8.4 Hz), 7.36 (t, 1H, J = 7.8 Hz), 7.17 (t, 1H, J = 7.6 Hz), 7.11 (dd, 1H, J = 1.7, 8.2 Hz), 6.79 (d, 1H, J = 1.7 Hz), 5.31−5.15 (m, 1H), 3.35 (s, 3H), 2.89−2.70 (m, 1H), 2.13 (d, 1H, J = 13.6 Hz), 2.03−1.70 (m, 5H), 1.68−1.46 (m, 1H), 1.35−1.12 (m, 2H); ESI-MS m/z 659.67 $(M + H)^+$.

(3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-2″-oxo-*N*-(4-sulfamoylphenyl)dispiro- [cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamide (50). **50** was obtained according to general procedure D. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 7.60−7.50 (m, 3H), 7.39 (dd, 1H, J = 2.24, 8.22 Hz), 7.30 (t, 1H, J = 6.96 Hz), 7.12−7.02 (m, 2H), 6.73 (d, 1H, J = 1.59 Hz), 6.64 (d, 2H, J = 8.68 Hz), 4.80 (d, 1H, J = 10.19 Hz), 4.62 (d, 1H, J = 10.28 Hz), 2.24 (d, 1H, J = 13.09 Hz), 1.94 (d, 1H, J = 13.01 Hz), 1.88−1.41 (m, 6H), 1.20−0.93 (m, 2H); ESI-MS m/z 617.17 $(M + H)^+$.

(3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-*N*-(4-(methylsulfonyl)phenyl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamide (51). **51** was obtained according to general procedure D. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 7.91 (d, 2H, J = 8.80 Hz), 7.82 (d, 2H, J = 8.85 Hz), 7.70 (t, 1H, J = 6.71 Hz), 7.52 (dd, 1H, J = 2.42, 8.17 Hz), 7.35 (t, 1H, J = 7.58 Hz), 7.16 (t, 1H, J = 8.05 Hz), 7.10 (dd, 1H, J = 1.84, 8.21 Hz), 6.78 (d, 1H, J = 1.80 Hz), 5.20 (d, 1H, J = 14.03 Hz), 4.94 (d, 1H, J = 10.48 Hz), 3.09 (s, 3H), 2.80−2.63 (m, 1H), 2.11 (d, 1H, J = 13.18 Hz), 2.01−1.46 (m, 6H), 1.31−1.08 (m, 2H); ESI-MS m/z 616.58 $(M + H)^+$.

(3′*R*,4′*S*,5′*R*)-*N*-(4-Carbamoylphenyl)-6″-chloro-4′-(3-chloro-2-fluorophenyl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamide (52). Starting with compound **39** in place of **66**, compound **52** was obtained according to general procedure A. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.85 (d, 2H, J = 8.9,Hz), 7.70 (t, 1H, J = 7.9 Hz), 7.65 (d, 2H, J = 8.8 Hz), 7.51 (dd, 1H, J = 2.4, 8.2 Hz), 7.34 (t, 1H, J = 7.1 Hz), 7.15 (t, 1H, J = 8.0 Hz), 7.09 (dd, 1H, J = 1.9, 8.2 Hz), 6.78 (d, 1H, J = 1.9 Hz), 5.22−5.10 (m, 1H), 4.93 (d, 1H, J = 9.6 Hz), 2.81−2.58 (m, 1H), 2.09 (d, 1H, J = 12.4 Hz), 1.99−1.67 (m, 5H), 1.67−1.50 (m, 1H), 1.28−1.10 (m, 2H); ESI-MS m/z 581.33 $(M + H)^+$.

(3′*R*,4′*S*,5′*R*)-*N*-(4-Carbamoyl-2-methoxyphenyl)-6″-chloro-4′-(3-chloro-2-fluorophenyl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamide (53). Starting with compound **44** in place of **66**, compound **53** was obtained according to general procedure A. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.24 (d, 1H, J = 8.4 Hz), 7.75 (t, 1H, J = 7.0 Hz), 7.55−7.45 (m, 3H), 7.35 (t, 1H, J = 7.0 Hz), 7.15 (t, 1H, J = 7.9 Hz), 7.06 (dd, 1H, J = 1.9, 8.2 Hz), 6.76 (d, 1H, J = 1.9 Hz), 5.34−5.10 (m, 1H), 3.85 (s, 3H), 2.57−2.31 (m, 1H), 2.18−1.96 (m, 2H), 1.91−1.57 (m, 5H), 1.25−1.05 (m, 2H); ESI-MS m/z 611.25 $(M + H)^+$.

