Journal Pre-proofs

Discovery of a Novel Selective Water-Soluble SMAD3 Inhibitor as an Antitumor Agent

Nannan Wu, Guangyu Lian, Jingyi Sheng, Dan Wu, Xiyong Yu, Huiyao Lan, Wenhui Hu, Zhongjin Yang

PII: S0960-894X(20)30507-2
DOI: https://doi.org/10.1016/j.bmcl.2020.127396
Reference: BMCL 127396

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 22 May 2020
Revised Date: 2 July 2020
Accepted Date: 6 July 2020

Please cite this article as: Wu, N., Lian, G., Sheng, J., Wu, D., Yu, X., Lan, H., Hu, W., Yang, Z., Discovery of a Novel Selective Water-Soluble SMAD3 Inhibitor as an Antitumor Agent, *Bioorganic & Medicinal Chemistry Letters* (2020), doi: https://doi.org/10.1016/j.bmcl.2020.127396

Discovery of a Novel Selective Water-Soluble SMAD3 Inhibitor as an Antitumor Agent

Nannan Wu, [†, #] *Guangyu Lian,* [‡,#] *Jingyi Sheng,* [‡,#] *Dan Wu*[†]*, Xiyong Yu,* [†] *Huiyao Lan,*[‡,*] *Wenhui Hu,* [†,*] *and Zhongjin Yang* [†,*]

[†]*Key Laboratory of Molecular Target & Clinical Pharmacology and State Key Laboratory of Respiratory Disease, School of Pharmaceutical Sciences & the Fifth Affiliated Hospital, Guangzhou Medical University, Guangzhou, Guangdong, 511436, China.*

[‡] *Department of Anatomical and Cellular Pathology, The Chinese University of Hong Kong, Hong Kong SAR 999077, China.*

[#] These authors contributed equally to this work.

* Corresponding authors:

For Zhongjin Yang: phone: +86 20 31100920; E-mail: yzj23@163.com.

For Wenhui Hu: phone: +86 20 31100920; E-mail: huwenhui@gzhmu.edu.cn.

For Huiyao Lan: E-mail: email: hylan@cuhk.edu.hk.

Abstract

Targeting the SMAD3 protein is an attractive therapeutic strategy for treating cancer, as it avoids the potential toxicities due to targeting the TGF-β signaling pathway upstream. Compound SIS3 was the first selective SMAD3 inhibitor developed that had acceptable activity, but its poor water solubility limited its development. Here, a series of SIS3 analogs was created to investigate the structure−activity relationship for inhibiting the activation of SMAD3. On the basis of this SAR, further optimization generated a water-soluble compound, **16d**, which was capable of effectively blocking SMAD3 activation *in vitro* and had similar NK cell-mediated anticancer effects *in vivo* to its parent SIS3. This study not only provided a preferable lead compound, **16d,** for further drug discovery or a potential tool to study SMAD3 biology, but also proved the effectiveness of our strategy for water-solubility driven optimization.

KEYWORDS: SMAD3 inhibitor, tumor, NK cell, tumor microenvironment.

TGF-β /SMAD signaling plays an important role in cancer progression, switching from tumor suppression to tumor promotion.[1,2] In fact, TGF-β signaling is often overactivated in many advanced tumor types.[3-9] This can cause cancer cells to lose their epithelial characteristics, such as cell-cell adhesion and cell polarity, and acquire migratory and invasive properties.[2] Moreover, TGF-β/SMAD signaling can fuel heterogeneity in cancer stem cells and drug resistance,[10, 11] and is associated with poor prognosis in patients.[3] It is noted that TGF-β receptors not only activate SMAD-independent canonical pathways, but also allow SMAD-independent signaling responses and crosstalk with different signaling pathways (Figure 1).[12]

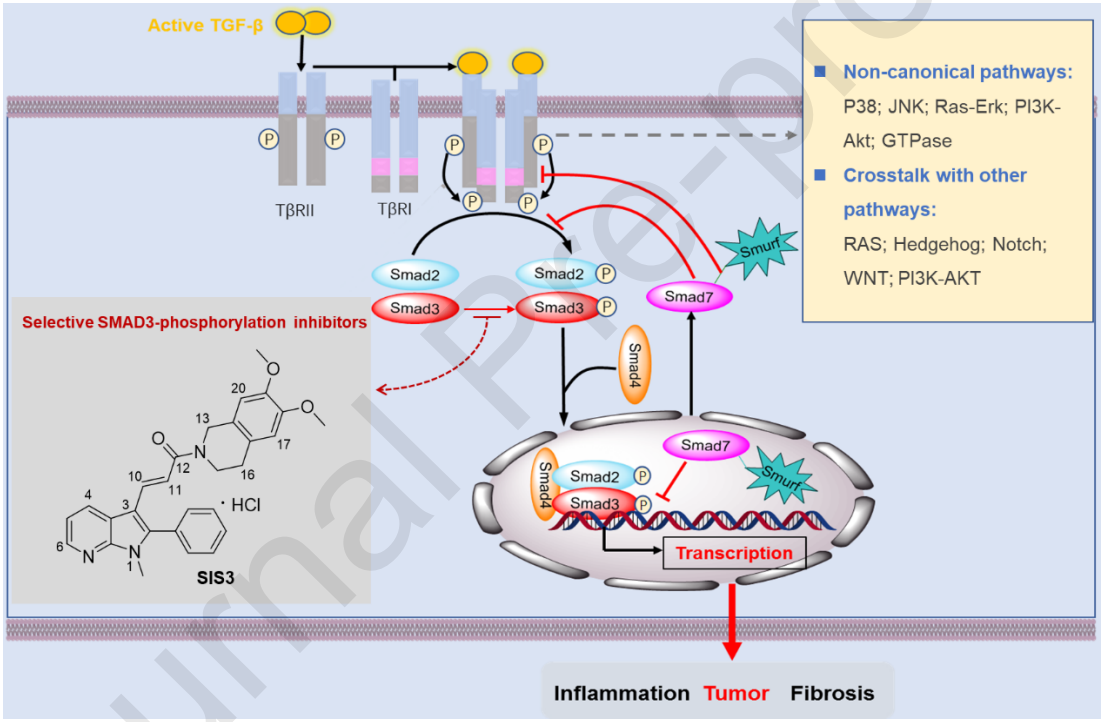


Figure 1. Overview of TGF-β/SMAD signaling.

