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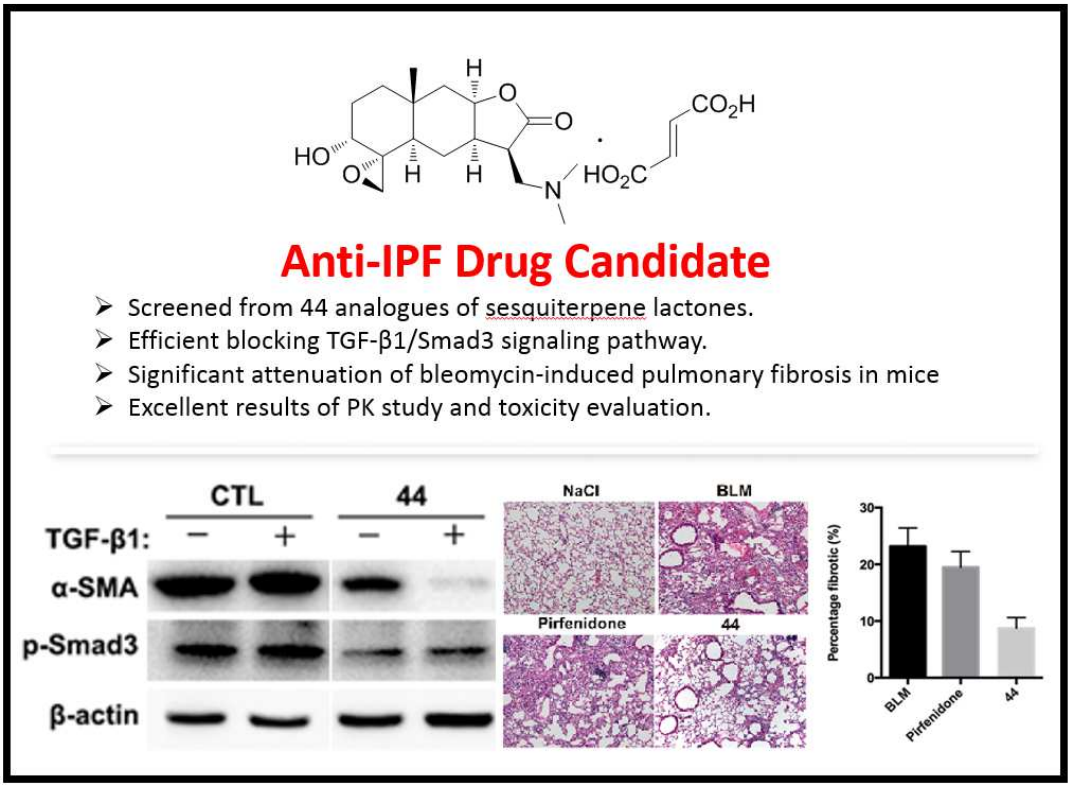

Synthesis and Discovery of a Drug Candidate for Treatment of Idiopathic Pulmonary Fibrosis through Inhibition of TGF-β1 Pathway

Xiaohe Li,[1, §] Cheng Lu,[1, §] Shuangwei Liu,[1, §] shuaishuai Liu,[1, §] Chengcheng Su,[2] Ting Xiao,[1] Zhun Bi,[1] Pengzhen Sheng,[1] Mengying Huang,[1] Xinhua Liu,[1] Yujiao Wei,[1] Lin Zhao,[1] Shengxiang Miao,[1] Jiahe Mao,[1] Kai Huang,[1] Shaoyan Gao,[1] Ning Liu,[1] Min Qi,[1] Tongtong Liu,[1] Shuanglin Qin,[1] Luqing Wei,[2] Tao Sun,[1] Wen Ning,[1] Guang Yang,[*,1] Honggang Zhou,[*,1] Cheng Yang[*,1]

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Keywords

Idiopathic Pulmonary Fibrosis

TGF-β1/p-Smad3 Signaling Pathway

Sesquiterpene Lactone

Isoalantolactone

High-throughput Screening

ABSTRACT

In this study, anti-IPF lead compounds **42** and **44**, derived from natural sesquiterpene lactones Isoalantolactone and alantolactone, were discovered by screening from a high-throughput TGF-β1 reporter luciferase assay. Notably, they could reduce the myofibroblast activation and extracellular matrix deposition both *in vitro* and *in vivo*. Additionally, compounds **42** and **44** could significantly attenuate bleomycin-induced pulmonary fibrosis in mice. Further validation of pharmacokinetics study and toxicity

evaluation indicated that compound **44** might be a promising anti-IPF drug candidate.

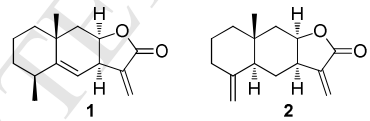
INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, and often fatal lung disease of unknown etiology. It is characterized by myofibroblasts accumulation and extracellular matrix (ECM) deposition. [1] A variety of causes could lead to IPF, while the mechanism of the underlying pathologic process remains poorly understood. IPF is associated with high morbidity and mortality, with a mean survival ranging from 3.2 to 5 years after diagnosis. [2] However, current therapies are largely ineffective against the disease progression, and most clinical trials of new therapies designed for IPF have obtained negative results in the past decades. [3]

Myofibroblasts are the key effector cells in pulmonary fibrogenesis and the primary cell type responsible for the synthesis and deposition of ECM including collagens I (COL1) and fibronectin (Fn). [4] Various cytokines and growth factors within the tissue microenvironment contribute to the activation of myofibroblasts. Among them, transforming growth factor (TGF)-β has been implicated as a "master switch" in the induction of lung fibrosis and myofibroblasts differentiation. [5, 6] The role of TGF-β1 is well described in IPF. To be specific, TGF-β1 is increased in tissue samples from both bleomycin-induced animal model [7] and IPF patients. [8] Moreover, over-expression of an adenovirus encoding active TGF-β1 leads to persistent pulmonary fibrosis, [9] while inhibition of TGF-β1 with soluble TGF-β receptor, [10] or a TGF-β receptor 1 (TβRI, ALK5) inhibitor, [11] ameliorates pulmonary fibrosis. In addition, mice null for the TGF-β1 downstream signaling p-Smad3 are protected from pulmonary fibrosis. [12] All together, TGF-β1 plays a crucial role in the pathogenesis of IPF. Based on this theory, we established a NIH3T3 fibroblasts cell line stably transfected with $(CAGA)_{12}$-Lux-reporter, which encoded 12 copies of canonical Smad3 DNA-binding sequence CAGA. This CAGA-NIH3T3 screening system was utilized for high-throughput screening compounds that can effectively inhibit the TGF-β signaling pathway and further attempt to discover the leading compounds for treating pulmonary fibrosis.

Previous studies have indicated that natural sesquiterpene lactone compounds harbored extensive connection with TGF- β1 signaling pathway. [13-15] Therefore, it might be a good resource to discover anti-IPF compounds from sesquiterpene lactones and their analogues.

Alantolactone (**1**) and isoalatolactone (**2**), belonging to a family of naturally occurring unique eudesmane-type sesquiterpene lactone, are enriched in the traditional Chinese medicine *Inula helenium L* (Shown in Scheme 1). Both of these two natural products exhibit considerable pharmacological activities, including antitumor, antibacterial, and anti-inflammatory effects. [16-20] Furthermore, isoalatolactone has been shown to attenuate the high glucose-stimulated activation of TGF-β1 and expression of monocyte chemoattractant protein-1 (MCP-1) and fibronectin (Fn) in mesangial cells of rats. [21] However, the relationship between isoalatolactone and TGF-β1 in the progression of IPF has not been investigated. Herein, semi-synthetic analogues were prepared based on the structure of alantolactone and isoalatolactone, followed by investigation of their inhibition on TGF-β1 signaling pathway and the potency for treatment of IPF.

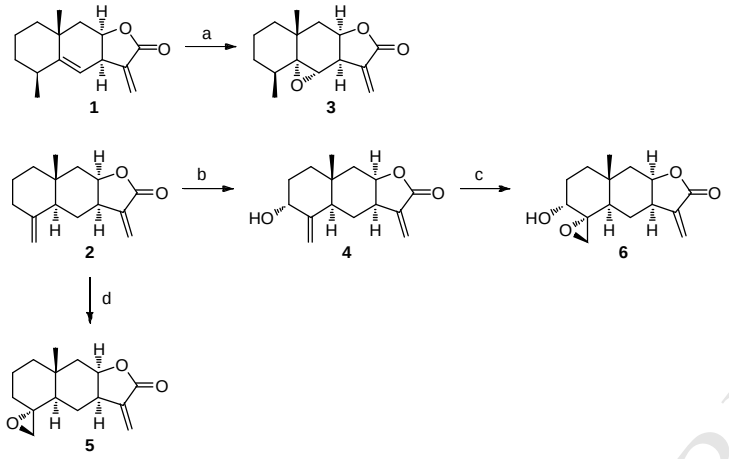


Scheme 1. Structure of Alantolactone (**1**) and Isoalatolactone (**2**)

RESULTS AND DISCUSSION

Chemistry

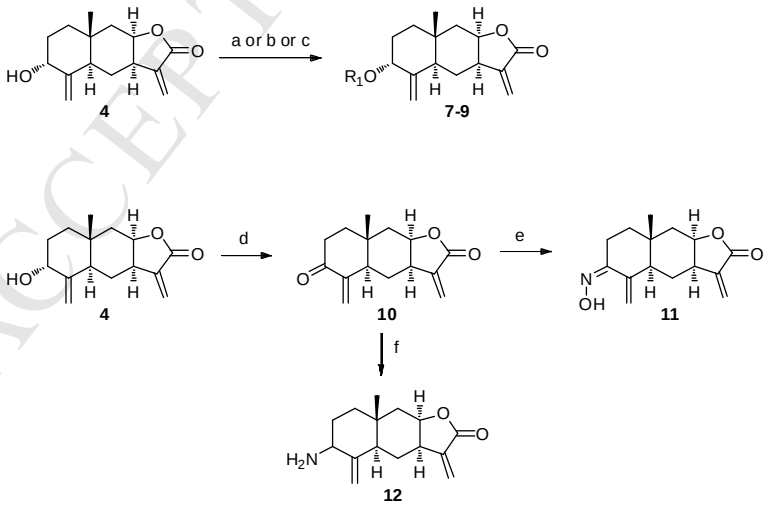
We took advantage of **1** and **2**, naturally abundant sesquiterpene lactones, as basic scaffold to synthesize the derivatives (Scheme 2). First of all, alantolactone (**1**) was introduced an epoxide ring via simple oxidization by m-CPBA to obtain a stable molecule **3**. Secondarily, isoalantolactone (**2**) was converted into intermediates **4** by mixed with SeO_2 in TBHP. Subsequently, the resulting allylic alcohol **4** was oxidized by m-CPBA to yield compound **6**.



Reagents and conditions: a) m-CPBA, CH_2Cl_2, rt, 90%; b) SeO_2, TBHP, reflux, 65%; c) m-CPBA, CH_2Cl_2, rt, 85%; d) m-CPBA, CH_2Cl_2, rt, 92%.

Scheme 2. Preparation of analogues **3**, **4**, **5** and **6**

With the key intermediates **4** in hands, functional groups transformation could provide more synthetic analogues **7**-**12** for biological investigation (Scheme 3). Specifically, pretreatment of compound **4** with NaH, following by reaction with BnBr to give rise to analogue **7** (Table 1, Entry 1). The secondary alcohol **4** was mixed with TBSCl and TBDPSCl, in the presence of imidazole, delivered the silyl ethers **8** and **9** (Table 1, Entries 2 and 3). This alcohol **4** was oxidized by Dess-Martin periodinane to furnish the enone **10**.



Reagents and conditions: a) NaH, DMF, BnBr, 0 ^{o}C to rt, 78%; b) TBSCl, imidazole, DMF, rt, 85%; c) TBDPSCl, imidazole, DMF, rt, 85%; d) Dess-Martin periodinane, CH_2Cl_2, 0 ^{o}C to rt, 65%; e) Py., $NH_2OH{\cdot}HCl$, MeOH, reflux, 72%; f) NH_4Cl, MeOH, $NaCNBH_3$, 0 ^{o}C to

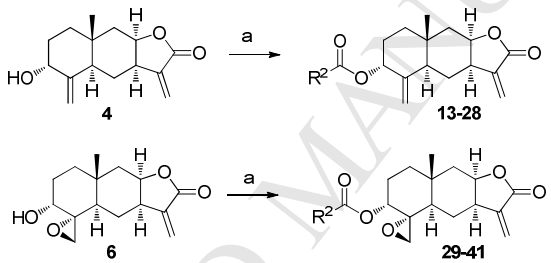
rt, 43%.