4-((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-4,4-dimethyl-2″-oxodispiro[cyclo- hexane-1,2'-pyrrolidine-3′,3″-indoline]-5'-carboxamido)benzoic Acid (54). **54** was obtained according to general procedure D followed by general procedure E. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.98 (d, 2H, J = 8.7 Hz), 7.70 (t, 1H, J = 7.7 Hz), 7.64 (d, 2H, J = 8.7 Hz), 7.57 (dd, 1H, J = 2.4, 8.2 Hz), 7.38 (t, 1H, J = 8.2 Hz), 7.19 (t, 1H, J = 8.1 Hz), 7.13 (dd, 1H, J = 1.9, 8.2 Hz), 6.81 (d, 1H, J = 1.9 Hz), 5.29 (d, 1H, J = 10.5 Hz), 4.97 (d, 1H, J = 10.8 Hz), 2.75 (d, 1H, J = 13.6 Hz), 2.13 (dt, 1H, J = 4.2, 14.0 Hz), 2.04 (d, 1H, J = 13.4 Hz), 1.87 (dt, 1H, J = 3.4, 14.2 Hz), 1.64 (d, 1H, J = 14.7 Hz), 1.53−1.34 (m, 3H), 1.02 (s, 3H), 0.77 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ ppm 178.48, 169.09, 168.51, 157.71 (d, J_{C-F} = 249.3 Hz), 145.30, 142.77, 136.94, 132.18, 131.88(2C), 129.50, 128.91, 128.25, 126.32 (d, J_{C-F} = 4.6 Hz), 123.29, 122.77, 122.28 (d, J_{C-F} = 19.1 Hz), 120.51(2C), 111.75, 72.59, 68.33, 62.99, 46.84, 35.75, 34.45, 32.21, 30.00, 28.07, 27.96, 23.63; ESI-MS m/z 610.25 $(M + H)^+$.

3-((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamido)bicyclo[1.1.1]pentane-1-carboxylic Acid (55). **55** was obtained using the same synthetic strategy described for **56**. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 7.61 (t, 1H, J = 6.55 Hz), 7.49 (dd, 1H, J = 2.34, 8.20 Hz), 7.39 (t, 1H, J = 6.90 Hz), 7.15 (t, 1H, J = 8.53 Hz), 7.10 (dd, 1H, J = 1.94, 8.22 Hz), 6.78 (d, 1H, J = 1.88 Hz), 4.98 (d, 1H, J = 10.87 Hz), 4.78 (d, 1H, J = 10.92 Hz), 2.84−2.71 (m, 1H), 2.26 (s, 6H), 2.14 (d, 1H, J = 13.90 Hz), 2.02−1.67 (m, 5H), 1.60−1.38 (m, 1H), 1.31−1.10 (m, 2H); ESI-MS m/z 572.25 $(M + H)^+$.

