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Aminopyridyl/Pyrazinyl Spiro[indoline-3,4′-piperidine]-2-ones As Highly Selective and Efficacious c-Met/ALK Inhibitors

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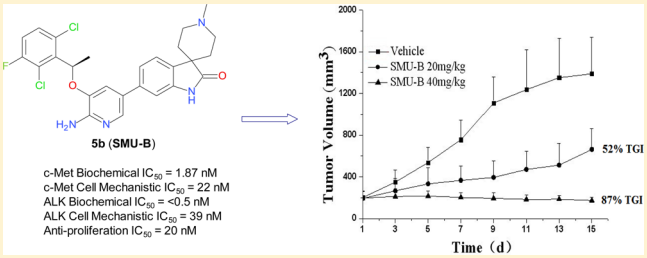
Supporting Information

ABSTRACT: A series of novel aminopyridyl/pyrazinyl-substituted spiro[indoline-3,4′-piperidine]-2-ones were designed, synthesized, and tested in various in vitro/in vivo pharmacological and antitumor assays. 6-[6-Amino-5-[(1*R*)-1-(2,6-dichloro-3-fluorophenyl)ethoxy]-3-pyridyl]-1′-methylspiro[indoline-3,4′-piperidine]-2-one (compound **5b** or **SMU-B**) was identified as a potent, highly selective, well-tolerated, and orally efficacious c-Met/ALK dual inhibitor, which showed pharmacodynamics effect by inhibiting c-Met phosphorylation in vivo and significant tumor growth inhibitions (>50%) in GTL-16 human gastric carcinoma xenograft models.



KEYWORDS: *Hepatocyte growth factor receptor (HGFR or c-Met), anaplastic lymphoma kinase (ALK), inhibitor, cancer, aminopyridine, aminopyrazine, spiro[indoline-3,4′-piperidine]-2-one, small molecule*

c-Met is a receptor tyrosine kinase known as hepatocyte growth factor receptor (HGFR). c-Met and its natural ligand hepatocyte growth factor (HGF), also known as the scatter factor, are often aberrantly expressed in a wide range of human cancers, including hereditary papillary renal cell carcinoma,[1] lung cancers,[2,3] head and neck cancers,[4] and gastric cancer.[5] Because of the role of dysregulated c-Met/HGF signaling in human oncogenesis, invasion, and metastasis, targeting the c-Met/HGF pathway becomes a powerful strategy for anticancer therapy. Currently half a dozen humanized monoclonal antibody and more than two dozens of small molecule c-Met/HGF inhibitors are being evaluated in preclinical and clinical studies as cancer treatments.[6−10] Two small molecule inhibitors, i.e., Pfizer's c-Met/ALK dual inhibitor Crizotinib (PF-02341066) and Exelixis' VEGFR2/c-Met multikinase inhibitor Cabozantinib (Figure 1), have been approved by the FDA, for treatments of late-stage non-small cell lung cancer[11] and progressive metastatic medullary thyroid cancer,[12] respectively.

In addition to c-Met, Crizotinib also inhibits anaplastic lymphoma kinase (ALK). ALK is a member of the insulin receptor superfamily of tyrosine kinases.[13] It plays a role in a number of human cancers, such as neuroblastoma,[14] pulmonary neuroendocrine carcinoma,[15] esophageal cancer,[16] diffuse large cell lymphoma,[17] and certain types of non-small cell lung cancer (NSCLC).[18] Targeting oncogenic c-Met and ALK simultaneously has proven to be beneficial in glioblastoma[19] and Ewing's sarcomas.[20] Although dual inhibition of c-Met and ALK sounds like attractive strategy for cancer therapy, there has not been a lot of known c-Met/ALK dual inhibitors in the literature. Crizotinib is the first and currently only approved c-Met/ALK dual inhibitor for treating EML4-ALK fusion protein-positive NSCLC patients. Other c-Met/ALK dual inhibitors include Xcovery's aminopyridazine-based X-376 and X-396.[21] In continuation of our interest in pursuing therapeutically useful c-Met inhibitors (Figure 2), we report herein a class of novel aminopyridyl/pyrazinyl-substituted spiro[indoline-3,4′-piperidine]-2-ones as highly selective and orally efficacious c-Met/ALK dual inhibitors.