Scheme 3. Synthetic Approach to analogues **7**-**12**.

Table 1. Structures of Analogues **7-9**

Entry	No. of Compounds	R^1
1	**7**	Bn
2	**8**	TBS
3	**9**	TBDPS

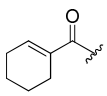
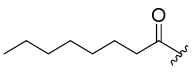
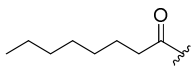
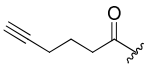
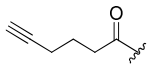
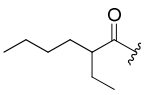
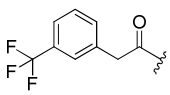
Also, we tried to prepare various esters analogues **13-40** on the secondary alcohols of the intermediates **4** and **6** (Table 2). Under our optimized conditions (Scheme 4), esterification could process efficiently in the presence of DIC and catalytic amount of DMAP in polar solvents (Such as MeCN or DMF).

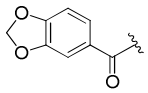
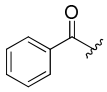
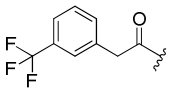
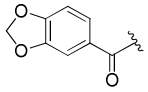
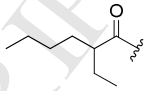
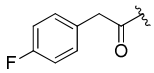
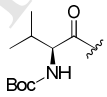
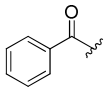
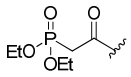
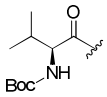
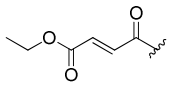
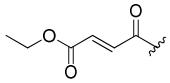
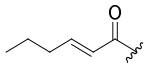
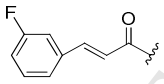
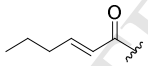
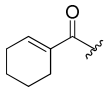
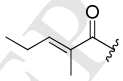


Reagents and conditions: a) RCO_2H, DIC, DMAP, DMF or MeCN, rt, 28%-95% yield.

Scheme 4. Preparation of the Ester Analogues **13-41**

Table 2. Structures of Analogues **7-9**

Entry	No. of Compounds	R^2	Yield/%	Entry	No. of Compounds	R^2	Yield/%
1	**13**		55	16	**28**		40
2	**14**		96	17	**29**		85
3	**15**		84	18	**30**		63
4	**16**		93	19	**31**		55

5	**17**		92	20	**32**		28
6	**18**		77	21	**33**		71
7	**19**		47	22	**34**		56
8	**20**		56	23	**35**		91
9	**21**		68	24	**36**		58
10	**22**		75	25	**37**		46
11	**23**		84	26	**38**		39
12	**24**		38	27	**39**		44
13	**25**		29	28	**40**		42
14	**26**		35	29	**41**		51
15	**27**		39				

Biology

Screening of functional compounds targeting TGF-β1/Smad3 signaling pathway in fibroblasts. The inhibitory potency of Alantolactone and Isoalatolactone analogues on TGF-β1/Smad3 signaling pathway in fibroblasts was evaluated by CAGA-luciferase report gene assay as described in experimental section. Fold changes of luciferase were shown in Figure 1, and the inhibition ratios of compounds were summarized in Table 3. Firstly, the natural products alantolactone **1** and isoalantolactone **2** were not found to exert any inhibition effect on TGF-β1/Smad3 signaling. Compared to natural

alantolactone **1**, the exposide analogue **3** exhibited a remarkable growth inhibition on TGF-β1/Smad3 pathway, with an inhibitory rate of 77%, which was the most potent analogues. Hydroxylated analogue **4** and **6** of isoalantolactone also exerted a stronge effect on blocking the TGF-β1/Smad3 pathway (61% and 46% of inhibitory rate, respectively). However, the analogues **5**, **10**, **11** and **12** of isoalantolactone did not shown significant inhibitory effect against the signaling pathway activated by TGF-β1, indicating that the hydroxyl group at C-3 might be the essential group to maintain the activity on this signaling pathway. Further modification was focused on the fuctionalization of this hydroxyl group. An ether and silyl ether analogues **7**, **8** and **9** reduced the inhibitory acvitivity. And the inhibition activity of most ester analogues was abolished except compound **38** and **41**. These two ester analogues harbored potent inhibitory rate on Smad3-activation (71% and 34% of inhibitory rate, respectively).

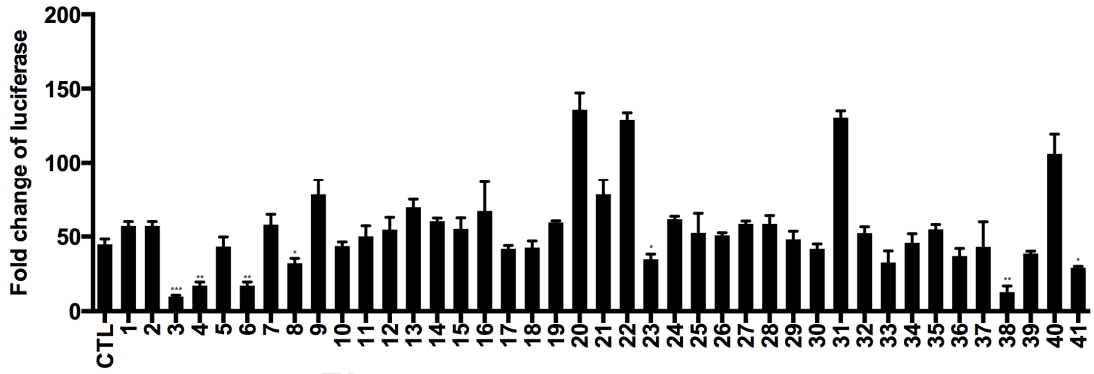


Figure 1. Screening of functional compounds targeting TGF-β1/Smad3 signaling pathway in fibroblasts.

CAGA-NIH3T3 cells were treated with/without TGF-β1 (5 ng/mL) accompanied with compounds (10μM) for 18h. Fold change of luciferase were divided TGF-β1-stimulated luciferase by luciferase in TGF-β1 non-stimulated group. Experiments were performed in triplicate, and the statistical significance was obtained with one-way ANOVA. Error bars indicate mean ± SEM. *P<0.05, * *P<0.01, * **P<0.001.

Table 3. Inhibition ratio of compounds on TGF-β1/Smad3 signaling pathway in fibroblasts

compound	**Inhibition**	**compound**	**Inhibition**	**compound**	**Inhibition**

	Ratio		Ratio		Ratio
1	-27.73%	**15**	-23.10%	**29**	-7.65%
2	-35.36%	**16**	-49.83%	**30**	6.65%
3	77.72%	**17**	6.58%	**31**	-190.46%
4	61.85%	**18**	4.63%	**32**	-16.96%
5	3.08%	**19**	-32.69%	**33**	26.69%
6	46.00%	**20**	-202.26%	**34**	-2.36%
7	-29.47%	**21**	-74.69%	**35**	-22.34%
8	27.79%	**22**	-187.25%	**36**	17.11%
9	-74.69%	**23**	21.97%	**37**	3.67%
10	2.24%	**24**	-37.58%	**38**	71.29%
11	-11.95%	**25**	-17.25%	**39**	13.46%
12	-21.86%	**26**	-13.63%	**40**	-136.64%
13	-55.44%	**27**	-30.63%	**41**	34.79%
14	-34.58%	**28**	-30.71%		

CAGA-NIH3T3 cells were treated with/without TGF-β1 (5 ng/mL) and compounds (10 μM) for 18h. The inhibition ratio was calculated by the TGF-β1-stimulated luciferase value of compounds-treated group compared with control group. All values are the mean of three independent experiments.

Selected Compounds Inhibited TGF-β1-induced Proliferation of Lung Fibroblasts. According to the results of luciferase screening, five compounds (**3**, **4**, **6**,

38 and **41**) were selected for further investgation of anti-proliferative effects on lung fibroblasts. MTT assays indicated that compounds **3**, **4**, **6** and **38** suppressed TGF-β1-stimulated proliferation of fibroblasts (Figure 2).

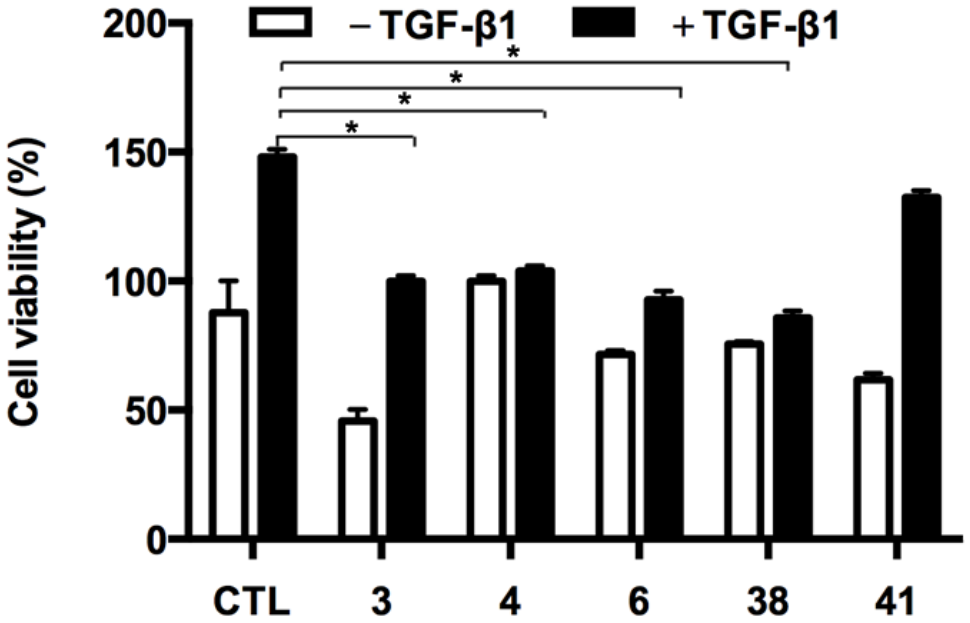


Figure 2. Selected compounds inhibited TGF-β1-induced proliferation of lung fibroblasts.

Mlg cells were treated with/without TGF-β1 (5 ng/mL) and compounds (10 μM) for 24h. The cells treated with DMSO were served as control. The five compounds, selected in the luciferase reporter screening system, were measured by MTT assays at 24h to determine the inhibitory effect of compounds on fibroblast proliferation, which were activated by TGF-β1. All values were normalized to the control, representing 100% cell viability. Experiments were performed in triplicate, and the statistical significance was obtained with one-way ANOVA. Error bars indicate mean ± SEM. *P<0.05.

Selected compounds inhibited TGF-β1-induced migration of lung fibroblasts. In addition, motility potential of lung fibroblasts under TGF-β1 and compounds treatment was further evaluated. Wound healing assays indicated that compounds **3**, **4**, **6** and **38** could inhibit TGF-β1-induced migration of lung fibroblasts, while the compound **41** exhibited weaker inhibition ability in comparison to other compounds (Figure 3).

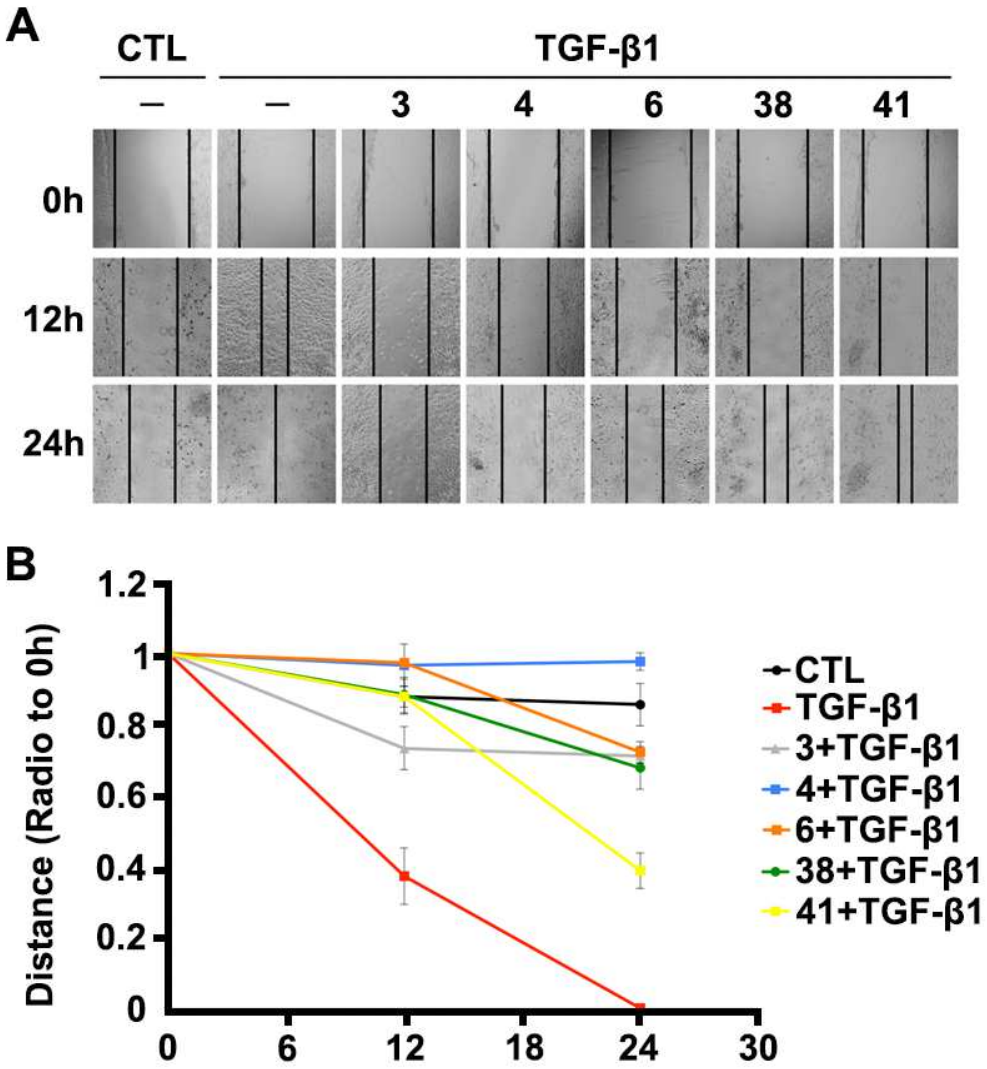


Figure 3. Selected compounds inhibited TGF-β1-induced migration of lung fibroblasts.