4-((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamido)bicyclo[2.2.2]octane-1-carboxylic Acid (56). HATU (616 mg, 1.62 mmol) and DIEA (0.550 mL, 3.24 mmol) were added to a suspension of acid **29** (500 mg, 1.08 mmol) in DCM (15 mL) and stirred. After 10 min, methyl 4-aminobicyclo[2.2.2]-octane-1-carboxylate (396 mg, 2.16 mmol) and DMAP (132 mg, 1.08 mmol) were added to the reaction. After overnight, the solvent was removed in vacuo and the crude was purified by column chromatography to give 549 mg of interemediate **56-ester**. LiOH·H_2O (110 mg, 2.62 mmol) and sodium hydroxide (105 mg, 2.62 mmol) were added to a solution of interemidiate **56-ester** (549 mg, 0.873 mmol) dissolved in a mixture of THF (3 mL), H_2O (3 mL), and MeOH (3 mL). After the hydrolysis was complete, as determined by TLC, the reaction was quenched with TFA (3 mL) and stirred. After 5 min, the solution was concentrated in vacuo (not to dryness) and the resulting oil was redissolved in CH_3CN and H_2O (1:1) and the solution was purified by preparative HPLC. The purified fractions were combined, concentrated in vacuo, redissolved in H_2O, frozen, and lyophilized to give **56** (TFA salt) as a white powder. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 7.63 (t, 1H, J = 6.84 Hz), 7.45 (d, 1H, J = 6.76 Hz), 7.35 (t, 1H, J = 7.21 Hz), 7.18−7.04 (m, 2H), 6.77 (dd, 1H, J = 1.26 Hz), 4.68 (d, 1H, J = 10.61 Hz), 2.73−2.48 (m, 1H), 2.16−1.98 (m, 1H), 1.98−1.43 (m, 18H), 1.27−1.02 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ ppm 180.79, 178.22, 167.86, 157.78 (d, J_{C-F} = 248.9 Hz), 145.23, 137.07, 132.32, 129.39, 128.99, 126.45 (d, J_{C-F} = 4.9 Hz), 123.39, 122.50, 122.17, 122.15 (d, J_{C-F} = 19.3 Hz), 111.77, 73.22, 67.72, 62.58, 52.82, 46.96 (d, J_{C-F} = 3.5 Hz), 38.96, 32.17, 31.36, 30.41(3C), 29.45(3C), 25.35, 23.08, 21.74; ESI-MS m/z 614.92 $(M + H)^+$.

4-((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-4,4-difluoro-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamido)bicyclo[2.2.2]octane-1-carboxylic Acid (57). **57** was obtained using the same synthetic strategy described for **56**. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 7.71 (s, 1H), 7.63 (t, 1H, J = 6.61 Hz), 7.50 (dd, 1H, J = 2.08, 8.18 Hz), 7.36 (t, 1H, J = 7.54 Hz), 7.18−7.05 (m, 2H), 6.79 (d, 1H, J = 1.83 Hz) 4.96 (d, 1H, J = 10.48 Hz), 4.71 (d, 1H, J = 10.51 Hz), 2.78 (d, 1H, J = 14.25 Hz), 2.59−1.91 (m, 6H), 1.91−1.70 (m, 12H), 1.53−1.33 (m, 1H); ESI-MS m/z 650.92 $(M + H)^+$.

(3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-*N*-(4-((methylsulfonyl)carbamoyl)-bicyclo[2.2.2]octan-1-yl)-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamide (58). CDI (49 mg, 0.303 mmol), DIEA (88 μL, 0.505 mmol), and DMAP (cat.) were added to a solution of **56** (62 mg, 0.101 mmol) in 1,2-dichloroethane (10 mL), and the reaction was heated to 40 °C. After 20 min, methanesulfonamide (96 mg, 1.01 mmol) was added and the reaction was refluxed. After overnight, the sovent was removed in vacuo and the crude was purified by prepartive HPLC to give **58** (TFA salt) as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 7.64 (t, 1H, J = 7.23 Hz), 7.45 (dd, 1H, J = 1.93, 8.22 Hz), 7.36 (t, 1H, J = 7.23 Hz), 7.18−7.04 (m, 2H), 6.77 (d, 1H, J = 1.66 Hz), 4.69 (d, 1H, J = 10.70 Hz), 3.19 (s, 3H), 2.75−2.52 (m, 1H), 2.21−1.99 (m, 1H), 1.99−1.44 (m, 17H), 1.41−1.27 (m, 1H), 1.27−1.03 (m, 2H); ESI-MS m/z 691.42 $(M + H)^+$.

4-((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-1′-methyl-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamido)bicyclo[2.2.2]octane-1-carboxylic Acid (59). Paraformaldehyde (15 mg, 0.506 mmol) was added to a