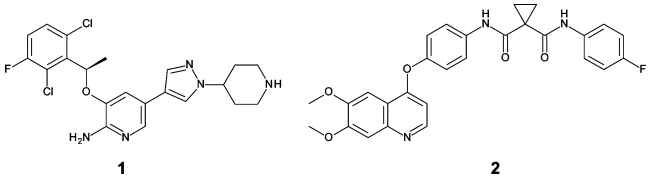


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Figure 1. Crizotinib (**1**) and Cabozantinib (**2**).

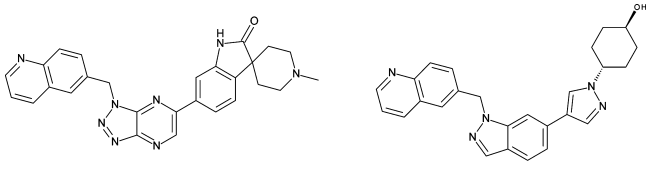

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Figure 2. Compounds **3**[22] and **4**[23] as c-Met inhibitors.

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It was found previously that the 1′-methyl-2-oxo-spiro-[indoline-3,4′-piperidine] moiety when attached to the triazolo-[4,5-*b*]pyrazine core was well tolerated in c-Met kinase.[22] We decided to introduce this group to the typical kinase hinge binding group aminopyridyl, which presented in the chemical structure of Crizotinib (**1**). Therefore, a series of novel 1′-methyl-2-oxo-spiro[indoline-3,4′-piperidine]-substituted aminopyridines and aminopyrazines[24] were designed, computationally modeled, synthesized, and tested in in vitro and in vivo assays. Compounds **5a**−**b**, **6a**−**b**, and **7a**−**b**, shown in Figure 3 were selected examples to be discussed in this letter.

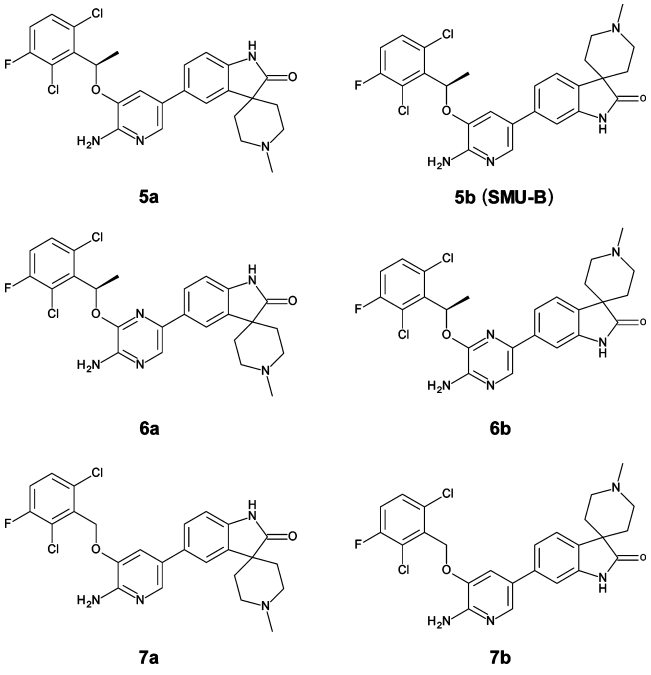

Figure 3. Novel aminopyridyl/pyrazinyl-substituted spiro compounds (**5a**−**b**, **6a**−**b**, and **7a**−**b**).

Docking studies revealed that compounds of **5a**−**b** and **6a**−**b** types fit very well in the crystal structure of c-Met kinase domain (PDB code, 2WGJ), Figure 4 showed the docking model of compound **5b** in the cocrystal structure of Crizotinib bound to the c-Met kinase. It was found that most parts of the molecules including the 2,6-dichloro-3-fluorobenzyloxyl and 2-aminopyridyl overplayed perfectly except for the piperidinyl-pyrazolyl group in Crizotinib and the 1′-methyl-2-oxo-spiro-[indoline-3,4′-piperidine] moiety in compound **5b**. The piperidinyl of Crizotinib and the spiro portion of **5b** were solvent exposed and therefore should not have a significant impact on potency even though they adopted a different conformations. Figure 5 showed the detailed interaction of **5b**