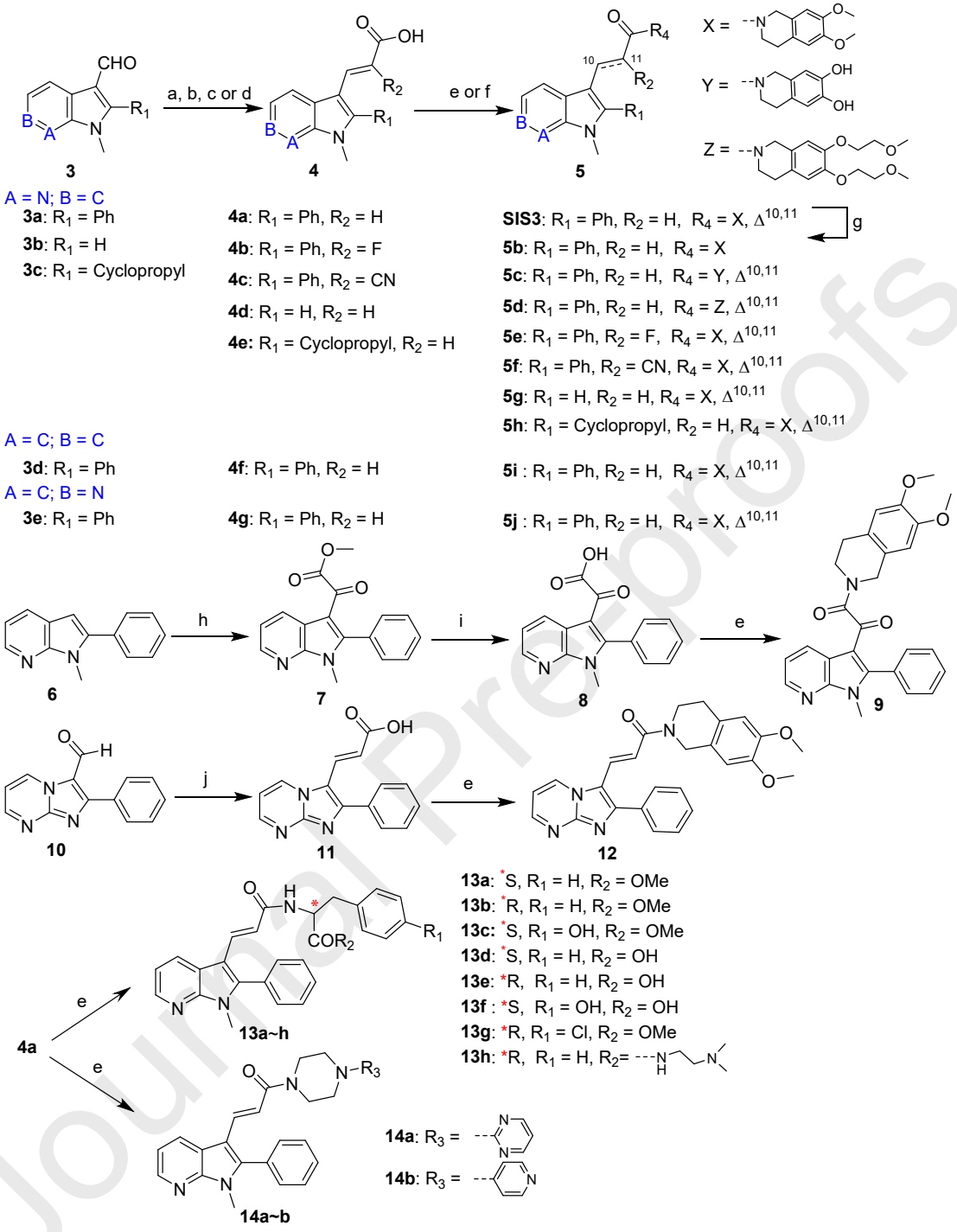
Due to TGF-β's pleiotropic roles and complex functions, its inhibition can lead to side effects.[13, 14] SMAD3, but not SMAD2 and SMAD4, is a key downstream mediator of TGFβ signaling, because it contains DNA binding domains that allow for it to bind directly to the promoters in its target genes and thereby modulate transcription.[15,16] Notably, SMAD3 has attracted an extraordinary amount of research interest due to its ability to modulate the expression of a key protein that is a target for the

immunotherapeutic treatment of cancer. More specifically, SMAD3 can upregulate the expression of programmed death-1 (PD-1) on T cells, which is a coinhibitory receptor that inhibits the activity of tumor-infiltrating lymphocytes (TILs) in cancer. It should be noted that SMAD2 cannot upregulate the expression of PD-1.[17] In addition, it has been shown that silencing of SMAD3 can suppress cancer cell growth and metastasis by enhancing the cancer-killing activity of NK cells.[18,19] Thus, the selective inhibition of the SMAD3 protein with a potent, low toxicity, drug could provide a promising anticancer treatment.

SIS3, a selective SMAD3-phosphorylation inhibitor (Figure 1)[20], contains a Michael acceptor, but our experiment showed it had no addition reactivity with nucleophilic groups from glutathione. Therefore, SIS3 cannot covalently bind to the targets, and not belong to Pan Assay INterference compoundS (PAINS). It has been proven to effectively enhance the anticancer activities of NK cells via an E4BP4-dependent mechanism and significantly suppress tumor growth and invasion.[18] However, SIS3 suffers from insolubility in water, actually dissolved in a mixed solution of 2% DMSO, 2% Tween-80 and 96% H_2O for in-vivo animal assays. The poor water solubility can also cause unstable results of biological assay, poor pharmacokinetic properties,[21,22] and lead to real problems in developing acceptable formulations in the later stages of development.[23]

These potential risks directed us to improve the water solubility of SIS3. As a drug-like parameter reflecting water solubility, the calculated lipophilicity (cLogP) of SIS3 (4.7) is high. Generally, a lower cLogP, as well as a lower molecular weight (MW), are favored to achieve good pharmacokinetic properties.[24] However, both cLogP and MW tend to rise during lead compound optimization.[25] From this perspective, it is preferable to develop a lead compound that has both a low cLogP and a low MW.