DOI: 10.1021/acs.jmedchem.6b01665
J. Med. Chem. 2017, 60, 2819−2839

solution of compound **56** (20 mg, 0.028 mmol) dissolved in AcOH (1 mL). After 15 min sodium triacetoxyborohydride (59 mg, 0.28 mmol) was added, and after reacting overnight, the reaction was quenched with saturated ammonium chloride solution and extracted with EtOAc. The EtOAc solvent was evaporated in vacuo, and the resulting oil was redissolved in a solution of MeCN and H_2O (1:1 with 0.1% TFA) and purified by preparative HPLC. The pure **59** fractions were combined, concentrate in vacuo, redissolved in H_2O (with minimum amount of MeCN), frozen, and lyophilized to give **59** as the TFA salt as a white powder. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 7.94 (s, 1H), 7.61−7.52 (m, 2H), 7.40 (t, 1H, J = 7.32 Hz), 7.19−7.08 (m, 2H), 6.78 (d, 1H, J = 1.56 Hz), 4.99 (d, 1H, J = 11.86 Hz), 4.63 (d, 1H, J = 12.06 Hz), 3.27 (s, 3H), 2.61−2.48 (m, 1H), 2.32−2.14 (m, 2H), 1.88−1.40 (m, 18H), 1.37−1.12 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ ppm 180.74, 178.21, 166.55, 157.74 (d, J_{C-F} = 249.6 Hz), 144.90, 137.11, 132.39, 129.80, 129.09, 126.30 (d, J_{C-F} = 4.7 Hz), 124.00, 123.43, 122.31 (d, J_{C-F} = 19.2 Hz), 121.63 (d, J_{C-F} = 13.6 Hz), 111.77, 77.98, 73.06, 67.67, 52.98, 46.56, 40.34, 38.97, 34.28, 30.38(3C), 29.44(3C), 28.97, 24.95, 23.57, 22.07; ESI-MS m/z 628.83 $(M + H)^+$.

4-((3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-1′-ethyl-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carboxamido)bicyclo[2.2.2]octane-1-carboxylic Acid (60). **60** was obtained using the same synthetic strategy described for **59**. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 7.63 (t, 1H, J = 7.04 Hz), 7.56−7.48 (m, 2H), 7.42 (t, 1H, J = 7.39 Hz), 7.18 (t, 1H, J = 7.96 Hz), 7.10 (d, 1H, J = 8.06 Hz), 6.79 (s, 1H), 5.08−4.96 (m, 1H), 4.57 (d, 1H, J = 11.85 Hz), 4.18−3.99 (m, 1H), 3.87−3.69 (m, 1H), 2.70−2.54 (m, 1H), 2.36−2.13 (m, 2H), 1.94−1.45 (m, 18H), 1.39 (t, 3H, J = 6.65 Hz), 1.32−1.14 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ ppm 180.95, 180.06, 177.01, 157.71 (d, J_{C-F} = 247.1 Hz), 144.91, 135.39, 130.37, 129.74 (d, J_{C-F} = 1.6 Hz), 129.34 (d, J_{C-F} = 2.5 Hz), 126.86 (d, J_{C-F} = 12.5 Hz), 126.47, 125.46 (d, J_{C-F} = 4.6 Hz), 122.27, 121.50 (d, J_{C-F} = 19.7 Hz), 110.69, 74.84, 72.58, 71.05, 51.51, 48.09, 47.36, 39.17, 37.56, 30.84(3C), 29.61(3C), 29.25, 26.37, 24.25, 23.14, 17.04; ESI-MS m/z 642.50 $(M + H)^+$.