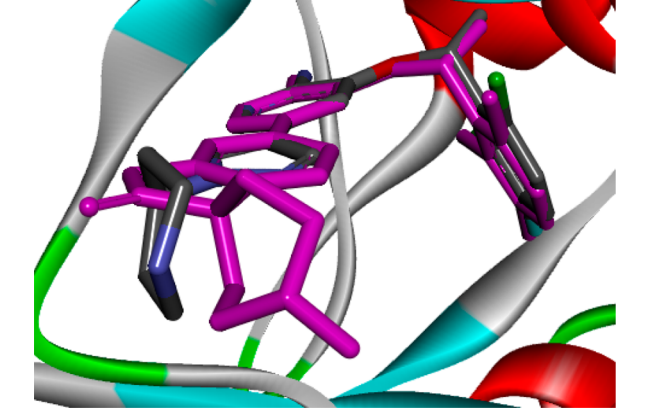

Figure 4. Overlay of Crizotinib (magenta) and compound **5b** (gray) in c-Met kinase domain (PDB code: 2WGJ).

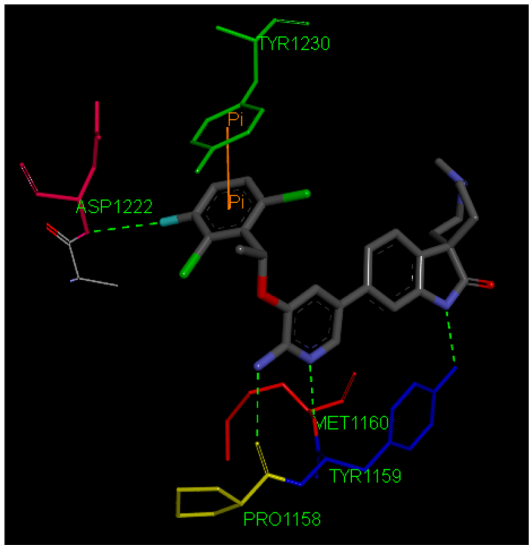

Figure 5. Interactions of compound **5b** with the amino acid residues in the ATP binding pocket of c-Met kinase.

with the c-Met kinase. The 2-aminopyridyl group formed a pair of bidentate hydrogen bonds with hinge residues Pro1158 (yellow) and Met1160 (red). The hydroxyl oxygen of Tyr1159 (blue) formed a hydrogen bond with the indolin-2-one NH. In the lipophilic back pocket, where the 2,6-dichloro-3-fluoro phenyl ring sat, the fluoro group formed a hydrogen bond with Asp1222 NH (magenta). The phenyl ring formed a $\pi-\pi$ stacking interaction with the phenyl ring of Tyr1230 (green). To test the modeling results, aminopyridyl compound **5b** and its regioisomer **5a** as well as their close analogues of aminopyrazines **6a**−**b** were synthesized. Achiral analogues **7a**−**b** were also prepared for comparison purpose.

Scheme 1 showed the synthetic methods for compounds **5a**−**b**, **6a**−**b**, and **7a**−**b**. Compounds **5a**−**b** were prepared by Suzuki coupling of bromide **10a**[25] and boronates **11m** or **11n**.[22] Compounds **6a**−**b** were synthesized in a similar fashion except that bromide **10b** was used. The required intermediate **10b** was obtained by reaction of commercially available 3,5-dibromopyrazin-2-amine (**8**) and (1*R*)-1-(2,6-dichloro-3-fluorophenyl)-ethanol (**9**) under basic condition. Compounds **7a**−**b** were generated by Suzuki coupling of intermediate **13** with **11m** or **11n**. Compound **13** was made in 4 steps from 2,6-dichloro-3-fluorobenzoic acid (**12**) by reduction of acid into alcohol, Mitsunobu reaction of the alcohol with 3-hydroxyl-2-nitropyridine, reduction of the nitro into amino group, and bromination of the pyridine ring with NBS.

The compounds were first screened in MKN45 cell mechanistic assay by measuring the inhibition of phosphorylation of c-Met signals. Crizotinib was used as the reference compound. The results are listed in Table 1.