Scheme 1. Synthesis of SIS3 and its derivatives **5b~j**, **9**, **12**, **13a~h** and **14a~b**[a]

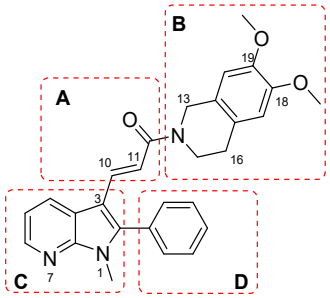


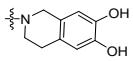
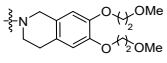
[a]Reagents and conditions: (a) methyl diethylphosphonoacetate, NaH, DMF, 0°C to r.t., 8 h; then add NaOH, MeOH, and H_2O; (b) malonic acid, piperidine, pyridine, 110°C, 6 h (c) Ethyl fluoroacetate, $TiCl_4$, TEA, CH_2Cl_2, r.t., 4 h; then add LiOH, THF, H_2O, 3 h; (d) t-butyl cyanoacetate; piperidine, AcOH, toluene, 100°C, 15 h; then add TFA, CH_2Cl_2, r.t., 12 h; (e) HATU, TEA, corresponding amines, DMF, 2 h. (f) oxalyl chloride, DMF, THF; then add DIPEA and amine **X**. (g) H_2, Pd/C, MeOH, r.t. overnight. (h) methyl chlorooxoacetate, $AlCl_3$, CH_2Cl_2, 0°C to r.t.,

overnight; (i) LiOH, MeOH, H_2O, r.t. overnight. (j) t-butyl diethylphosphonoacetate, then add TFA, CH_2Cl_2, r.t., 12 h.

To achieve this objective, we firstly designed and synthesized SIS3 analogs to study its structure−activity relationships (SAR) to determine the impact of molecular fragment on anti-SMAD3-activation. As shown in Scheme 1, the synthesis started with the known aldehydes **3a**~**e**, which were readily obtained from indoles, according to similar reported procedures.[26] A Horner−Wadsworth−Emmons (HWE) reaction of compounds **3a**, **3b,** or **3d** and methyl diethylphosphonoacetate was followed by ester hydrolysis to generate the corresponding acids, **4a**, **4d,** or **4f**. Alternatively, compounds **4e** or **4g** were obtained by the Knoevenagel reaction of **3c** or **3e** and malonic acid.[27] Compound **3a** and ethyl fluoroacetate were treated with $TiCl_4$ and TEA in CH_2Cl_2 to afford the desired **4b**.[28] Similar to the synthetic method for **4e**, treatment of **3a** with t-butyl cyanoacetate in the presence of piperidine, followed by removal of the t-butyl group with TFA provided **4c**. Treatment of the acids **4a~d** with oxalyl chloride or HATU, and then condensation with the corresponding secondary amines gave amides SIS3 and **5c**~**j**.[29] Similarly, using aldehyde **10** as the substrate, amide **12** was obtained in two steps.[30] Reduction of the double bond in SIS3 with H_2 and Pd/C in MeOH yielded **5b**.[31] Finally, compound **9** was prepared in three steps as follows: 7-azaindole **6** was treated with $AlCl_3$ in CH_2Cl_2 at room temperature followed by the addition of methyl oxalyl chloride to yield compound **7**;[32] Next, compound **7** was hydrolyzed to the acid, and then condensed with 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline to obtain the desired compound **12**. With **4a** in hand, compounds **13a-h** and **14a-b** were easily obtained by a condensation reaction with the corresponding amines in the presence of HATU.

Table 1. Inhibition of SMAD3-phosphorylation by **SIS3**, **4a**, **5b~h**, **9**, and **12**.

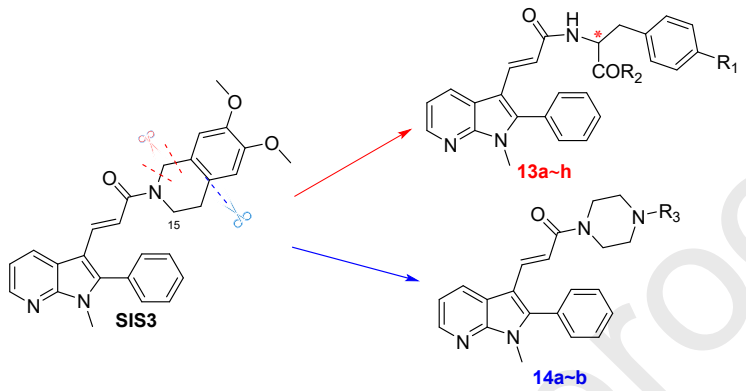


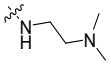
Cmpd	Region				Inhibition %[a]	
	A	**B**	**C**	**D**	5 μM	10 μM
SIS3	–	–	–	–	51.8±1.4	79.3±0.2
5b		–	–	–	10.2±13.0	12.3±4.9
9		–	–	–	-25.2±4.2	-40.7±1.5
5e		–	–	–	56.4±5.3	95.1±1.2
5f		–	–	–	-13.5±6.7	-2.6±9.7
4a	–		–	–	-9.2±0.3	2.6±7.9
5c	–		–	–	59.8±5.8	91.1±5.2
5d	–		–	–	44.6±5.9	78.5±3.2
5i	–	–		–	45.7±7.1	54.0±11.6
12	–	–		–	30.8±5.7	53.1±2.9
5j	–	–		–	19.1±7.8	52.1±6.3
5g	–	–	–		29.7±12.9	48.7±6.7
5h	–	–	–		18.5±14.3	53.7±5.2

[a] The % inhibition values are the means of three experiments.