Mlg cells were treated with/without TGF-β1 (5 ng/mL) and compounds (10 μM) for 12h or 24 h. (A) The images were photographed at 0h, 12h and 24h post scratching (40x). (B) The wound areas were measured at the three time points in every group, and distance ratio was compared with initial scratch area. Error bars indicate mean ± SEM.

Selected compounds inhibited TGF-β1-induced activation of lung fibroblasts. Progressive pulmonary fibrosis was characterized by the accumulation of activated fibroblasts (also named myofibroblasts) in the fibrotic foci. [22] Thus, TGF-β1 was used to activate lung fibroblasts, followed by investigation of whether the selected compounds could interfere with the activation of fibroblasts. Real-time PCR assays

were used to detect the mRNA levels of *α-SMA*, *type I collagen* (*Col1a1*) and *Fibronectin* (*Fn*), the makers of newly appearing myofibroblasts after TGF-β1 stimulation (Shown in Figure 4). As shown in Figure 4, compounds **3** and **6** could inhibit TGF-β1–induced mRNA expression of *α-SMA*; compounds **3**, **4**, **6** and **38** could decresed mRNA levels of *Col1a1*; compounds **3**, **4** and **6** could significantly suppress mRNA expression levels of *Fn* in lung fibroblasts upon 24 h co-incubation. Together, these data suggested that compounds **3**, **4**, **6**, **38** and **41** were able to inhibit the activation of lung fibroblasts.

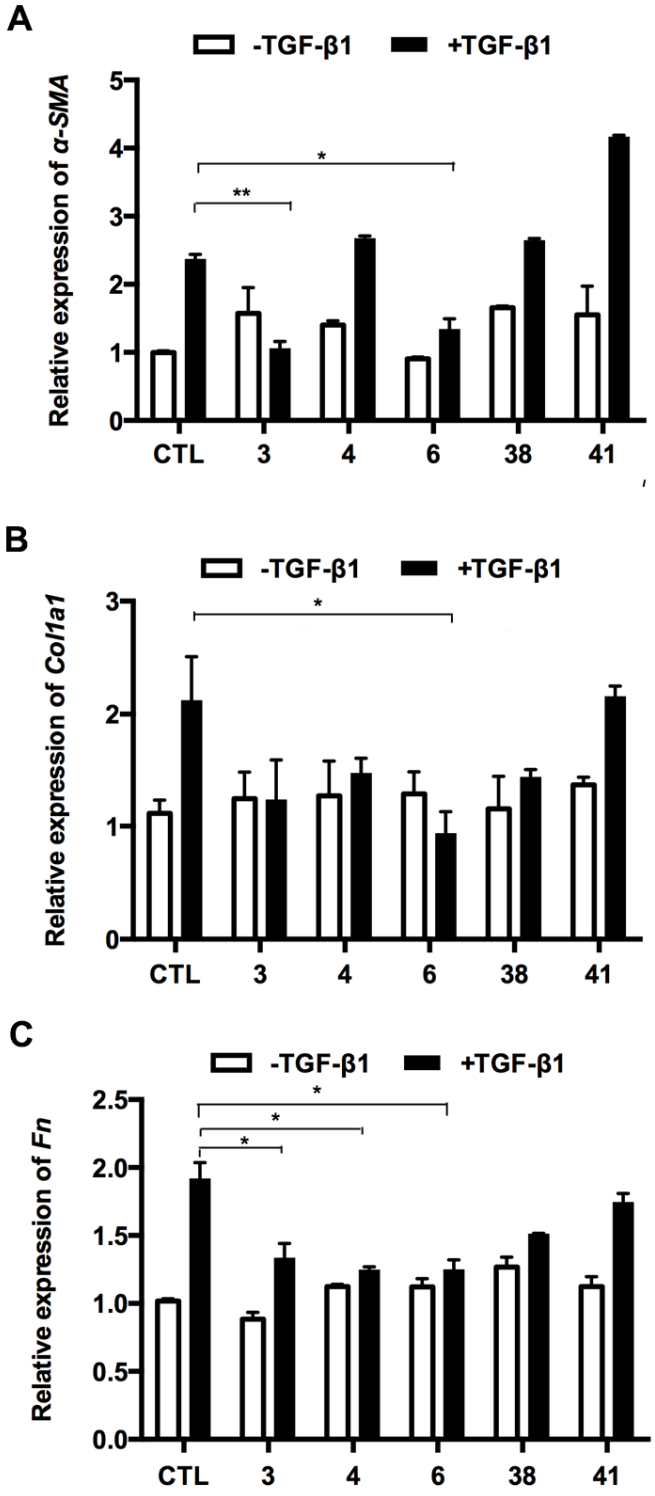
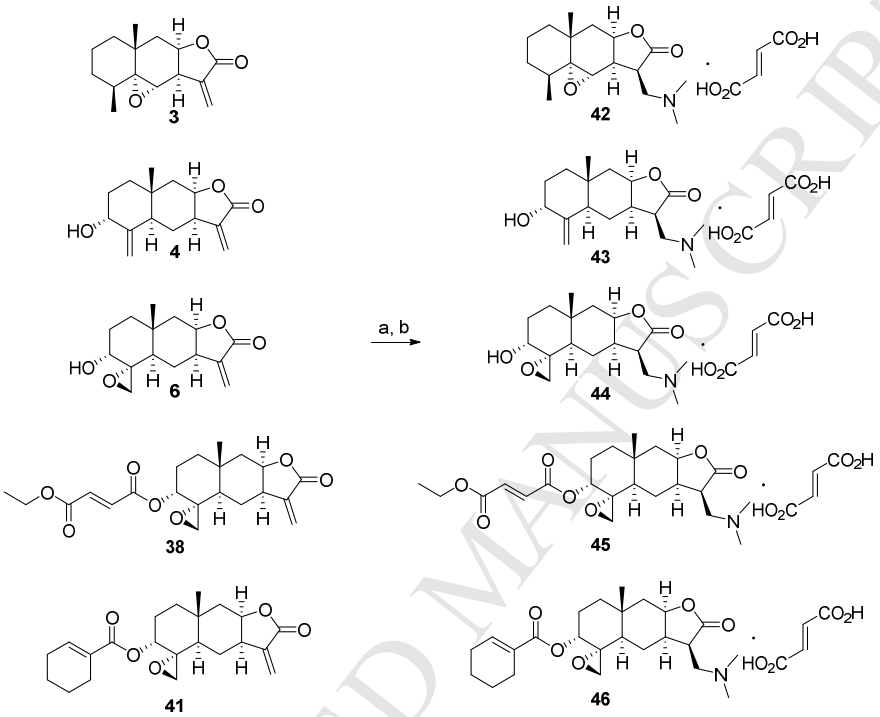


Figure 4. Selected compounds inhibited TGF-β1-induced activation of lung fibroblasts. Lung fibroblasts were treated with/without TGF-β1 (5 ng/mL) and compounds (10 μM) for 24 h.

(A-C) Real-time PCR was performed to examine the mRNA level of *α-SMA*, *Col1a1* and *Fn*. Data are expressed as mean ±SEM, n=3, *P< 0.05, **P< 0.01.

Preparation of Water-soluble Prodrug Molecules 42-46

Due to the poor PK data of compound **3**, **4**, **6**, **38** and **41**, water-soluble prodrugs **42-46** were synthesized based on previous strategy, which could improve the PK properties, dramaticly. [23-25] As shown in scheme 5, compounds **3**, **4**, **6**, **38** and **41** reacted with dimethylamine *via* michael addition, followed by mixture with maleic acid to gain stable salts **42-46** (shown in Scheme 5).



Reagents and conditions: a) $Me_2NH.HCl$, K_2CO_3, CH_2Cl_2, reflux; b) Fumaric acid, EtOAc, rt, 58-89% over 2 steps.

Scheme 5. Preparation of the Water-soluble Analogues **42-46**.

Water-soluble prodrugs 42-46 dose-dependently inhibited TGF-β1/Smad3 signaling pathway in fibroblasts. To investigate whether the water-soluble analogues **42**-**46** exerted an inhibitory effect on TGF-β1/smad3 signaling pathways, compounds **42-46** were gradient screened by a CAGA-luciferase reporter system.Consequently, the luciferase assay illustrated that compounds **42** and **44** could significantly inhibit TGF-β1/smad3 signaling in a dose-dependent manner (Figure 5A and 5C); compounds **45** displayed high suppression ability at a concentration of 40 μM (Figure 5D); compounds **43** and **46** showed moderate inhibition during gradiant screening (Figure 5B and 5E). Collectively, these results suggested that water-soluble analogues

42, **44** and **45** have relative higher biological potency compared with compound **43** and **46**.

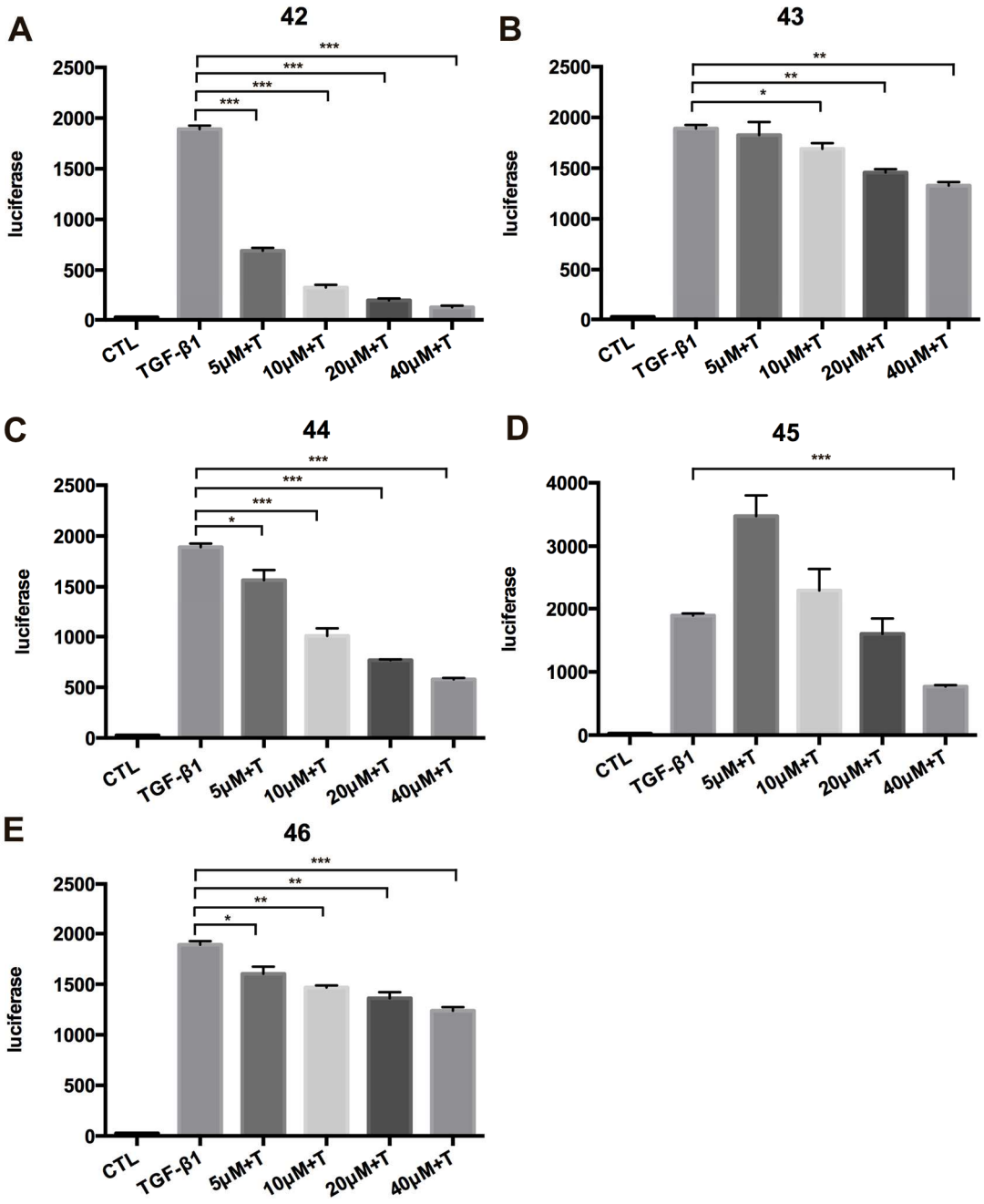


Figure 5. Inhibition activity detection of water-soluble analogues **42-46** on TGF-β1/Smad3 signaling pathway in fibroblasts.