It was found that compounds **5a**−**b** and **6a**−**b** were very potent in cells with IC_{50} values in the same range with

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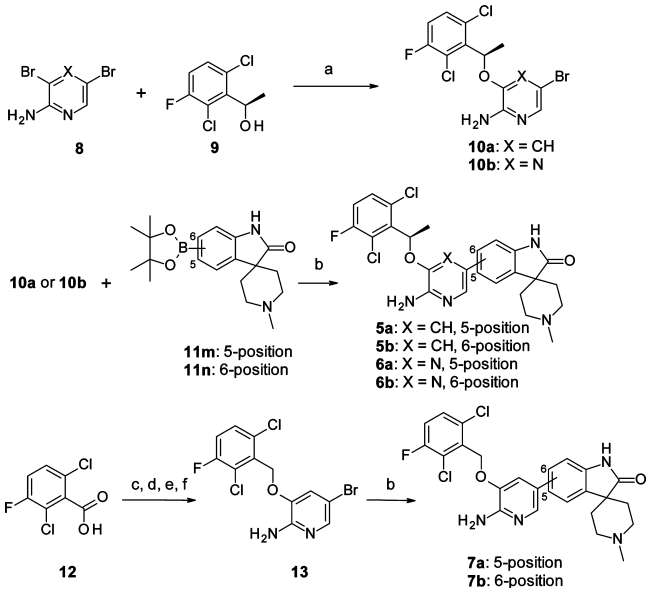
Scheme 1. Synthesis of Compounds 5a−b, 6a−b, and 7a−b[a]



[a]Reagents and conditions: (a) NaH, THF, reflux, 20 h, 78%. (b) $Pd(PPh_3)_4$, DME−H_2O (4:1), K_2CO_3, 80 °C. Yields: 86% for **5a**; 82% for **5b**; 54% for **6a**; 67% for **6b**; 85% for **7a**; 78% for **7b**. (c) BH_3, THF, reflux, 24 h, 75%. (d) 3-Hydroxyl-2-nitropyridine, DIAD, PPh_3, THF, 0 °C, 16 h, 90%. (e) Fe, HCl (aq, 20 mol %), EtOH, 80 °C, 30 min, 95%. (f) NBS, CH_3CN, 0 °C, 30 min, 60%.

Table 1. c-Met Cell Mechanistic IC_{50}s of Compounds 5a−b, 6a−b, and 7a−b in MKN-45 Cells and Modeling Scores

compd	c-Met cell mechanistic IC_{50} (μM)[a] in MKN-45 cells	modeling score[b]
Crizotinib	0.020	8.67
5a	0.020	8.68
5b (**SMU-B**)	0.019	8.67
6a	0.023	9.11
6b	0.012	8.95
7a	>10.0	6.70
7b	9.0	6.91

[a]Data generated in house. [b]Data generated using Surflex-Dock program.

Crizotinib. The high potencies were consistent with the docking results. To our surprise, however, the achiral compounds **7a−b** were much less potent with IC_{50} values ≥ 9 μM although their chemical structures were so similar to compounds **5a−b** and the only difference was with or without the α-methyl group at the benzylic position. To understand what factor had contributed to the differences in potency, modeling scores were generated using the Surflex-Dock program's empirical scoring function (Table 1).[26,27] The results showed that compounds **7a−b** received lower scores of <7.0 than all other compounds, which had scores >8.6. The α-methyl group at the benzylic position must have been required to help maintain the bioactive U-shaped binding mode.

Compound **5b**, i.e., **SMU-B**, was further studied in various in vitro and in vivo assays. In DiscoveRx's *scan*EDGE^{SM} panel of 97 kinases,[28,29] compound **5b** showed high selectivity and only hit three kinases, i.e., c-Met, ALK, and AXL, with percent control <10% at 100 nM drug concentration (Figure 6). To verify this result, compound **5b** was tested in Reaction Biology Corp's nanoliter kinase assay[30] and ProQinase's cell mechanistic assay against c-Met, ALK, and AXL. The results are summarized in Table 2.