(3′*R*,4′*S*,5′*R*)-6″-Chloro-4′-(3-chloro-2-fluorophenyl)-1′-methyl-2″-oxodispiro[cyclohexane-1,2′-pyrrolidine-3′,3″-indoline]-5′-carbothioamide (68). Intermediate **64**[21] (1.0 g, 2.09 mmol) was dissolved in a mixture of DCM (10 mL) and AcOH (10 mL). Paraformaldehyde (1.13 g, 37.62 mmol) was added, and after 10 min $NaBH(OAc)_3$ (4.43 g, 20.9 mmol) was added in portions. After 4 h, as determined by TLC, the reaction was complete. Saturated NH_4Cl (aq) was added to the reaction which was then extracted with EtOAc. The EtOAc solution was washed with NaOH (1 M) and brine, then dried over Na_2SO_4, filtered, concentrated, and purified by column chromatography to give 764 mg of intermediate **65**. Sodium hydroxide (320 mg, 8.0 mmol) was added to a solution of intermediate **65** (764 mg, 1.6 mmol) dissolved in a mixture of THF (3 mL), H_2O (3 mL), and MeOH (3 mL), and the solution was heated to 45−50 °C. After hydrolysis was complete, as determined by TLC, the reaction was cooled, neutralized with 2 M HCl, and extracted with EtOAc. The EtOAc solution was dried over Na_2SO_4, filtered, and concentrated to give the acid intermediate **66** as a solid that was used without further purification. CDI (315 mg, 1.94 mmol) and DIEA (0.450 mL, 2.59 mmol) were added to a solution of **66** (300 mg, 0.648 mmol) in THF (15 mL) and heated to 50 °C. After 30 min ammonium hydroxide was was added. After standing overnight, H_2O was added and the reaction was extracted with EtOAc. The EtOAc solution was dried over sodium sulfate, filtered, concentrated, and purified by column chromatography to give 240 mg of compound **67**. Lawesson's reagent (102 mg, 0.252 mmol) was added to a solution of compound **67** (60 mg, 0.126 mmol) in DCM (3 mL) and refluxed overnight. Then the reaction was cooled, the solvent was removed, and the crude was column chromatographed to produce 60 mg of **68**. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.66 (ddd, 1H, J = 1.4, 6.4, 7.9 Hz), 7.53 (dd, 1H, J = 2.5, 8.3 Hz), 7.38 (t, 1H, J = 7.3 Hz), 7.15 (t, 1H, J = 8.0 Hz), 7.10 (dd, 1H, J = 1.9, 8.2 Hz), 6.77 (d, 1H, J = 1.9 Hz), 5.48 (br s, 1H), 4.71 (d, 1H, J = 11.2 Hz), 3.37 (s, 3H), 2.38 (d, 1H, J = 14.2 Hz), 2.19−2.02 (m, 1H), 1.99−1.85 (m, 1H), 1.83−1.61 (m, 4H), 1.50−1.36 (m, 1H), 1.35−1.16 (m, 2H); ESI-MS m/z 492.67 $(M + H)^+$.

Fluorescence Polarization-Based (FP-Based) Protein Binding Assay, Cell Growth Inhibition Assay, Pharmacodynamics (PD) Study in SJSA-1 Xenograft Model, and in Vivo Efficacy Study. These assays were performed according to the previously reported procedures.[21] All the in vivo studies were performed under an animal protocol (PRO00005315) approved by the University Committee on Use and Care of Animals of the University of Michigan, in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Computational Methods. Docking studies were performed using our previously reported structure of MDM2 in complex with **2** (PDB code 5TRF)[18] with the GOLD[33,34] program (version 5.2). For each genetic algorithm (GA) run, a maximum number of 200 000 operations were performed on a population of five islands of 100 individuals. Operator weights for crossover, mutation, and migration were set to 95, 95, and 10, respectively, and the Chemscore[35] fitness function was used to evaluate the docked conformations. Docking was terminated after 10 runs for each ligand, and the top 10 conformations were saved for analysis of the predicted binding modes.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.6b01665.

Molecular formula strings and some data (CSV)
Structure representation (PDB)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: Shaomeng@umich.edu.

ORCID

Ding Du: 0000-0002-4615-5433
Shaomeng Wang: 0000-0002-8782-6950

Notes

The authors declare the following competing financial interest(s): The University of Michigan has filed a patent on AA-115 and its analogues, and Shaomeng Wang, Angelo Aguilar, Jianfeng Lu, Liu Liu, and Donna McEachern are co-inventors of the patent. The patent application covering AA-115 and its analogues has been licensed by Ascentage Pharma Group for clinical development in which Shaomeng Wang is a co-founder, owns stock, and serves as a paid consultant. Shaomeng Wang and the University of Michigan also receive a research grant from Ascentage Pharma Group.

■ ACKNOWLEDGMENTS

This study is supported in part by grants from the National Institutes of Health (Grant R01CA121279 to S.W.), the National Cancer Institute Cancer Center Core grant (P30CA046592) to the University of Michigan Comprehensive Cancer Center, a grant from Ascentage Pharma Group to the University of Michigan (to S.W.), the Key New Drug Creation project of the major science and technology program (Grant 2012ZX09401005, China), National "863" Grant (2012AA020305, China), and Science & Technology Innovation Team Grant of Guangdong Province (Grant 2013Y114) to Jiangsu Ascentage Biomed Development Inc.