To evaluate the ability of the compounds to inhibit SMAD3 activation, all the synthesized compounds were tested for their ability to inhibit SMAD3 phosphorylation at two different concentrations (5 μM and 10 μM) using a CAGA-based luciferase reporter assay.[33] As shown in Table 1, the parent compound SIS3 inhibited SMAD3 phosphorylation by 51.8% at 5 μM and 79.3% at 10 μM. To confirm the tolerability of substitution in the A-region, various functional groups were introduced into this region. Among these, compound **5b** or **9**, derived from a reduction or carbonyl substitution of the 10,11-double bond in SIS3, exhibited diminished or lost inhibitory activity. A higher reactive Michael acceptor α-cyanoacrylamide (**5f**) resulted in a complete loss of activity, which indicated they were reversible small molecule inhibitors. In stark contrast, fluorination of the 11-position in SIS3 (**5e**, 95% inhibition at 10 μM) slightly improved activity, possibly due to the fact that fluorine is more lipophilic than hydrogen and significantly more lipophilic than C=O or C≡N substituents. In the B-region, a complete loss of activity was observed following the removal of the tetrahydroisoquinoline moiety (**4a**), while elimination or substitution of the two methyl groups on the phenyl ring (**5c** or **5d**) of SIS3 had either a slightly beneficial effect or no effect on activity, respectively. For the C-region and D-region modified compounds, a moderate decrease was observed in their inhibition of SMAD3 phosphorylation. For example, **5i**, **5j,** and **12** all showed a reduced inhibition of SMAD3 phosphorylation (approximately 50% at 10 μM for all three compounds). A similar result was seen upon replacement of the phenyl group with a hydrogen atom (**5g**, 48.7% inhibition at 10 μM) or a cyclopropyl group (**5h**, 53.7% inhibition at 10 μM). These findings indicate that the 10,11-double bond is essential for inhibitory activity towards SMAD3 phosphorylation, and increasing lipophilicity in the A-region can have a beneficial effect on activity. Notably, the C-18 or C-19 positions in the tetrahydroisoquinoline moiety can tolerate certain types of groups, such as hydroxyl and ether groups.

Table 2. Inhibition of SMAD3 phosphorylation by compounds **13a**~**h** and **14a**~**b**



Cmpd	Group				Inhibition, %[a]	
	Chirality	R_1	R_2	R_3	5 μM	10 μM
13a	S	H	OMe	n.a. [b]	32.4±3.0	67.1±6.9
13b	R	H	OMe	n.a. [b]	47.6±8.0	51.4±3.3
13c	S	OH	OMe	n.a. [b]	41.9±10.4	62.6±9.5
13d	S	H	OH	n.a. [b]	1.8±29.3	6.6±9.6
13e	R	H	OH	n.a. [b]	19.6±12.3	31.2±10.5
13f	S	OH	OH	n.a. [b]	< 0%	< 0%
13g	R	Cl	OMe	n.a. [b]	44.8±3.1	55.3±8.7
13h	R	H		n.a. [b]	n.d.[c]	< 0%
14a	n.a. [b]	n.a. [b]	n.a. [b]		26.4±17.0	37.3±0.6

14b	n.a. [b]	n.a. [b]	n.a. [b]		35.4±8.0	69.0±5.2

[a] The % inhibition values are the means of three experiments. [b]n.a. means not applicable.

On the basis of the above analysis, we chose the well-tolerated tetrahydroisoquinoline moiety as a region for further modification. By utilizing drug design strategies including ring variation, scaffold hopping, and bioisosteres, two series of compounds were designed, synthesized, and evaluated for their inhibitory activity towards SMAD3 phosphorylation (Table 2). To begin with, a polar carboxyl group was introduced at the C-15 position, with the six-membered ring being opened, to construct commercially available phenylalanine fragments. As shown in Table 2, compound **13d** (1.8% inhibition at 5 μM), **13e** (19.6% inhibition at 5 μM), and **13f** (< 0% inhibition at 5 μM), with the introduction of a carboxyl group at the C-15 position, exhibited a significant drop in inhibition compared to SIS3. Unsurprisingly, compound **13h** with a polar and hydrophilic fragment at the C-15 position lost inhibition, even at 10 μM. When the carboxyl group was changed to a lipophilic ester group, such as in compounds **13a**, **13b**, and **13c**, a recovery was observed in the inhibition of anti-SMAD3 phosphorylation (Table 2). Comparing the % inhibition of **13a** (32.4%) with **13b** (47.6%), and **13d** (1.8%) with **13e** (19.6%), all at 5 μM, the R configuration at the C-15 position appeared to have a weak advantage over the S configuration. Similar to the data shown in Table 1, substitution of the phenyl ring with various groups (**13a**, **13b**, and **13g**) had no obvious effects on inhibitory activity. In another series of compounds, conversion of the benzene ring to an electron-deficient pyridine or pyrimidine ring, **14a** and **14b**, resulted in reduced inhibitory activity at 5 μM.

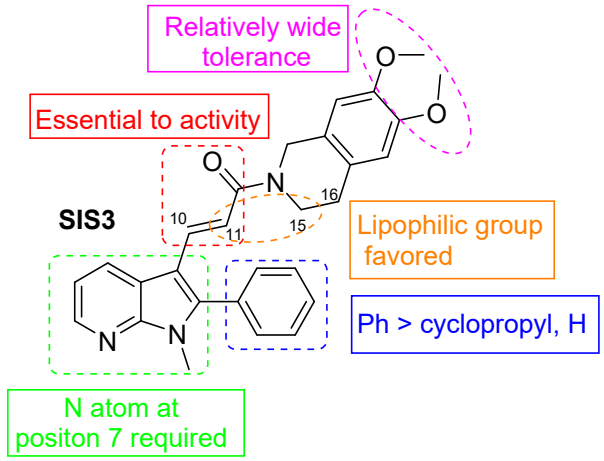
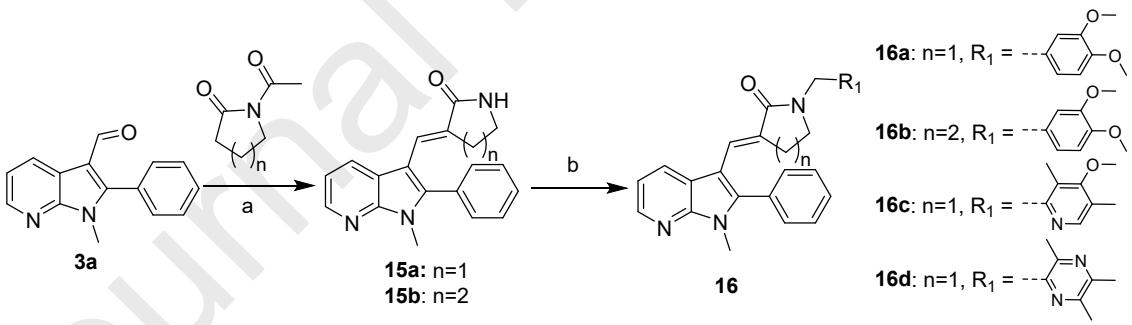