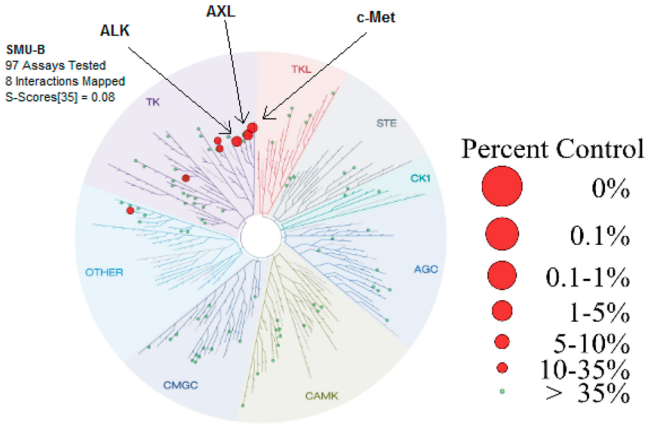


Figure 6. Kinase dendrogram of compound **5b** (**SMU-B**) at [drug] = 100 nM in DiscoveRx's *scan*EDGE^{SM} against 97 wild-type and mutant kinases.

Table 2. Biochemical and Cell Mechanistic IC_{50}s of Compound 5b (SMU-B)

	biochemical IC_{50} (nM)[a]		cell mechanistic IC_{50} (nM)[b]		
target	[ATP][c] (μM)	**5b**	cell line	**5b**	Crizotinib
c-Met	10	1.87	MKN45	22	20
ALK	50	<0.5	karpas 299	39	110
AXL	100	28.9	MEF	300	

[a]Data generated by Reaction Biology Corp. [b]Data generated by ProQinase GmbH. [c][ATP] = Km.

Compound **5b** potently inhibited c-Met, ALK, and AXL kinases with IC_{50}s of 1.87, <0.5, and 28.9 nM, respectively, in biochemical assays, and 22, 39, and 300 nM, respectively, in cellular kinase assays. It was equipotent against c-Met (22 vs 20 nM) and 2.8-fold more potent than Crizotinib against ALK (39 vs 110 nM) in cells. The c-Met cell IC_{50} data of compound **5b** generated by ProQinase in MKN45 cells matched well with our in house data (22 vs 19 nM, Tables 1 and 2). Encouraged by the high biochemical and cell mechanistic potencies against c-Met, we determined the antiproliferative activities of compound **5b** in four cell lines with different c-Met status (Table 3). Compound **5b** had 20 nM IC_{50} in inhibiting the growth of human gastric carcinoma cell line GTL-16 with Met gene amplification but was less potent in non-small cell lung cancer (NSCLC) lines H1993 and H441 with IC_{50}s of 1.58 and 2.82 μM, respectively. Compound **5b** was not potent in human glioblastoma cell line U87MG with HGF/Met autocrine formation.

The excellent in vitro profile of compound **5b** warranted further studies of this compound in vivo including pharmacokinetics (PK), pharmacodynamics (PD), and tumor growth inhibition (TGI) in xenograft models. The PK studies were carried out in BALB/c mice following i.v. and p.o. routes of administration, and PD studies conducted in GTL-16 nu/nu mouse xenografts following a single oral dose at 20, 40, and 80 mg/kg, respectively. The results were summarized in Table 4. It was found that compound **5b** was orally bioavailable with 48% bioavailability in mice. In PD studies, compound **5b** significantly inhibited phosphorylation of c-Met kinase at 20 mg/kg, and completely stopped the phospho-c-Met signals in

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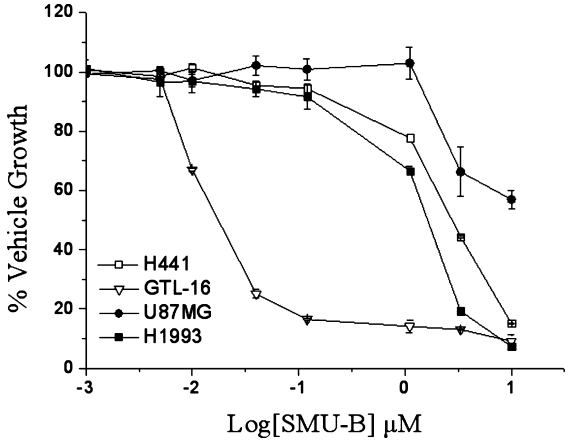
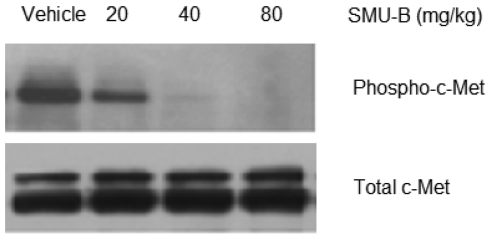
Table 3. Antiproliferative IC_{50}s of Compound 5b (SMU-B) in Four Different Cell Lines