(A-E) CAGA-NIH3T3 fibroblasts cells were treated with/without TGF-β1 (5 ng/mL) and different doses of water-soluble analogues **42-46** for 18h. Concentration gradients of compounds were set as 5, 10, 20 and 40 μM. Experiments were performed in triplicate, and the statistical significance was obtained with one-way ANOVA. Error bars indicate mean ± SEM. *P<0.05, **P<0.01, ***P<0.001.

Compounds 42, 44 and 45 inhibited fibroblasts activation and ECM production *in vitro* through suppressing TGF-β1/Smad3 signaling pathway. According to the outcomes of luciferase assay, compounds **42**, **44** and **45** were chosen for further biological activity verification. The inhibitory capability of compounds **42**, **44** and **45** on TGF-β1 responsiveness was analyzed in lung fibroblasts. As expected, compounds **42**, **44** and **45** could decrease TGF-β1-induced α-SMA expression and ECM production in fibroblasts, accompanied by much lower phosphorylation levels of Smad3 (Figure 6). These results suggested that the water-soluble analogues **42**, **44** and **45** inhibited fibroblasts activation possibly through suppressing TGF-β1/Smad3 signaling pathway.

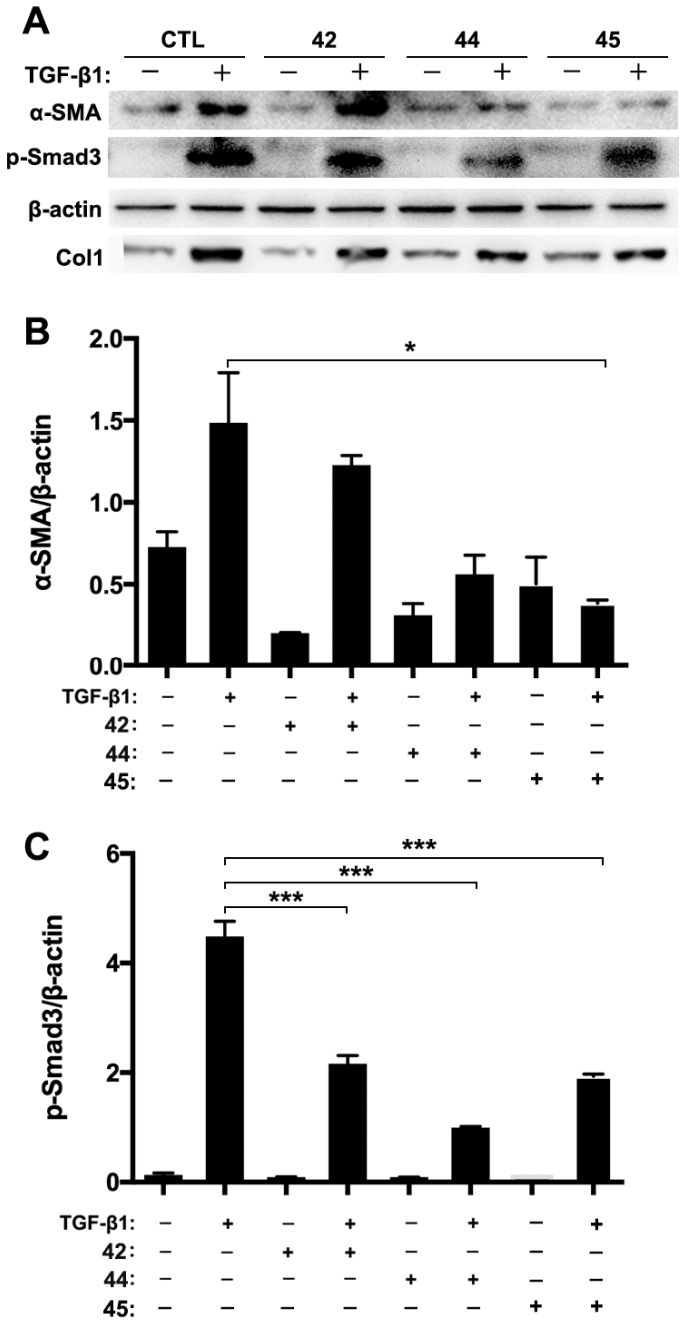


Figure 6. Compounds **42**, **44** and **45** inhibited fibroblasts activation and ECM production through suppressing TGF-β1/Smad3 signaling pathway.

(A) Lung fibroblasts were treated with/without TGF-β1 (5 ng/mL) and compounds **42**, **44** and **45** (10 μM) for 24 h. The expression of α-SMA in cell extracts, Col1 in the medium (supernatant) were examined by Western blot. Lung fibroblasts were treated with/without TGF-β1 (5 ng/mL) and compound **42**, **44** and **45** (10 μM) for 30min, the expression of p-Smad3 was examined by Western blot. β-Actin was used as a loading control. (B-C) The relative density of the bands of α-SMA and p-Smad3 normalized to β-actin is represented in the graphs. Data are presented as mean ± SD (n = 4). *P < 0.05, ***P < 0.001.

Compound 42, 44 and 45 attenuated bleomycin-induced pulmonary fibrosis in mice. To further determine the theraputic impact of the compounds **42**, **44** and **45** on lung fibrosis *in vivo*, bleomycin (BLM)-induced pulmonary fibrosis mice model was established, which were treated with compounds **42**, **44** and **45** after bleomycin injury. Pirfenidone, a listed drug for the treatment of IPF, was used as a positive control. The pathologic changes in the lung was shown in Figure 7A. The sections of the NaCl group displayed clear lung structures while the alveolar structures were destroyed in the model group. Compound **42**, **44** and **45** treatments improved the alveolar structure compared with that in the model group and Pirfenidone group. The percentage of fibrotic area was reduced by approximately 71.8%, 60.5% and 53.5% following the treatment of compounds **42**, **44** and **45**, respectively (Figure 7B). Collagen content was decreased in the mice treated with compounds **42**, **44** and **45**, compared with those treated with Pirfenidone. Among three compounds, compounds **42** and **44** harbored a preferable effect on decreasing ECM production (Figure 7C). In summary, the histopathological analysis and collagen content suggested that compounds **42**, **44** and **45** exerted a promising theraputic impact on BLM-induced pulmonary fibrosis in mice.

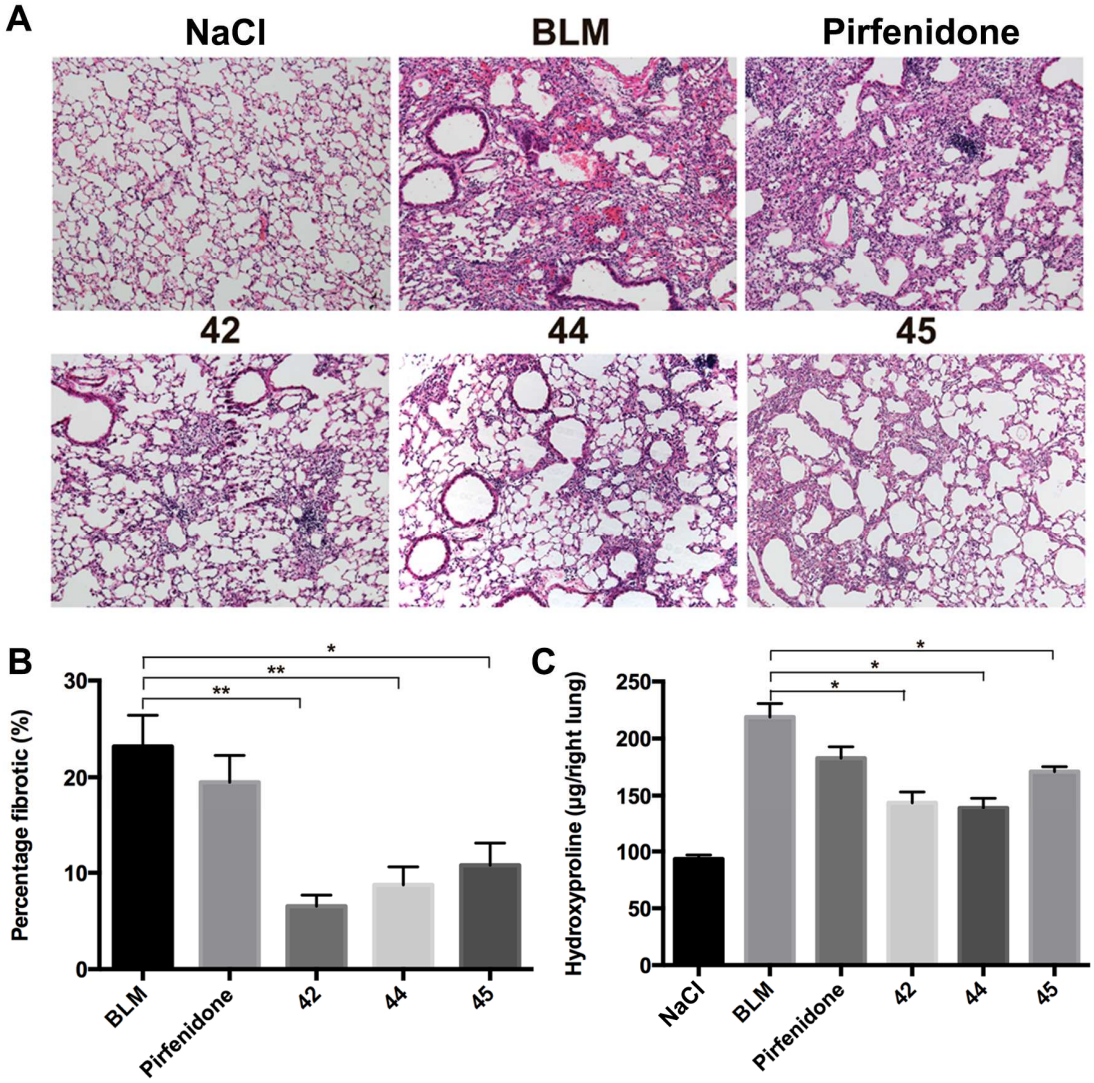


Figure 7. In vivo effects of compounds **42**, **44** and **45** on bleomycin-induced pulmonary fibrosis in mice.

C57BL/6 male mice were intratracheal instillated with BLM (2 U/kg) to induce pulmonary fibrosis. Compounds **42**, **44**, **45** and Pirfenidone (all 100 mg/kg) were given orally once daily from day 7-14 after BLM treatment and lungs were harvested at day 14 for the following analysis (n = 10 per group). (A) Body weight of each group. (B) Hydroxyproline contents in lung tissues of each group were measured as decribed in the methods (*P < 0.05). (C) Lung fibrotic score analysis of the lung sections. The fibrotic area is presented as a percentage. The lung fibrosis areas of each group were calculated using Image-Pro Plus Version 6.0 software (Media Cybernetic, Inc. American). ** P < 0.01. (D) 14 days after BLM instillation, lung histology of each group mice was determined by hematoxylin-eosin (H&E) (200X).