Figure 2. The preliminary structure−activity relationship of **SIS3**

Figure 2 summarizes the conclusions drawn from these studies on the effect of structural changes within the four domains on the ability to inhibit SMAD3 phosphorylation. These SARs provide further guidance for the design of new optimized SIS3 analogues.

Scheme 2. Synthesis of SIS3 derivatives **16a~d**[a]

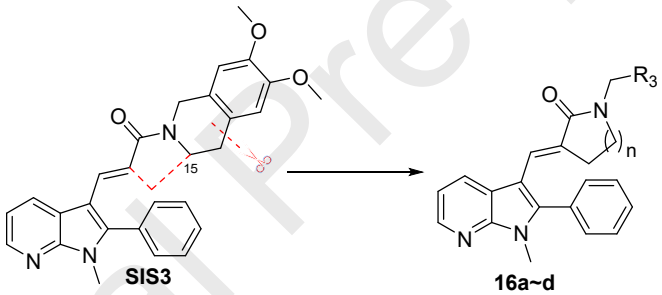


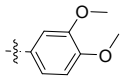
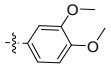
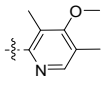
[a] Reagents and conditions: (a) LDA, THF, 0°C to 45°C, 48 h; (b) NaH, DMF, 0°C to r.t., overnight.

Based on the above SAR findings, we reasoned that in order to improve the water-solubility properties, the introduction of polar or hydrophilic substituents around the tolerant region A might improve solubility. Considering that increasing the lipophilicity at the C-11 and C-15 positions had beneficial effects, the 15, 16- ethylene group was transferred to the C-11 position to form new skeleton compounds **16a-d**. Their synthesis route is shown in Scheme 2: the 3-alkylidenelactams **15a** and **15b** were prepared by

aldol condensation of aldehyde **3a** and lactams (acetamide protecting group),[34] and then treatment of **15a** or **15b** with the corresponding alkyl halides or methanesulfonate in the present of NaH resulted in the desired products **16a**~**16d**.[35]

Table 3. Inhibition of SMAD3-phosphorylation by compounds **16a~d**



Cmpd	Group		Inhibition %[a]		clogP[b]
	n	R_3	5 μM	10 μM	
SIS3	n.a.[c]	n.a.[c]	51.8±1.4	79.3±0.2	4.7
16a	1		54.2±1.9	91.2±1.1	4.5
16b	2		57.2±5.9	86.7±2.1	5.0
16c	1		-10.2±3.4	3.8±10.3	4.7
16d	1		44.4±7.6	62.6±3.4	3.7

[a] The % inhibition values are the means of three experiments. [b]Calculated logarithm of the octanol/water distribution coefficient using ChemBioDraw Ultra 14.0. [c] n.a. means not applicable.

As shown in Table 3, **16a** did show a slight increase in inhibitory activity (91.2%) at 10 μM while maintaining activity at 5 μM (54.2%). However, compound **16b**, which contained three carbon atoms in the new linker region, did not show increases in inhibitory activity at either 5 μM or 10 μM. While replacing the dimethoxybenzyl in **16a** with a polar bioisostere appeared to be a good strategy to improve water-solubility, unfortunately, when the dimethoxybenzyl group was replaced with 4-methoxy-3,5-dimethylpyridyl in compound **16c**, the % inhibition dropped to 3.8% at 10 μM. As another bioisosteric replacement, the introduction of 2,3,5-trimethylpyrazyl in place of the dimethoxybenzyl group (**16d**), resulted in slight reductions in the % inhibition values, being 44.4% at 5 μM and 62.6% at 10 μM. In spite of this, its cLogP value was remarkably improved from 4.7 to 3.7.

Table 4. Drug-like properties comparison of **SIS3** and **16d**

Cmpd	MW	clogP[a]	ED_{50}[b,c] μM	TD_{50}[b,d] μM	Solubility	
					in PBS (pH 7.4)	in water (HCl)
SIS3	453	4.7	4.53	18.66	0.006 mg/mL	0.141 mg/mL
16d	437	3.7	6.63	22.04	0.014 mg/mL	> 94 mg/mL

[a] Calculated logarithm of the octanol/water distribution coefficient using ChemBioDraw Ultra 14.0. [b]The inhibition concentration are the means of three experiments. [c]Concentration required to inhibit SMAD3 phosphorylation by 50% relative to controls. [d]Concentration required to inhibit A549 cell growth by 50% relative to controls as determined by MTT assay.

Compared with SIS3, our lead compound **16d** showed similar inhibitory activity towards SMAD3 phosphorylation and had similar cytotoxicity towards A549 cells as determined by an MTT assay (Table 4). Furthermore, **16d** had a decreased MW and a reduced cLogP value of 3.7, which could better meet Lipinski's "rule of five". The solubilities of compound **16d** or its HCl salt were determined in both pure water and phosphate buffer (pH 7.4) at room temperature by HPLC (Table 4)[36]. It was found that the solubility of **16d** as a free base (14 μg/mL) was 2-fold greater than SIS3 in PBS (6

μg/mL), while the solubility of **16d** as the HCl salt (> 94 mg/mL) was 666-fold greater than SIS3 (0.141 mg/mL). Obviously, the improvement in aqueous solubility is attributable to the pyrazyl ring, which is more water soluble than the tetrahydroisoquinoline moiety in SIS3.