■ ABBREVIATIONS USED

THF, tetrahydrofuran; CAN, ceric ammonium nitrate; DCM, dichloromethane; MeOH, methanol; DCE, 1,2-dichloroethane; CDI, 1,1′-carbonyldiimidazole; AcOH, acetic acid; DIEA, *N*,*N*′-

DOI: 10.1021/acs.jmedchem.6b01665
J. Med. Chem. 2017, 60, 2819−2839

diisopropylethylamine; DME, 1,2-dimethoxyethane; TFA, trifluoroacetic acid; TFAA, trifluoracetic anhydride; HPLC, high performance liquid chromatography; UPLC, ultraperformance liquid chromatography; DMAP, 4-(dimethylamino)-pyridine; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; rt, room temperature; HATU, 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate

REFERENCES

(1) Hainaut, P.; Hollstein, M. p53 and human cancer: the first ten thousand mutations. *Adv. Cancer Res.* **1999**, *77*, 81−137.

(2) Vousden, K. H.; Lane, D. P. p53 in health and disease. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 275−283.

(3) Momand, J.; Zambetti, G. P.; Olson, D. C.; George, D.; Levine, A. J. The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. *Cell* **1992**, *69*, 1237−1245.

(4) Freedman, D. A.; Wu, L.; Levine, A. J. Functions of the MDM2 oncoprotein. *Cell. Mol. Life Sci.* **1999**, *55*, 96−107.

(5) Bond, G. L.; Hu, W. W.; Levine, A. J. MDM2 is a central node in the p53 pathway: 12 years and counting. *Curr. Cancer Drug Targets* **2005**, *5*, 3−8.

(6) Wu, X.; Bayle, J. H.; Olson, D.; Levine, A. J. The p53-mdm-2 autoregulatory feedback loop. *Genes Dev.* **1993**, *7*, 1126−1132.

(7) Juven-Gershon, T.; Oren, M. Mdm2: the ups and downs. *Mol. Med.* **1999**, *5*, 71−83.

(8) Momand, J.; Wu, H. H.; Dasgupta, G. MDM2−master regulator of the p53 tumor suppressor protein. *Gene* **2000**, *242*, 15−29.

(9) Vassilev, L. T.; Vu, B. T.; Graves, B.; Carvajal, D.; Podlaski, F.; Filipovic, Z.; Kong, N.; Kammlott, U.; Lukacs, C.; Klein, C.; Fotouhi, N.; Liu, E. A. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science* **2004**, *303*, 844−848.

(10) Vassilev, L. T. p53 Activation by small molecules: application in oncology. *J. Med. Chem.* **2005**, *48*, 4491−4499.

(11) Shangary, S.; Wang, S. Targeting the MDM2-p53 interaction for cancer therapy. *Clin. Cancer Res.* **2008**, *14*, 5318−5324.

(12) Wang, S. M.; Zhao, Y. J.; Bernard, D.; Aguilar, A.; Kumar, S. Targeting the MDM2-p53 protein-protein interaction for new cancer therapeutics. *Top. Med. Chem.* **2012**, *8*, 57−79.

(13) Carry, J. C.; Garcia-Echeverria, C. Inhibitors of the p53/hdm2 protein-protein interaction-path to the clinic. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 2480−2485.

(14) Ray-Coquard, I.; Blay, J. Y.; Italiano, A.; Le Cesne, A.; Penel, N.; Zhi, J.; Heil, F.; Rueger, R.; Graves, B.; Ding, M.; Geho, D.; Middleton, S. A.; Vassilev, L. T.; Nichols, G. L.; Bui, B. N. Effect of the MDM2 antagonist RG7112 on the P53 pathway in patients with MDM2-amplified, well-differentiated or dedifferentiated liposarcoma: an exploratory proof-of-mechanism study. *Lancet Oncol.* **2012**, *13*, 1133−1140.

(15) Zhao, Y.; Aguilar, A.; Bernard, D.; Wang, S. Small-molecule inhibitors of the MDM2-p53 protein-protein interaction (MDM2 Inhibitors) in clinical trials for cancer treatment. *J. Med. Chem.* **2015**, *58*, 1038−1052.