c-Met status	cell line	antiproliferative IC_{50} (μM), **5b** (**SMU-B**)
Met amplification	GTL-16	0.020
Met amplification	H1993	1.58
Met overexpression	H441	2.82
HGF/Met autocrine	U87MG	>10

Table 4. Pharmacokinetics and Pharmacodynamics Results of Compound 5b (SMU-B); Inhibition of Phospho-c-Met in GTL-16 Xenografts after 4 h Following a Single Oral Dose



pharmacokinetics in BALB/c mice				
dose (mg/kg)	dosing route	C_{max} (μM)	$AUC_{0-\infty}$ (ng.h/mL)	*F* %
5	i.v.	1.36	1229.1	100
20	p.o.	1.45	2348.5	48

40 and 80 mg/kg dose groups. These results demonstrated that the in vitro c-Met potencies could be successfully translated into in vivo activities.

To assess the antitumor potential of compound **5b** in vivo, efficacy studies were carried out in GTL-16 xenograft models. Tumor-bearing nu/nu mice were dosed orally at 20 and 40 mg/kg, qd, for 14 days. Tumor sizes and body weights were measured every other day. The results were shown in Figure 7.

Significant antitumor efficacies of compound **5b** (**SMU-B**) were observed in this model, i.e., 52% and 87% TGI's for the 20 and 40 mg/kg dose groups, respectively. There was no significant body weight loss in all experiment groups (data not shown), which suggested low toxicity of this compound.

In summary, a series of novel aminopyridyl/pyrazinyl-substituted spiro[indoline-3,4′-piperidine]-2-ones were designed, synthesized, and tested in various in vitro and in vivo assays. Selected compounds, especially compound **5b** (**SMU-B**), showed single-digit nM biochemical and less than 100 nM cellular potencies against c-Met and ALK kinases. Compound **5b** (**SMU-B**) was highly selective and only hit very few targets in the 97-kinase panel screening. It was orally bioavailable and

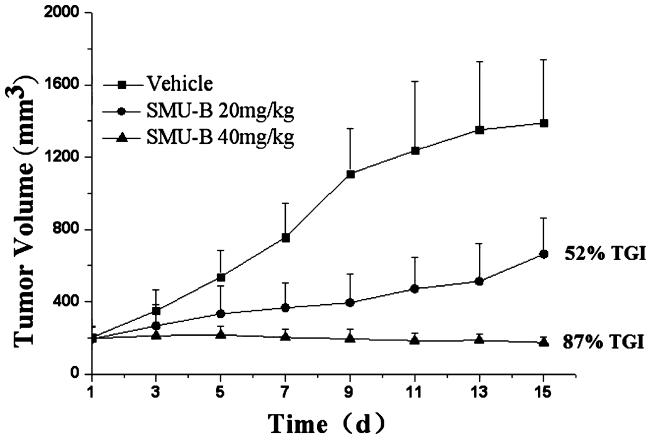


Figure 7. Tumor growth inhibition (TGI) by compound **5b** (SMU-B) in GTL-16 xenograft models.

showed pharmacodynamics effect and significant tumor growth inhibitions in GTL-16 human gastric carcinoma xenograft models. Compound **5b** (**SMU-B**) represented one of the very few selective and highly efficacious c-Met/ALK dual inhibitors with clear mechanism of action (MoA) known to date. The superior antitumor properties of compound **5b** (**SMU-B**) justified further studies.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthetic procedures and analytical data for compounds reported in this letter and procedures for in vitro and in vivo assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

§(J.L. and N.W.) These authors contributed equally to this work.

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Notes

The authors declare no competing financial interest.

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