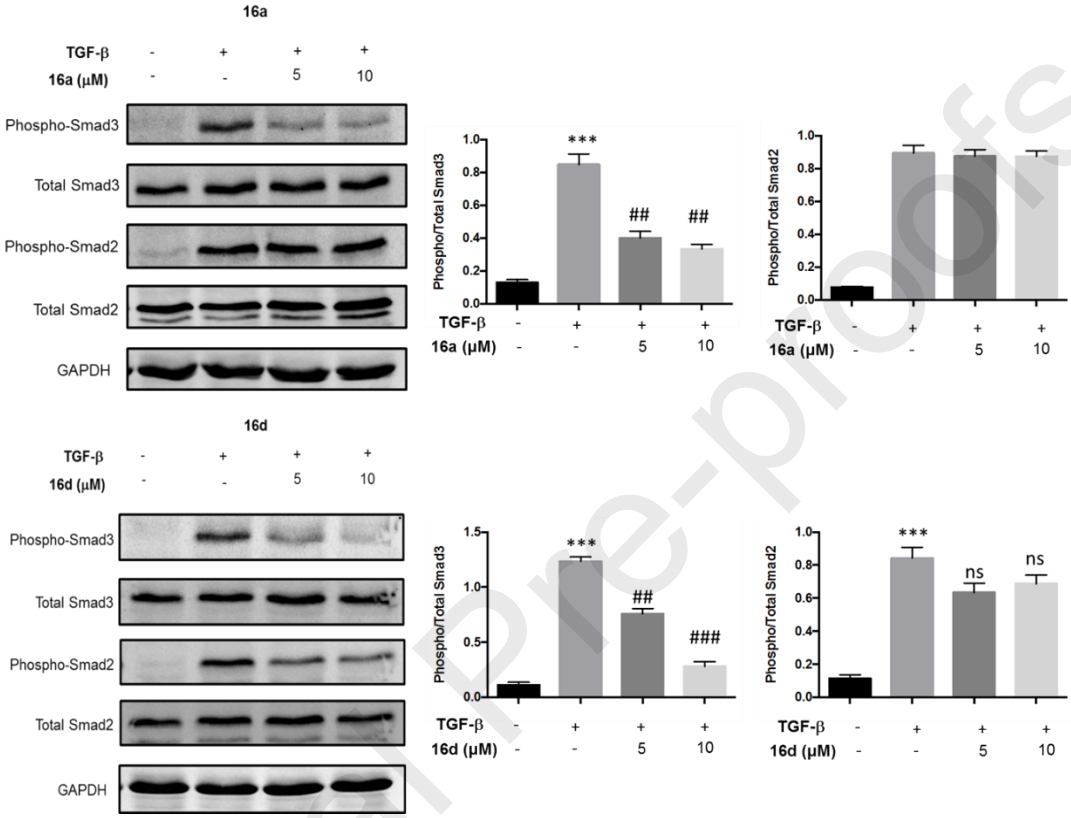


Figure 3. Compounds **16a** and **16d** reduce the TGF-β-dependent increase in phosphorylation of SMAD3. A549 cells were stimulated with 1 ng/mL TGF-β for 30 minutes with or without 2 hours pre-treatment with **16a** or **16d** at both 5 and 10 μM. Cells were lysed and the levels of phospho-SMAD3, SMAD3, phospho-SMAD2, and SMAD2 were determined by western blot. GAPDH levels were used to ensure equal protein loading.

In order to accurately assess selectivity, we chose two compounds, namely **16a** and **16d,** to examine their effect on the TGF-β-induced phosphorylation of SMAD3 and SMAD2. As shown by the results of a western blot analysis (Figure 3), the phosphorylation of SMAD3, but not of SMAD2, was inhibited by both **16a** and **16d**.

These data suggest that compound **16d** and its analog **16a** are specific inhibitors of SMAD3 phosphorylation.