Compounds 42, 44 and 45 inhibited ECM production *in vivo* through suppressing TGF-β1/Smad3 signaling pathway. To explore the mechanism of

compounds **42**, **44** and **45** on BLM-induced fibrogenic changes, the protein levels of collagen1 and p-Smad3 in lung tissues were assesses in each group. Consistent with the hydroxyl-proline assay, the expression levels of col1 were significantly reduced in compounds-treatment group (Figure 8A and 8B). The phosphorylation levels of Smad3 in lung tissues of compounds **42** and **44** treated group were apparently declined compared with those in other groups (Figure 8A and 8C). Consistently, the immunohistochemical analysis of α-SMA, Col1 and p-Smad3 in lung sections indicated the same results (Figure 8D and 8E). In consideration of the luciferase results of compound **45**, the lower anti-fibrosis potency of compound **45** may be associated with the moderate inhibitory ability on TGF-β1/Smad3 signaling pathway and a higher administration dose may improve the anti-fibrosis efficacy.

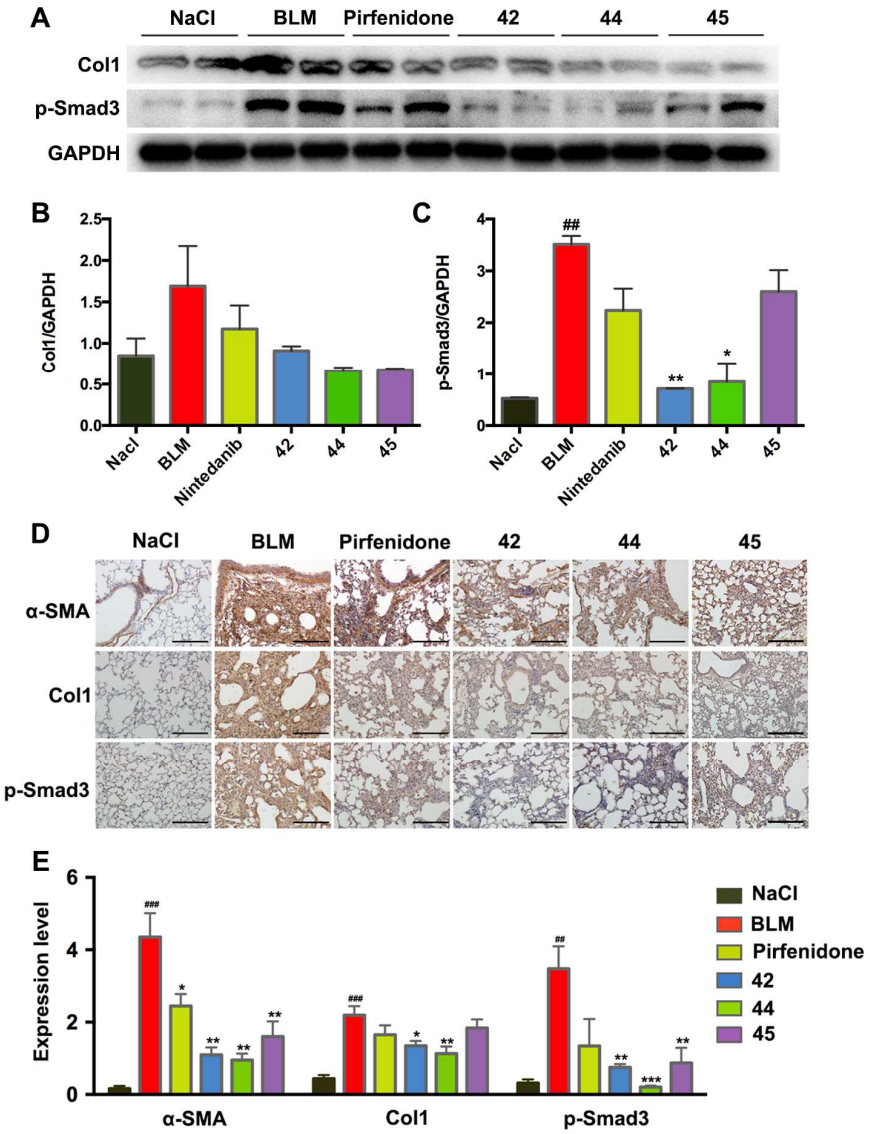


Figure 8. Compounds **42**, **44** and **45** inhibited ECM production *in vivo* through suppressing TGF-β1/Smad3 signaling pathway.

C57BL/6 male mice were intratracheal instillated with BLM (2 U/kg) to induce pulmonary fibrosis. Compounds **42**, **44**, **45** and Pirfenidone (all 100 mg/kg) were given orally once daily from day 7-14 after BLM treatment and lungs were harvested at day 14 for the following analyses (n = 10 per group). (A-C) The expression of p-Smad3 and Col1 in lung tissues were determined by Western blot analysis, GAPDH was used as a loading control. Relative density of the bands of p-Smad3 and Col1 normalized to GAPDH were represented in the graphs. (D-E) Immunohistochemical analysis of α-SMA, Col1 and p-Smad3 in lung sections. Representative images of the staining are shown (n = 10 per group). Error bars indicate mean ± SEM.

Compounds 42 and 44 inhibited human lung fibroblasts activation and ECM production through suppressing TGF-β1/Smad3 signaling pathway.

According to the results of *in vivo* experiments, compounds **42** and **44** showed a significant anti-fibrosis potency in bleomycin-induced mice model. Afterwards, we evaluated the impact of compounds **42** and **44** on human lung fibroblasts. As shown in Figure 9, the administration with compounds **42** and **44** resulting in a blunted TGF-β1-induced phosphorylation of Smad3, protein expression of α-SMA and production of type I collagen in human lung fibroblasts. The above-described results were consistent with both *in vitro* and *in vivo* experiments on mice, suggesting that compounds **42** and **44** may be useful in clinical experiments.

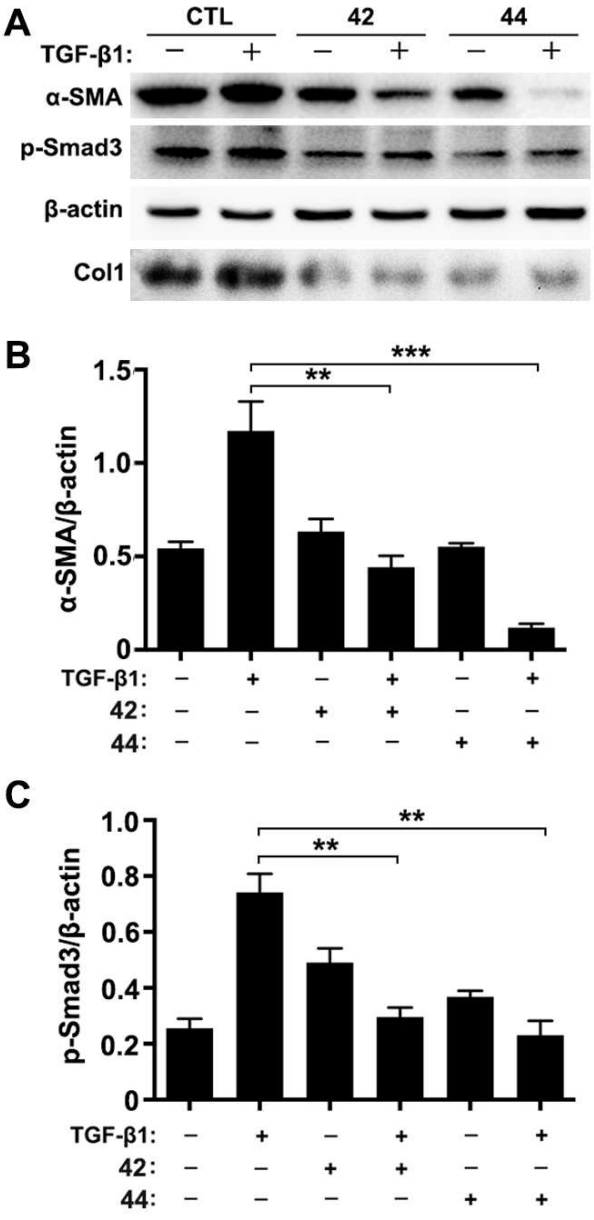


Figure 9. Compounds **42** and **44** inhibited human lung fibroblasts activation and ECM production through suppressing TGF-β1/Smad3 signaling pathway.

(A) Human lung fibroblasts (HFL1) were treated with/without TGF-β1 (5 ng/mL) and compounds **42** and **44** (10 μM) for 24 h. The expression of α-SMA in cell extracts, Col1 in the medium (supernatant) were examined by Western blot. Fibroblasts were treated with/without TGF-β1 (5 ng/mL) and compounds **42** and **44** (10 μM) for 30min, the expression of p-Smad3 was examined by Western blot. β-Actin was used as a loading control. (B-C) The relative density of the bands of α-SMA and p-Smad3 normalized to β-actin is represented in the graphs. Data are presented as mean ± SD (n = 3). **P < 0.01, ***P < 0.001.

Stability in Mouse Plasma. The plasma stability of compounds **42** and **44** in rat plasma for 12 h was analyzed (Shown in Figure 10). After incubation with plasma at 37℃ for 3 h, the remaining ratio of compound **42** and **44** was more than 50% compared to the initial concentration, indicating that both compounds were stable in rat plasma. Besides, within 12 h, compounds **42** and **44** continuously transformed into **3** and **6**, respectively, through a retro Michael reaction (Scheme 6). These results showed that compounds **42** and **44** had superior kinetic properties as prodrugs.

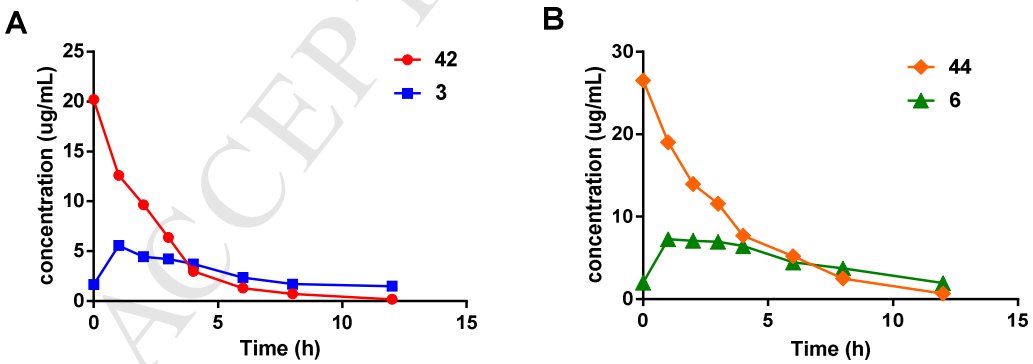
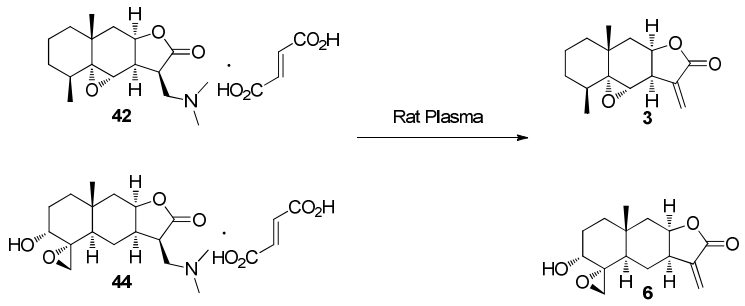
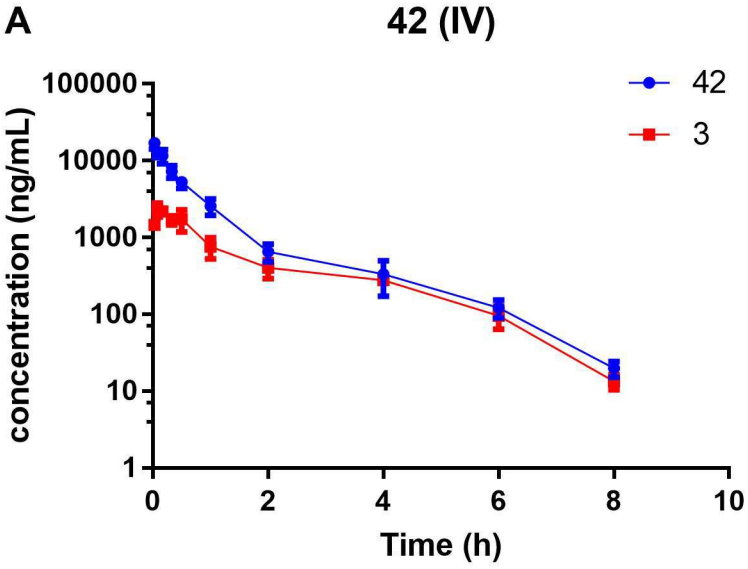


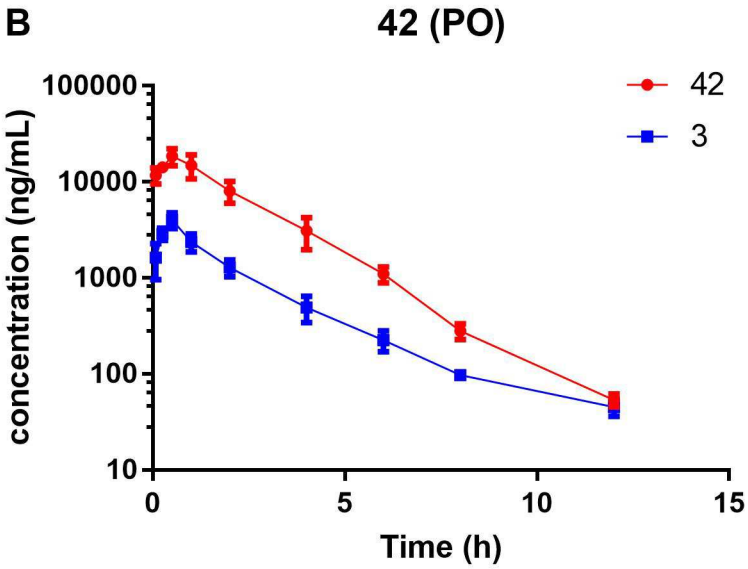
Figure 10. Concentration–time curve of compounds **42** (A) and **44** (B) in rat plasma.