(16) Vu, B.; Wovkulich, P.; Pizzolato, G.; Lovey, A.; Ding, Q.; Jiang, N.; Liu, J. J.; Zhao, C.; Glenn, K.; Wen, Y.; Tovar, C.; Packman, K.; Vassilev, L.; Graves, B. Discovery of RG7112: A small-molecule MDM2 inhibitor in clinical development. *ACS Med. Chem. Lett.* **2013**, *4*, 466−469.

(17) Ding, Q.; Zhang, Z.; Liu, J. J.; Jiang, N.; Zhang, J.; Ross, T. M.; Chu, X. J.; Bartkovitz, D.; Podlaski, F.; Janson, C.; Tovar, C.; Filipovic, Z. M.; Higgins, B.; Glenn, K.; Packman, K.; Vassilev, L. T.; Graves, B. Discovery of RG7388, a potent and selective p53-MDM2 inhibitor in clinical development. *J. Med. Chem.* **2013**, *56*, 5979−5983.

(18) Wang, S.; Sun, W.; Zhao, Y.; McEachern, D.; Meaux, I.; Barriere, C.; Stuckey, J. A.; Meagher, J. L.; Bai, L.; Liu, L.; Hoffman-Luca, C. G.; Lu, J.; Shangary, S.; Yu, S.; Bernard, D.; Aguilar, A.; Dos-Santos, O.; Besret, L.; Guerif, S.; Pannier, P.; Gorge-Bernat, D.; Debussche, L. SAR405838: an optimized inhibitor of MDM2-p53 interaction that induces complete and durable tumor regression. *Cancer Res.* **2014**, *74*, 5855−5865.

(19) Sun, D.; Li, Z.; Rew, Y.; Gribble, M.; Bartberger, M. D.; Beck, H. P.; Canon, J.; Chen, A.; Chen, X.; Chow, D.; Deignan, J.; Duquette, J.; Eksterowicz, J.; Fisher, B.; Fox, B. M.; Fu, J.; Gonzalez, A. Z.; Gonzalez-Lopez De Turiso, F.; Houze, J. B.; Huang, X.; Jiang, M.; Jin, L.; Kayser, F.; Liu, J. J.; Lo, M. C.; Long, A. M.; Lucas, B.; McGee, L. R.; McIntosh, J.; Mihalic, J.; Oliner, J. D.; Osgood, T.; Peterson, M. L.; Roveto, P.; Saiki, A. Y.; Shaffer, P.; Toteva, M.; Wang, Y.; Wang, Y. C.; Wortman, S.; Yakowec, P.; Yan, X.; Ye, Q.; Yu, D.; Yu, M.; Zhao, X.; Zhou, J.; Zhu, J.; Olson, S. H.; Medina, J. C. Discovery of AMG 232, a potent, selective, and orally bioavailable MDM2-p53 inhibitor in clinical development. *J. Med. Chem.* **2014**, *57*, 1454−1472.

(20) Holzer, P.; Masuya, K.; Furet, P.; Kallen, J.; Valat-Stachyra, T.; Ferretti, S.; Berghausen, J.; Bouisset-Leonard, M.; Buschmann, N.; Pissot-Soldermann, C.; Rynn, C.; Ruetz, S.; Stutz, S.; Chene, P.; Jeay, S.; Gessier, F. Discovery of a dihydroisoquinolinone derivative (NVP-CGM097): a highly potent and selective MDM2 inhibitor undergoing phase 1 clinical trials in p53wt tumors. *J. Med. Chem.* **2015**, *58*, 6348−6358.

(21) Aguilar, A.; Sun, W.; Liu, L.; Lu, J.; McEachern, D.; Bernard, D.; Deschamps, J. R.; Wang, S. Design of chemically stable, potent, and efficacious MDM2 inhibitors that exploit the retro-mannich ring-opening-cyclization reaction mechanism in spiro-oxindoles. *J. Med. Chem.* **2014**, *57*, 10486−10498.

(22) Zhao, Y.; Liu, L.; Sun, W.; Lu, J.; McEachern, D.; Li, X.; Yu, S.; Bernard, D.; Ochsenbein, P.; Ferey, V.; Carry, J. C.; Deschamps, J. R.; Sun, D.; Wang, S. Diastereomeric spirooxindoles as highly potent and efficacious MDM2 inhibitors. *J. Am. Chem. Soc.* **2013**, *135*, 7223−7234.