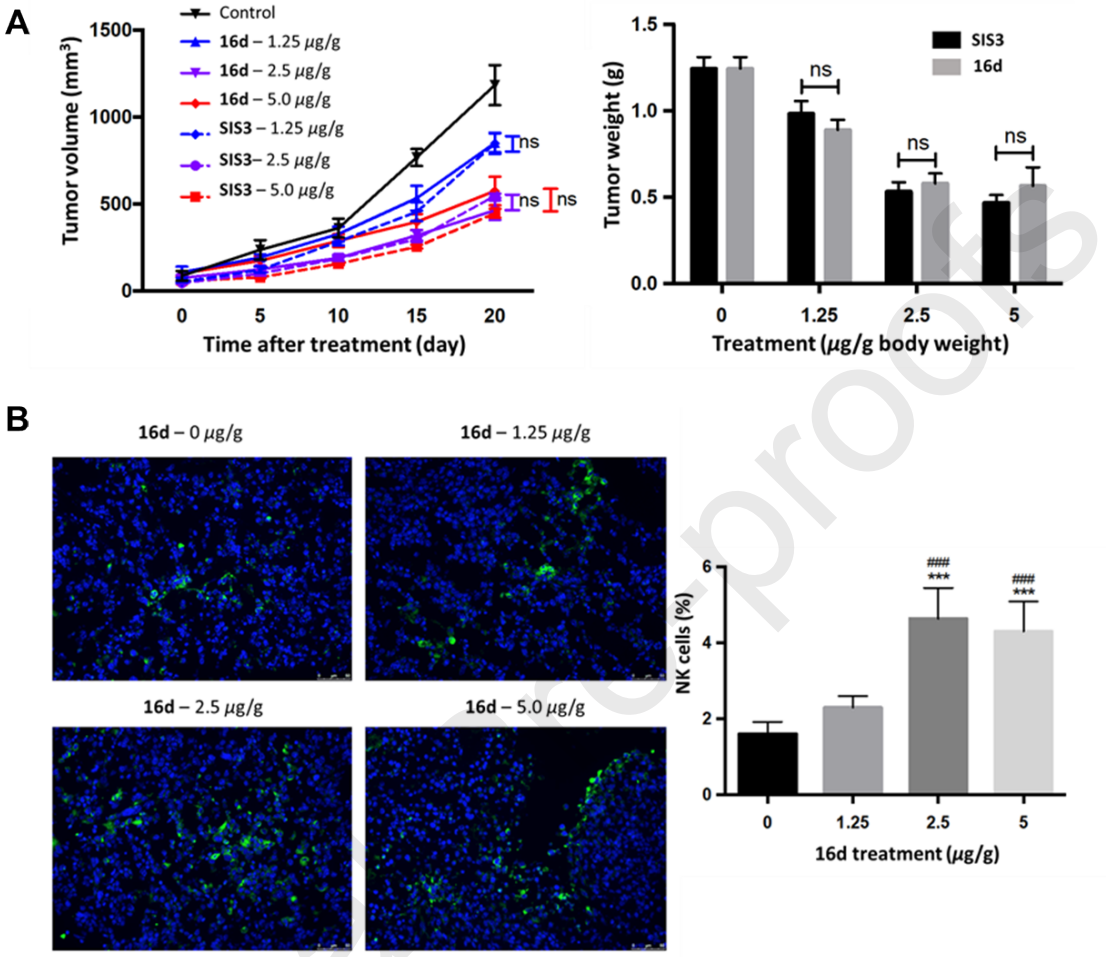


Figure 4. Compound **16d** suppresses cancer progression by enhancing NK cell accumulation in the syngeneic Lewis lung carcinoma (LLC) model mice. (A)Tumor volumes were measured every five days, and tumor weights were measured at the end of the experiment. Data are expressed as the mean ± SD for groups of five mice. ns, no significant difference, *$p < 0.05$, ***$p < 0.001$. (B) Representative images showing immunofluorescent staining of NK1.1 (green) are shown. The nuclei were counterstained with DAPI (blue). The percentage of NK cells in the tumor microenvironment are shown in the right panel. Each bar represents the mean ± SD for groups of three mice. *** $p < 0.001$ vs **16d** – 0 μg/g, $^{###}$ $p < 0.001$ vs **16d** –1.25 μg/g.

The anticancer activity of **16d** *in vivo* was evaluated in a syngeneic Lewis lung carcinoma (LLC) model in C57BL/6 mice. Tumor bearing mice were randomized and placed into different treatment groups and then treated intraperitoneally with SIS3 (a solution of 2% DMSO, 2% Tween-80 and 96% H_2O) or **16d** (a solution of 2% Tween-80 and 98% H_2O) at the dosage of 0, 1.25, 2.5 or 5.0 $\mu g \cdot g^{-1} \cdot day^{-1}$ (i.p.) for 20 consecutive days (Figure 4A). *In vivo* treatment with **16d** significantly reduced the volume and weight of the LLC tumors in mice in a dose-dependent manner. Moreover, there was no significant difference in the tumor suppressive effect between SIS3 and **16d** in terms of the size and weight of the lung tumors in mice. Next, we investigated the mechanisms through which the SMAD3 signaling inhibitor **16d** attenuates LLC progression. As previously reported, deletion or inactivation of Smad3 largely promotes NK cell mediated immunity against tumors.[17,37] Therefore, we examined the impact of **16d** on NK cell accumulation in the tumor microenvironment. As shown in Figure 4B, treatment with **16d** increased the number of tumor-infiltrating NK cells in a dose-dependent manner. Compared with the control mice, treatment with **16d** at 2.5 or 5.0 μg/g significantly increased NK cell accumulation, which indicated the presence of an enhanced antitumor immune response. These results suggest that compound **16d** may markedly suppress cancer progression by enhancing NK cell-mediated anticancer immunity in LLC model mice.

In conclusion, modifications based on SIS3 resulted in the discovery of a library of anti-SMAD3-phosphorylation agents. According to these results, the following SARs were found: (1) the 10,11-double bond is essential for anti-SMAD3 phosphorylation activity; (2) diverse substitutions on C-18 and C-19 can be tolerated; (3) variations in the 7-azaindole ring and the 2-phenyl group can affect the inhibitory activity. Compared with SIS3, the novel lead compound **16d** has higher water solubility, and demonstrates a similar potency for the inhibition of SMAD3-phosphorylation. In addition, **16d** also had a high anticancer effect by enhancing NK cell-mediated immunity *in vivo*. The work reported herein provides a potential tool to study SMAD3 biology or a desirable lead compound for further drug discovery.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (NSFC) (NO. 81803364 to Z. Yang and NO. 81872743 to W. Hu).

Appendix A. Supplementary data

The following are the Supplementary data to this article. Supporting data to this article can be found online.

References

1. Christofori, G. *Nature* **2006,** *441*, 444.
2. Massague, J. *Cell* **2008,** *134*, 215.
3. Bruna, A.; Darken, R. S.; Rojo, F.; Ocana, A.; Penuelas, S.; Arias, A.; Paris, R.; Tortosa, A.; Mora, J.; Baselga, J.; Seoane, J. *Cancer cell* **2007,** *11*, 147.
4. Watanabe, T.; Wu, T. T.; Catalano, P. J.; Ueki, T.; Satriano, R.; Haller, D. G.; Benson, A. R.; Hamilton, S. R. *N Engl J Med* **2001,** *344*, 1196.
5. Drabsch, Y.; Ten, D. P. *J Mammary Gland Biol Neoplasia* **2011,** *16*, 97.
6. Wiercinska, E.; Naber, H. P. H.; Pardali, E.; Pluijm, G.; Dam, H.; Dijke, P. *Breast Cancer Res. Treat* **2011,** *128*, 657.
7. Arteaga, C. L. *Curr Opin Genet Dev* **2006,** *16*, 30.
8. Mundy, G. R. *Nat Rev Cancer* **2002,** *2*, 584.
9. Guise, T. A.; Chirgwin, J. M. *Clin Orthop Relat Res* **2003,** S32.
10. Oshimori, N.; Oristian, D.; Fuchs, E. *Cell* **2015,** *160*, 963.
11. Tripathi, V.; Sixt, K. M.; Gao, S.; Xu, X.; Huang, J.; Weigert, R.; Zhou, M.; Zhang, Y. E. *Mol cell* **2016,** *64*, 549.
12. Akhurst, R. J.; Hata, A. *Nat Rev Drug Discov* **2012,** *11*, 790.
13. Shull, M. M.; Ormsby, I.; Kier, A. B.; Pawlowski, S.; Diebold, R. J.; Yin, M.; Allen, R.; Sidman, C.; Proetzel, G.; Calvin, D.; Annunziata, N.; Doetschman, T. *Nature* **1992,** *359*, 693.
14. Bierie, B.; Chung, C. H.; Parker, J. S.; Stover, D. G.; Cheng, N.; Chytil, A.; Aakre, M.; Shyr, Y.; Moses, H. L. *J Clin Invest* **2009,** *119*, 1571.
15. Meng, X. M.; Nikolic-Paterson, D. J.; Lan, H. Y. *Nat Rev Nephrol* **2016,** *12*, 325.
16. Bae, E.; Sato, M.; Kim, R. J.; Kwak, M. K.; Naka, K.; Gim, J.; Kadota, M.; Tang, B.; Flanders, K. C.; Kim, T. A.; Leem, S. H.; Park, T.; Liu, F.; Wakefield, L. M.; Kim, S. J.; Ooshima, A. *Cancer Res* **2014,** *74*, 6139.