Scheme 6. Compounds **42** and **44** Transformed into **3** and **6** through a Retro Michael Reaction in Rat Plasma

Pharmacokinetic (PK) Study of compounds 42 and 44 in Mice. Pharmacokinetic (PK) Study of compounds 42 and 44 in Mice. The plasma concentration–time curves for compounds **42** and **44** after a single dose in rats were shown in Figure 11. The pharmacokinetic parameters determined with a non-compartmental model were shown in supplementary material. The plasma concentrations of both compounds **42** and **44** rapidly reached peak, and gradually declined after PO administration. Besides, compounds **42** and **44** were constantly releasing **3** and **6** *in vivo*, resulting in an effective concentration range of the blood concentration of **3** and **6** for hours. The pharmacokinetics of both compounds were investigated by tail intravenous injection (IV, Figure 10A and figure 10B), and the pharmacokinetic parameters were shown in supplementary material. The oral bioavailability of compound **42** was determined to be 75%, while compound **44** had a better bioavailability of 78%. The pharmacokinetic properties of compound **42** and **44** made them drug-like molecules for further development.





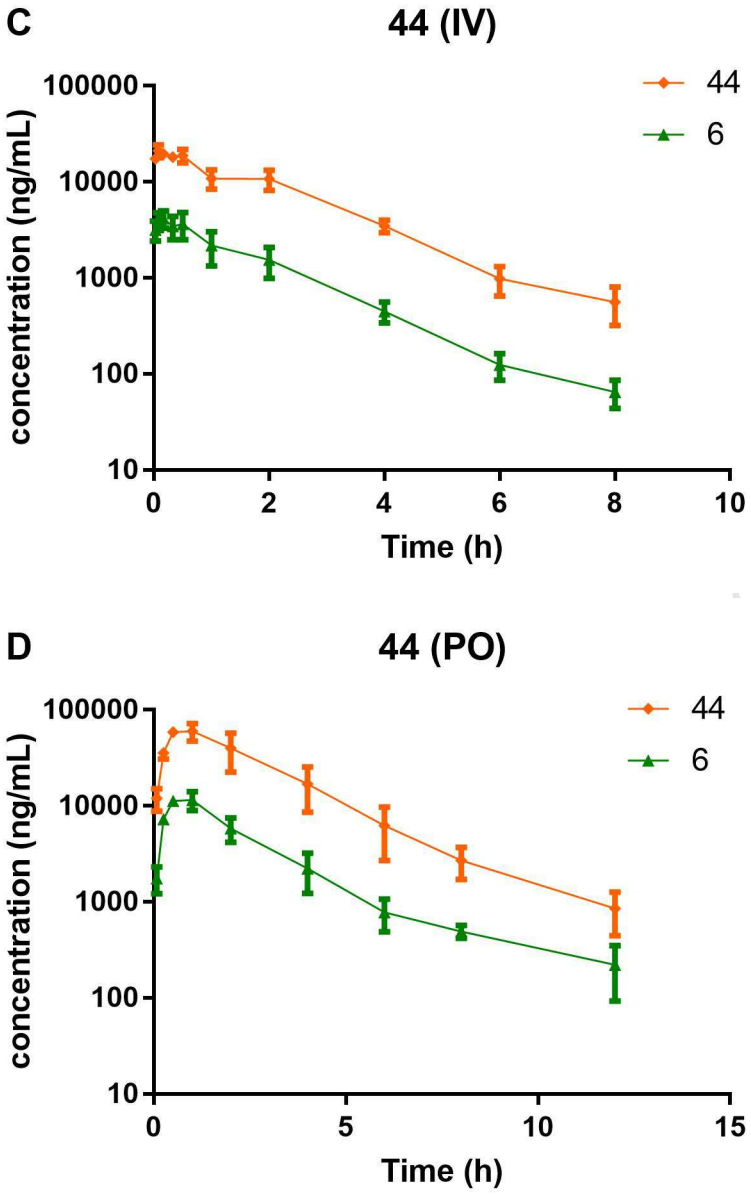


Figure 11. PK study of compounds **42** and **44** in rats. (A) compound **42** plasma concentration–time curve in SD rats after a single IV administration of drug (30 mg/kg, mean ± SD, n = 5); (B) compounds **42** plasma concentration–time curve in SD rats after a single PO administration of drug (150 mg/kg, mean ± SD, n = 5); (C) compounds **44** plasma concentration–time curve in SD rats after a single IV administration of drug (30 mg/kg, mean ± SD, n = 5); (D) compound **42** plasma concentration–time curve in SD rats after a single PO administration of drug (150 mg/kg, mean ± SD, n = 5);

Toxicity Evaluation of Compounds 42 and 44. As a preliminary toxicology study,

C57/BL6 mice were treated with compounds **42** and **44**. Firstly, animals were administered with **42** or **44** by gavage in a single dose (n = 10). For both compound **42** and **44**, no animals died at 1, 2, 3, 4 and 5 g/kg. Then a 7-day oral chronic toxicity study was conducted at 2 g/kg with both compounds **42** and **44** (dosed one daily, n = 10). All animals survived in both compounds **42** and **44** groups. Throughout another 30-day dosing period (dosed one daily, 2 g/kg, PO, n = 10), no animal displayed any systemic symptoms during the observation period in the compound **42** group, while two animals died in the compound **42** group. Together, these data indicated that compound **42** had superior safety *in vivo* by oral administration.

CONCLUSION

In this report, 44 semi-synthetic analogues of natural Sesquiterpene Lactones were prepared, whose anti-IPF potency was evaluated with the high-throughput TGF-β1 reporter luciferase assay *in vitro*. The lead compounds **42** and **44** exerted high inhibitory activities on TGF-β1 signaling pathway and inhibited TGF-β1-induced α-SMA expression and ECM production in *vitro*. Additionally, they exhibited promising efficacy in a bleomycin-induced pulmonary fibrosis mice model. Furthermore, the compounds **42** and **44** could significantly inhibit human lung fibroblasts activation *in vitro*. Further PK study and toxicity evaluation illustrated that lead compound **44** might be a promising anti-IPF drug candidate, which deserved further investigation.

EXPERIMENTAL SECTION

The Synthesis of the compound **3-46**.

Chemistry.

General Methods. Before their use, the solvents were distilled and dried using standard methods (Purification of laboratory chemicals (Six edition), Wilfred L. F. Armarego and Christina L. L. Chai). ^{1}H NMR spectra were obtained by using a Bruker AV 400. Chemical shifts are reported in parts per million (ppm) relative to either a

tetramethylsilane internal standard or solvent signals. ^{13}C NMR spectra were recorded using a Bruker AV 400 spectrometer (100 MHz). Chemical shifts (δ) are reported in parts per million measured relative to the solvent peak. High-resolution mass spectra (HRMS) were obtained with a FTICR-MS (Ionspec 7.0T) spectrometer. The purity of all of the tested compounds was greater than 95%, which was identified by HPLC.

*(1aR,2S,5aR,6aR,9aR,9bS)-2,5a-Dimethyl-9-methyleneoctahydro-2H-oxireno[2',3':4,4a]naphtho[2,3-b]furan-8(9H)-one (**3**).* Alantolactone (2.0 g, 8.6 mmol) was dissolved in CH_2Cl_2 (10 mL). m-CPBA (2.1 g, 10 mmol) in dichloromethane (10 mL) was added dropwise to this solution. The mixture was stirred at room temperature for 3 h. Then the reaction mixture was diluted with 30 mL saturated $Na_2S_2O_3$ solution, washed with saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, filtered, and evaporated to afford a crude product, which was purified by column chromatography [petroleum ether−EtOAc (5:2)] to obtain compound **3** as a white solid (1.9 g, 90%). mp 164-166℃. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.40 (s, 1H), 5.77 (s, 1H), 4.78 – 4.55 (m, 1H), 3.66 (dt, J = 8.9, 2.8 Hz, 1H), 2.89 (d, J = 2.5 Hz, 1H), 1.92 – 1.64 (m, 3H), 1.59 – 1.28 (m, 6H), 1.11 (s, 3H), 1.04 (dd, J = 7.8, 2.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 169.5, 136.6, 123.6, 75.0, 67.4, 61.1, 39.4, 37.6, 37.3, 37.0, 32.5, 29.4, 23.8, 18.0, 16.4. HR-MS (ESI) m/z: calcd for $C_{15}H_{20}O_3Na^+$ $[M + Na]^+$ 271.1305, found 271.1306.

*(3aR,4aR,6R,8aR,9aR)-6-Hydroxy-8a-methyl-3,5-dimethylenedecahydronaphtho[2,3-b]furan-2(3H)-one (**4**).* To a solution of SeO_2 (87.0 mg, 0.74 mmol) in CH_2Cl_2 (5 mL) was added TBHP (0.37 mL), and the mixture was stirred at 0 ℃ for 30 min. Isoalantolactone (500 mg, 2.15 mmol) in CH_2Cl_2 (5 mL) was then added dropwise to the reaction mixture, and the mixture was stirred at room temperature for 24 h. Then the reaction mixture was diluted with 8 mL saturated $Na_2S_2O_3$ solution, and extracted with CH_2Cl_2. The combined organic layers were washed with saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, and evaporated under vaccum. The

crude product was purified by column chromatography [petroleum ether−EtOAc (3:1)] to obtain compound **4** (white solid, 347 mg, 65%). mp 144-145℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 5.96 (s, 1H), 5.75 (s, 1H), 4.88 (d, J = 1.6 Hz, 1H), 4.72 (s, 1H), 4.53 (td, J = 4.8, 1.6 Hz, 1H), 4.45 (t, J = 1.7 Hz, 1H), 4.12 (d, J = 2.9 Hz, 1H), 3.13 (ddd, J = 12.0, 6.9, 5.1 Hz, 1H), 2.45 – 2.34 (m, 1H), 2.01 (dd, J = 15.4, 1.8 Hz, 1H), 1.73 – 1.52 (m, 5H), 1.27 – 1.04 (m, 2H), 0.69 (s, 3H).^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 170.5, 152.0, 142.8, 120.6, 108.1, 77.0, 71.8, 40.9, 40.0, 39.8, 35.8, 34.2, 29.9, 27.1, 17.4. HR-MS (ESI) m/z: calcd for $C_{15}H_{20}O_3Na^+$ $[M + Na]^+$ 271.1305, found 271.1304.

*(3aR,4aR,5R,8aR,9aR)-8a-Methyl-3-methylenedecahydro-2H-spiro[naphtho[2,3-b]furan-5,2'-oxiran]-2-one (**5**).* Isoalantolactone (1.00 g, 4.30 mmol) was dissolved in CH_2Cl_2 (16 mL). m-CPBA (890 mg, 5.16 mmol) was added to this solution in batches. The mixture was stirred at room temperature for 3 h. Then the reaction mixture was diluted with 16 mL saturated $Na_2S_2O_3$ solution, washed with saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, filtered, and evaporated to afford a crude product, which was purified by column chromatography [petroleum ether−EtOAc (20:1)] to obtain compound **5** (white solid, 984 mg, 92%). mp 124-126℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.03 (s, 1H), 5.48 (d, J = 2.2 Hz, 1H), 4.41 (t, J = 5.0 Hz, 1H), 2.83 (dt, J = 11.6, 5.7 Hz, 1H), 2.60 (d, J = 4.2 Hz, 1H), 2.48 (t, J = 3.1 Hz, 1H), 2.12 (d, J = 15.6 Hz, 1H), 1.83 -1.09 (m, 9H), 0.95 – 0.70 (m, 4H).^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.4, 141.8, 120.4, 76.5, 58.5, 50.7, 44.2, 41.9, 41.4, 40.4, 35.3, 34.3, 23.1, 20.3, 18.6. HR-MS (ESI) m/z: calcd for $C_{15}H_{20}O_3$ Na^+ $[M + Na]^+$ 271.1305, found 271.1307.