(23) Zhang, Z.; Chu, X. J.; Liu, J. J.; Ding, Q.; Zhang, J.; Bartkovitz, D.; Jiang, N.; Karnachi, P.; So, S. S.; Tovar, C.; Filipovic, Z. M.; Higgins, B.; Glenn, K.; Packman, K.; Vassilev, L.; Graves, B. Discovery of potent and orally active p53-MDM2 inhibitors RO5353 and RO2468 for potential clinical development. *ACS Med. Chem. Lett.* **2014**, *5*, 124−127.

(24) Stepan, A. F.; Subramanyam, C.; Efremov, I. V.; Dutra, J. K.; O'Sullivan, T. J.; DiRico, K. J.; McDonald, W. S.; Won, A.; Dorff, P. H.; Nolan, C. E.; Becker, S. L.; Pustilnik, L. R.; Riddell, D. R.; Kauffman, G. W.; Kormos, B. L.; Zhang, L.; Lu, Y.; Capetta, S. H.; Green, M. E.; Karki, K.; Sibley, E.; Atchison, K. P.; Hallgren, A. J.; Oborski, C. E.; Robshaw, A. E.; Sneed, B.; O'Donnell, C. J. Application of the bicyclo[1.1.1]pentane motif as a nonclassical phenyl ring bioisostere in the design of a potent and orally active gamma-secretase inhibitor. *J. Med. Chem.* **2012**, *55*, 3414−3424.

(25) Kerns, E. H.; Di, L. Plasma Protein Binding. In *Drug-like Properties: Concepts, Structure Design and Methods*; Academic Press: San Diego, CA, 2008; Chapter 14, pp 187−196.

(26) Gillis, E. P.; Eastman, K. J.; Hill, M. D.; Donnelly, D. J.; Meanwell, N. A. Applications of fluorine in medicinal chemistry. *J. Med. Chem.* **2015**, *58*, 8315−8359.

(27) Smart, B. E. Fluorine substituent effects (on bioactivity). *J. Fluorine Chem.* **2001**, *109*, 3−11.

(28) Huchet, Q. A.; Kuhn, B.; Wagner, B.; Fischer, H.; Kansy, M.; Zimmerli, D.; Carreira, E. M.; Muller, K. On the polarity of partially fluorinated methyl groups. *J. Fluorine Chem.* **2013**, *152*, 119−128.

(29) Moody, C. J.; Bagley, M. C. Total synthesis of (+)-nostocyclamide. *J. Chem. Soc., Perkin Trans. 1* **1998**, 601−607.

(30) Phillips, A. J.; Uto, Y.; Wipf, P.; Reno, M. J.; Williams, D. R. Synthesis of functionalized oxazolines and oxazoles with DAST and Deoxo-Fluor. *Org. Lett.* **2000**, *2*, 1165−1168.

(31) Borg, S.; Estennebouhtou, G.; Luthman, K.; Csoregh, I.; Hesselink, W.; Hacksell, U. Synthesis of 1,2,4-oxadiazole-derived, 1,3,4-oxadiazole-derived, and 1,2,4-triazole-derived dipeptidomimetics. *J. Org. Chem.* **1995**, *60*, 3112−3120.

(32) Wipf, P.; Venkatraman, S. Total synthesis of (−)-muscoride A. *J. Org. Chem.* **1996**, *61*, 6517−6522.

DOI: 10.1021/acs.jmedchem.6b01665
J. Med. Chem. 2017, 60, 2819−2839

(33) Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. Development and validation of a genetic algorithm for flexible docking. *J. Mol. Biol.* **1997**, *267*, 727−748.
(34) Verdonk, M. L.; Cole, J. C.; Hartshorn, M. J.; Murray, C. W.; Taylor, R. D. Improved protein-ligand docking using GOLD. *Proteins: Struct., Funct., Genet.* **2003**, *52*, 609−623.
(35) Eldridge, M. D.; Murray, C. W.; Auton, T. R.; Paolini, G. V.; Mee, R. P. Empirical scoring functions: I. The development of a fast empirical scoring function to estimate the binding affinity of ligands in receptor complexes. *J. Comput.-Aided Mol. Des.* **1997**, *11*, 425−445.

DOI: 10.1021/acs.jmedchem.6b01665
J. Med. Chem. 2017, 60, 2819−2839