17. Park, B. V.; Freeman, Z. T.; Ghasemzadeh, A.; Chattergoon, M. A.; Rutebemberwa, A.; Steigner, J.; Winter, M. E.; Huynh, T. V.; Sebald, S. M.; Lee, S. J.; Pan, F.; Pardoll, D. M.; Cox, A. L. *Cancer Discov* **2016,** *6*, 1366.
18. Tang, P. M.; Zhou, S.; Meng, X. M.; Wang, Q. M.; Li, C. J.; Lian, G. Y.; Huang, X. R.; Tang, Y. J.; Guan, X. Y.; Yan, B. P.; To, K. F.; Lan, H. Y. *Nat Commun* **2017,** *8*, 14677.
19. Wang, Q. M.; Tang, P. M.; Lian, G. Y.; Li, C.; Li, J.; Huang, X. R.; To, K. F.; Lan, H. Y. *Cancer Immunol Res* **2018,** *6*, 965.
20. Jinnin, M.; Ihn, H.; Tamaki, K. *Mol Pharmacol* **2006,** *69*, 597.
21. Nassar, A. E.; Kamel, A. M.; Clarimont, C. *Drug Discov Today* **2004,** *9*, 1020.
22. Stella, V. J.; Nti-Addae, K. W. *Adv Drug Deliv Rev* **2007,** *59*, 677.
23. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv Drug Deliv Rev* **2001,** *46*, 3.
24. Gleeson, M. P. *J Med Chem* **2008,** *51*, 817.
25. Oprea, T. I.; Davis, A. M.; Teague, S. J.; Leeson, P. D. *J. Chem. Inf. Comput. Sci* **2001,** *41*, 1308.
26. Nyerges, M.; Pintér, Á.; Virányi, A.; Bitter, I.; Tőke, L. *Tetrahedron Lett* **2005,** *46*, 377.
27. Kaminska, K.; Ziemba, J.; Ner, J.; Schwed, J. S.; Lazewska, D.; Wiecek, M.; Karcz, T.; Olejarz, A.; Latacz, G.; Kuder, K.; Kottke, T.; Zygmunt, M.; Sapa, J.; Karolak-Wojciechowska, J.; Stark, H.; Kiec-Kononowicz, K. *Eur J Med Chem* **2015,** *103*, 238.
28. Fujii, Y.; Wasano, N.; Tamura, N.; Shindo, M.; Matsumoto, K., Cis-cinnamic Acid Analogs and Gravitropism Modifiers Containing Them. J.P. Patent 2016160246, 2016.
29. Yang, Z.; Kuang, B.; Kang, N.; Ding, Y.; Ge, W.; Lian, L.; Gao, Y.; Wei, Y.; Chen, Y.; Zhang, Q. *Eur J Med Chem* **2017,** *127*, 296.
30. Kamal, A.; Bharath Kumar, G.; Lakshma Nayak, V.; Reddy, V. S.; Shaik, A. B.; Rajender; Kashi Reddy, M. D. *Med. Chem. Comm* **2015,** *6*, 606.
31. Ma, Y.; Yin, D.; Ye, J.; Wei, X.; Pei, Y.; Li, X.; Si, G.; Chen, X.; Chen, Z.; Dong, Y.; Zou, F.; Shi, W.; Qiu, Q.; Qian, H.; Liu, G. *J. Med. Chem* **2020**, *63*, 10, 5458.
32. Colley, H. E.; Muthana, M.; Danson, S. J.; Jackson, L. V.; Brett, M. L.; Harrison, J.; Coole, S. F.; Mason, D. P.; Jennings, L. R.; Wong, M.; Tulasi, V.; Norman, D.; Lockey, P. M.; Williams, L.; Dossetter, A. G.; Griffen, E. J.; Thompson, M. J. *J. Med. Chem* **2015,** *58*, 9309.
33. Luwor, R. B.; Wang, B.; Nheu, T. V.; Iaria, J.; Tsantikos, E.; Hibbs, M. L.; Sieber, O. M.; Zhu, H. J. *Growth Factors* **2011,** *29*, 211.
34. Andreani, A.; Rambaldi, M.; Locatelli, A.; Leoni, A.; Bossa, R.; Chiericozzi, M.; Galatulas, I.; Salvatore, G. *J. Med. Chem* **1993,** *28*, 825.
35. Liu, X.; Han, Z.; Wang, Z.; Ding, K. *Angew Chem Int Ed Engl* **2014,** *53*, 1978.
36. Gross, G.; Tardio, J.; Kuhlmann, O. *Int J Pharm* **2012,** *437*, 103.
37. Lian, G. Y.; Wang, Q. M.; Tang, P. M.; Zhou, S.; Huang, X. R.; Lan, H. Y. *MOL THER* **2018,** *26*, 2255.

Graphical Abstract