*(3aR,4aR,5R,6R,8aR,9aR)-6-Hydroxy-8a-methyl-3-methylenedecahydro-2H-spiro[naphtho[2,3-b]furan-5,2'-oxiran]-2-one (**6**).* Compound **4** (112 mg, 0.45 mmol) was dissolved in CH_2Cl_2 (1 mL). m-CPBA (100 mg, 0.60 mmol) in CH_2Cl_2 (1 mL) was added to this solution dropwise. The mixture was stirred at room temperature for 2 h. Then the reaction mixture was diluted with 3 mL saturated $Na_2S_2O_3$ solution, washed with saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, filtered, and evaporated to afford a crude product, which was purified by column chromatography [petroleum

ether−EtOAc (1:1)] to obtain compound **6** (white solid, 97 mg, 85%). mp 167℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.11 (s, 1H), 5.56 (s, 1H), 4.50 (td, *J* = 4.9, 1.5 Hz, 1H), 3.41 (d, *J* = 2.9 Hz, 1H), 2.93 (dt, *J* = 11.9, 6.0 Hz, 1H), 2.80 (d, *J* = 4.1 Hz, 1H), 2.64 (d, *J* = 4.1 Hz, 1H), 2.36 (s, 1H), 2.23 (ddd, *J* = 27.7, 14.4, 2.1 Hz, 2H), 1.85 (dt, *J* = 10.9, 3.1 Hz, 2H), 1.68 (td, *J* = 11.7, 10.4, 8.1 Hz, 1H), 1.61 – 1.51 (m, 2H), 1.37 (dt, *J* = 13.2, 3.5 Hz, 1H), 1.02 – 0.88 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.5, 141.7, 120.6, 76.6, 72.8, 61.4, 50.1, 41.4, 40.4, 37.1, 34.8, 33.9, 26.8, 22.7, 18.1. HR-MS (ESI) m/z: calcd for $C_{15}H_{20}O_4Na^+$ $[M + Na]^+$ 287.1254, found 287.1253.

*(3aR,4aR,6R,8aR,9aR)-6-(Benzyloxy)-8a-methyl-3,5-dimethylenedecahydronaphtho[2,3-b]furan-2(3H)-one (**7**).* To a solution of compound **4** (300 mg, 1.21 mmol) in dry THF (5 mL) was added NaH (58.0 mg, 1.45 mmol). The mixture was stirred at 0 ℃ for 30 min. Then BnBr (269 mg, 1.57 mmol) and TBAI (44.6 mg, 0.12 mmol) were added to the reaction mixture, and the mixture was stirred at room temperature for 16 h. 1 N HCl was added dropwise to the mixture to adjust the pH to 1~2. Then the mixture was diluted in 10 mL saturated NaCl solution, and extracted with EA. The combined organic layers were dried over anhydrous Na_2SO_4, and evaporated under vaccum. The crude product was purified by column chromatography [petroleum ether−EtOAc (20:1)] to obtain compound **7** (white solid, 319 mg, 78%). mp 147.3-147.8℃. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.22 (d, *J* = 26.9 Hz, 5H), 6.07 (s, 1H), 5.52 (s, 1H), 4.91 (s, 1H), 4.66 (s, 1H), 4.50 – 4.37 (m, 2H), 4.21 (d, *J* = 12.1 Hz, 1H), 3.83 (d, *J* = 2.5 Hz, 1H), 2.99 – 2.83 (m, 1H), 2.16 (dd, *J* = 28.1, 14.2 Hz, 2H), 1.82 (dd, *J* = 9.7, 2.7 Hz, 1H), 1.61 (d, *J* = 7.1 Hz, 1H), 1.55 – 1.46 (m, 2H), 1.35 (t, *J* = 12.9 Hz, 1H), 1.27 (d, *J* = 8.3 Hz, 1H), 1.20 (d, *J* = 7.5 Hz, 1H), 0.77 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 169.6, 146.6, 141.2, 137.9, 127.3, 126.4, 126.3, 119.1, 110.3, 78.5, 75.8, 68.0, 40.1, 39.8, 39.5, 35.4, 33.1, 27.1, 26.1, 16.2. HR-MS (ESI) m/z: calcd for $C_{22}H_{26}O_3Na^+$ $[M + Na]^+$ 361.1774, found 361.1774.

*(3aR,4aR,6R,8aR,9aR)-6-((Tert-butyldimethylsilyl)oxy)-8a-methyl-3,5-dimethylenedecahydronaphtho[2,3-b]furan-2(3H)-one (**8**).* To a solution of compound **4** (300 mg, 1.21 mmol) in DMF (10 mL) was added TBSCl (452 mg, 3.00 mmol), DMAP (26.9 mg,

0.24 mmol) and imidazole (409 mg, 6.01 mmol). The reaction mixture was stirred at room temperature for 4 h. Then reaction mixture was diluted in 80 mL saturated NaCl solution, and 1 N HCl solution was adde to adjusted the pH 1~2. The aqueous layer was extracted with EA, and the combined organic layer was dried over Na_2SO_4, and evaporated under vacuum. The crude product was purified by column chromatography [petroleum ether−EtOAc (50:1)] to obtain compound **8** (colorless oil, 369 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.11 (d, J = 1.2 Hz, 1H), 5.59 (d, J = 1.0 Hz, 1H), 4.84 (d, J = 1.4 Hz, 1H), 4.50 (td, J = 4.9, 1.7 Hz, 1H), 4.46 (t, J = 1.5 Hz, 1H), 4.22 (d, J = 2.9 Hz, 1H), 3.00 (ddd, J = 12.1, 7.0, 5.2 Hz, 1H), 2.35 (ddt, J = 13.0, 3.0, 1.6 Hz, 1H), 2.16 (dd, J = 15.6, 1.7 Hz, 1H), 1.72 – 1.51 (m, 5H), 1.41 – 1.31 (m, 1H), 1.28 – 1.20 (m, 1H), 0.86 (s, 9H), 0.78 (s, 3H), 0.03 (s, 3H), -0.02 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.7, 151.0, 142.2, 120.0, 107.8, 77.0, 73.8, 41.2, 40.5, 40.4, 35.9, 34.1, 30.7, 27.1, 25.8, 18.1, 17.1, -4.7, -5.0. HR-MS (ESI) m/z: calcd for $C_{21}H_{34}O_3SiNa^+$ $[M + Na]^+$ 385.2169, found 385.2170.

*(3aR,4aR,6R,8aR,9aR)-6-((Tert-butyldiphenylsilyl)oxy)-8a-methyl-3,5-dimethylenedec ahydronaphtho[2,3-b]furan-2(3H)-one (**9**).* Following the procedure described for preparation of compound **8**, compound **9** was prepared from compound **4** as a colorless oil. (Yield 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.67 (ddd, J = 15.7, 8.0, 1.5 Hz, 5H), 7.50 – 7.43 (m, 2H), 7.42 (s, 1H), 7.37 (d, J = 6.8 Hz, 1H), 6.19 – 6.11 (m, 1H), 5.62 (s, 1H), 5.24 (d, J = 2.0 Hz, 1H), 4.64 – 4.55 (m, 1H), 4.36 (d, J = 2.8 Hz, 1H), 3.45 (dt, J = 12.2, 6.2 Hz, 1H), 2.15 (ddd, J = 30.1, 14.4, 5.0 Hz, 2H), 1.96 – 1.85 (m, 2H), 1.65 – 1.53 (m, 4H), 1.51 (dd, J = 5.0, 2.8 Hz, 1H), 1.33 – 1.26 (m, 2H), 1.08 (s, 9H), 0.87 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.7, 149.7, 142.2, 136.0, 135.8, 134.8, 134.3, 134.1, 129.6, 129.5, 127.5, 127.4, 120.1, 108.6, 77.0, 74.5, 41.3, 40.8, 40.6, 36.1, 34.2, 30.3, 27.1, 19.4, 17.1. HR-MS (ESI) m/z: calcd for $C_{31}H_{38}O_3SiNa^+$ $[M + Na]^+$ 509.2482 found 509.2483.

*(3aR,4aR,8aR,9aR)-8a-Methyl-3,5-dimethyleneoctahydronaphtho[2,3-b]furan-2,6(3H, 4H)-dione (**10**).* To a solution of compound **4** (2.0 g, 8.0 mmol) in CH_2Cl_2 (100 mL) was added Dess-martin periodinane (8.5g, 20 mmol) . The reaction mixture was stirred at room temperature for 3 h. Then the mixture was diluted with 100 mL saturated

$Na_2S_2O_3$ solution, washed with saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, and evaporated under vaccum. The crude product was purified by column chromatography [petroleum ether−EtOAc (5:1)] to obtain compound **10** (white solid, 1.3 g, 65%). mp 152.3-152.6℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 6.00 (d, J = 1.1 Hz, 1H), 5.78 (d, J = 1.0 Hz, 1H), 5.69 (dd, J = 2.5, 1.3 Hz, 1H), 5.12 (dd, J = 2.6, 1.3 Hz, 1H), 4.58 (td, J = 4.8, 1.8 Hz, 1H), 3.12 (ddd, J = 12.0, 7.0, 5.2 Hz, 1H), 2.46 – 2.38 (m, 2H), 2.34 (dq, J = 11.9, 2.7 Hz, 1H), 2.12 (dd, J = 15.7, 1.9 Hz, 1H), 2.01 – 1.89 (m, 1H), 1.77 – 1.54 (m, 3H), 1.11 (dt, J = 14.0, 12.0 Hz, 1H), 0.84 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 201.2, 170.4, 148.3, 142.5, 121.0, 118.6, 76.6, 44.0, 39.9, 39.0, 38.1, 36.2, 32.0, 27.2, 17.4. HR-MS (ESI) m/z: calcd for $C_{15}H_{18}O_3Na^+$ $[M + Na]^+$ 269.1148, found 269.1147.

*(3aR,4aR,8aR,9aR,Z)-6-(Hydroxyimino)-8a-methyl-3,5-dimethylenedecahydronaphtho [2,3-b]furan-2(3H)-one (**11**).* To a solution of compound **10** (100 mg, 0.40 mmol) in pyridine (4 mL) was added hydroxylamine hydrochloride (79.0 mg, 69.5 mmol). The mixture was stirred at room temperature for 4 h. Then the pH of the reaction mixture was adjusted to 1~2 with 1 N HCl solution. The aqueous was extracted with EA, and the combined organic layer was dried over Na_2SO_4, and evaporated under vaccum. The crude product was purified by column chromatography [CH_2Cl_2−MeOH (10:1)] to obtain compound **11** (colorless oil, 72 mg, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.10 (d, J = 2.4 Hz, 1H), 5.57 (d, J = 2.4 Hz, 1H), 5.34 (s, 1H), 4.71 (s, 1H), 4.45 (q, J = 4.8, 3.9 Hz, 1H), 3.18 (dt, J = 16.8, 3.3 Hz, 1H), 2.92 (p, J = 5.8 Hz, 1H), 2.22 – 1.19 (m, 8H), 0.89 – 0.85 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 169.3, 158.0, 142.2, 140.8, 119.6, 111.3, 75.2, 44.1, 39.6, 38.9, 38.0, 31.9, 26.28, 19.2, 16.0. HR-MS (ESI) m/z: calcd for $C_{15}H_{19}NO_3Na^+$ $[M + Na]^+$ 284.1257, found 284.1256.

*(3aR,4aR,8aR,9aR)-6-Amino-8a-methyl-3,5-dimethylenedecahydronaphtho[2,3-b]fur an-2(3H)-one (**12**).* To a solution of compound **10** (200 mg, 0.81 mmol) in dry MeOH (10 mL) was added NH_4Cl (433 mg, 8.10 mmol) and $NaBH_3CN$ (36.0 mg, 0.57 mmol). The mixture was stirred at room temperature overnight. Then the mixture was diluted in 10 mL saturated $NaHCO_3$ solution, and extracted with EA. The combined

organic layers were dried over anhydrous Na_2SO_4, and evaporated under vaccum. The crude product was purified by column chromatography [petroleum ether−EtOAc (10:1)] to obtain compound **12** (colorless oil, 86 mg, 43%).^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.15 (s, 1H), 5.61 (s, 1H), 5.13 (s, 1H), 4.63 (s, 1H), 4.50 (dt, *J* = 4.9, 3.0 Hz, 1H), 4.01 (dd, *J* = 11.6, 5.3 Hz, 1H), 2.96 (dt, *J* = 12.2, 6.0 Hz, 1H), 2.25 (dd, *J* = 15.6, 1.7 Hz, 1H), 1.97 (ddd, *J* = 12.3, 5.7, 3.1 Hz, 1H), 1.73 (dd, *J* = 7.1, 2.7 Hz, 1H), 1.64 – 1.56 (m, 2H), 1.50 – 1.42 (m, 3H), 1.32 (td, *J* = 13.5, 4.0 Hz, 1H), 0.82 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 169.4, 150.0, 140.9, 119.4, 102.5, 75.6, 72.1, 43.6, 40.0, 39.3, 39.0, 33.0, 31.2, 26.5, 16.6. HR-MS (ESI) m/z: calcd for $C_{15}H_{21}NO_2Na^+$ $[M + Na]^+$ 270.1465, found 270.1466.

*(3aR,6R,8aR,9aR)-8a-Methyl-3,5-dimethylene-2-oxododecahydronaphtho[2,3-b]furan-6-yl 2-ethylbutanoate (**13**).* Compound **4** (150 mg, 0.60 mmol) was dissovled in CH_2Cl_2 (3 mL). DMAP (29.0 mg, 0.24 mmol), DIC (182 mg, 1.44 mmol) and octanoic acid (261 mg, 1.81 mmol) were added to this solution. After stirred at room temperature overnight, the reaction mixture was filtered. The filtrate was then washed with saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The crude product was purified by column chromatography [petroleum ether−EtOAc (10:1)] to obtain compound **13** (colorless oil, 114 mg, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.14 (d, *J* = 1.2 Hz, 1H), 5.60 (d, *J* = 1.2 Hz, 1H), 5.40 (s, 1H), 5.13 (t, *J* = 1.2 Hz, 1H), 4.69 (dd, *J* = 1.9, 1.1 Hz, 1H), 4.53 – 4.51 (m, 1H), 3.02 – 3.01 (m, 1H), 2.24 (dd, *J* = 3.8, 1.9 Hz, 1H), 2.22 – 2.21 (m, 1H), 1.75– 1.24 (m, 12H), 0.88 (q, *J* = 7.4 Hz, 6H), 0.84 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm)174.1, 169.4, 144.6, 141.0, 119.3, 111.4, 75.6, 73.7, 48.1, 40.6, 40.0, 39.4, 35.5, 32.8, 26.1, 25.9, 24.0, 16.0, 10.7. HR-MS (ESI) m/z: calcd for $C_{21}H_{30}O_4Na^+$ $[M + Na]^+$ 369.2036, found 369.2036.

*(3aR,6R,8aR,9aR)-8a-Methyl-3,5-dimethylene-2-oxododecahydronaphtho[2,3-b]furan-6-yl octanoate (**14**).* Following the procedure described for preparation of compound **13**, compound **14** was prepared from compound **4** as a colorless oil. (Yield 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.13 (d, *J* = 1.2 Hz, 1H), 5.58 (s, 1H), 5.36 (t, *J* = 2.9 Hz, 1H), 5.12 (s, 1H), 4.72 – 4.62 (m, 1H), 4.51 (td, *J* = 4.8, 1.6 Hz, 1H), 3.00 (ddd, *J* = 12.0,

6.9, 5.2 Hz, 1H), 2.31 – 2.18 (m, 4H), 1.79 (dq, *J* = 8.0, 4.0, 3.6 Hz, 2H), 1.71 (ddd, *J* = 14.0, 7.1, 2.7 Hz, 1H), 1.60 (dd, *J* = 14.6, 6.6 Hz, 3H), 1.54 (d, *J* = 4.5 Hz, 1H), 1.41 (t, *J* = 3.9 Hz, 1H), 1.37 (d, *J* = 3.7 Hz, 1H), 1.28 (q, *J* = 4.4 Hz, 7H), 0.92 – 0.77 (m, 7H). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* (ppm) 172.8, 170.5, 145.6, 142.0, 120.4, 112.5, 76.6, 75.0, 41.5, 41.0, 40.4, 36.4, 34.7, 33.8, 31.7, 29.1, 29.0, 27.1, 27.0, 25.1, 22.6, 17.0, 14.1. HR-MS (ESI) m/z: calcd for $C_{23}H_{34}O_4Na^+$ $[M + Na]^+$ 397.2349, found 397.2350.

*(3aR,6R,8aR,9aR)-8a-Methyl-3,5-dimethylene-2-oxododecahydronaphtho[2,3-b]furan -6-yl hex-5-ynoate (**15**).* Following the procedure described for preparation of compound **13**, compound **15** was prepared from compound **4** as a colorless oil. (Yield 84%). ^{1}H NMR (400 MHz, $CDCl_3$) *δ* (ppm) 6.08 (d, *J* = 1.2 Hz, 1H), 5.53 (s, 1H), 5.32 (s, 1H), 5.07 (s, 1H), 4.67 – 4.61 (m, 1H), 4.46 (td, *J* = 4.9, 1.6 Hz, 1H), 2.96 (dt, *J* = 12.0, 6.0 Hz, 1H), 2.39 (t, *J* = 7.4 Hz, 2H), 2.18 (tt, *J* = 13.2, 2.2 Hz, 4H), 1.91 (t, *J* = 2.6 Hz, 1H), 1.80-1.18(m, 9H), 0.77 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* (ppm) 171.0, 169.4, 144.5, 140.9, 119.4, 111.6, 82.3, 75.6, 74.3, 68.1, 40.5, 40.0, 39.4, 35.4, 32.8, 32.3, 26.0, 25.9, 22.7, 16.8, 16.0. HR-MS (ESI) m/z: calcd for $C_{21}H_{26}O_4Na^+$ $[M + Na]^+$ 365.1723, found 365.1724.

*(3aR,6R,8aR,9aR)-8a-Methyl-3,5-dimethylene-2-oxododecahydronaphtho[2,3-b]furan -6-yl 2-ethylhexanoate (**16**).* Following the procedure described for preparation of compound **13**, compound **16** was prepared from compound **4** as a colorless oil. (Yield 93%). ^{1}H NMR (400 MHz, $CDCl_3$) *δ* (ppm) 6.08 (s, 1H), 5.53 (d, *J* = 2.3 Hz, 1H), 5.32 (d, *J* = 2.9 Hz, 1H), 5.07 (s, 1H), 4.63 (s, 1H), 4.46 (dt, *J* = 4.8, 3.0 Hz, 1H), 3.01 – 2.84 (m, 1H), 2.26 – 2.05 (m, 3H), 1.74 (dt, *J* = 6.7, 2.8 Hz, 2H), 1.64 (ddd, *J* = 14.2, 7.2, 2.6 Hz, 1H), 1.57 – 1.14 (m, 12H), 0.85 – 0.75 (m, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* (ppm) 175.3, 170.4, 145.6, 145.6, 141.9, 120.4 112.4, 76.6, 74.7, 47.5, 41.6, 41.1, 40.5, 36.6, 33.8, 31.9, 29.6, 27.1, 25.6, 22.7, 17.0, 14.0, 11.8. HR-MS (ESI) m/z: calcd for $C_{23}H_{34}O_4Na^+$ $[M + Na]^+$ 397.2349, found 397.2350.

*(3aR,6R,8aR,9aR)-8a-Methyl-3,5-dimethylene-2-oxododecahydronaphtho[2,3-b]furan -6-yl acetate (**17**).* Following the procedure described for preparation of compound **13**, compound **17** was prepared from compound **4** as a colorless oil. (Yield 92%). ^{1}H NMR (400 MHz, $CDCl_3$) *δ* (ppm) 6.08 (d, *J* = 1.2 Hz, 1H), 5.53 (d, *J* = 1.1 Hz, 1H), 5.29

(d, *J* = 2.9 Hz, 1H), 5.07 (d, *J* = 1.6 Hz, 1H), 4.69 – 4.59 (m, 1H), 4.46 (td, *J* = 4.9, 1.6 Hz, 1H), 3.06 – 2.88 (m, 1H), 2.27 – 2.13 (m, 2H), 1.99 (s, 3H), 1.80 – 1.61 (m, 3H), 1.57 – 1.44 (m, 2H), 1.38 – 1.23 (m, 2H), 0.77 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* (ppm) 169.4, 169.0, 144.5, 140.9, 119.3, 111.5, 75.6, 74.3, 40.4, 39.9, 39.4, 35.3, 32.8, 26.0, 25.9, 20.5, 16.0. HR-MS (ESI) m/z: calcd for $C_{17}H_{22}O_4Na^+$ $[M + Na]^+$ 313.1410, found 313.1410.

*(3aR,6R,8aR,9aR)-8a-Methyl-3,5-dimethylene-2-oxododecahydronaphtho[2,3-b]furan-6-yl cyclopropanecarboxylate (**18**).* Following the procedure described for preparation of compound **13**, compound **18** was prepared from compound **4** as a colorless oil. (Yield 77%). ^{1}H NMR (400 MHz, $CDCl_3$) *δ* (ppm) 6.15 (d, *J* = 1.2 Hz, 1H), 5.67 – 5.54 (m, 1H), 5.35 (t, *J* = 2.9 Hz, 1H), 5.13 (s, 1H), 4.75 – 4.67 (m, 1H), 4.53 (td, *J* = 4.9, 1.7 Hz, 1H), 3.11 – 2.94 (m, 1H), 2.25 (ddd, *J* = 15.6, 8.8, 2.0 Hz, 2H), 1.77 (dddd, *J* = 24.1, 13.9, 6.5, 2.8 Hz, 3H), 1.59 – 1.54 (m, 1H), 1.47 – 1.36 (m, 2H), 1.35 – 1.21 (m, 2H), 1.02 – 0.95 (m, 2H), 0.87 – 0.82 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* (ppm) 172.8, 169.5, 144. 6, 140.9, 119.4, 111.5, 75.6, 74.2, 40.5, 40.0, 39.4, 35.4, 32.8, 25.9, 21.6, 20.1, 16.0, 12.2, 7.5. HR-MS (ESI) m/z: calcd for $C_{19}H_{24}O_4Na^+$ $[M + Na]^+$ 339.1567, found 339.1568.

*(3aR,4aR,6R,8aR,9aR)-8a-Methyl-3,5-dimethylene-2-oxododecahydronaphtho[2,3-b]furan-6-yl 2-(3-(trifluoromethyl)phenyl)acetate (**19**).* Following the procedure described for preparation of compound **13**, compound **19** was prepared from compound **4** as a colorless oil. (Yield 47%). ^{1}H NMR (400 MHz, $CDCl_3$) *δ* (ppm) δ 7.51 – 7.38 (m, 4H), 6.05 (d, *J* = 2.2 Hz, 1H), 5.51 (d, *J* = 2.1 Hz, 1H), 5.28 (q, *J* = 2.6 Hz, 1H), 5.04 (s, 1H), 4.61 (d, *J* = 2.1 Hz, 1H), 4.43 – 4.36 (m, 1H), 3.61 (d, *J* = 2.1 Hz, 2H), 2.82 (td, *J* = 9.5, 7.0, 4.0 Hz, 1H), 2.08 (dt, *J* = 15.4, 2.0 Hz, 1H), 1.87 (dt, *J* = 12.9, 2.3 Hz, 1H), 1.69 (tt, *J* = 7.3, 3.4 Hz, 2H), 1.54 (ddd, *J* = 9.2, 6.0, 2.6 Hz, 1H), 1.35 – 1.19 (m, 4H), 0.72 (d, *J* = 2.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* (ppm) 170.4, 169.4, 145.0, 141.9, 135.3, 132.8, 129.0, 126.1, 126.1, 123.9, 123.9, 120.4, 112.9, 76.5, 76.1, 41.7, 41.3, 40.8, 40.2, 36.2, 33.7, 26.9, 26.8, 17.0. HR-MS (ESI) m/z: calcd for $C_{24}H_{25}F_3O_4Na^+$ $[M + Na]^+$ 457.1597, found 457.1596.

(3aR,4aR,6R,8aR,9aR)-8a-Methyl-3,5-dimethylene-2-oxododecahydronaphtho[2,3-b]f

*uran-6-yl benzo[d][1,3]dioxole-5-carboxylate (**20**).* Following the procedure described for preparation of compound **13**, compound **20** was prepared from compound **4** as a colorless oil. (Yield 56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.64 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.45 (d, *J* = 1.8 Hz, 1H), 6.85 (d, *J* = 8.2 Hz, 1H), 6.05 (s, 2H), 5.67 – 5.51 (m, 2H), 5.22 (s, 1H), 4.76 (d, *J* = 1.9 Hz, 1H), 4.54 (dt, *J* = 5.0, 2.4 Hz, 1H), 3.04 (dt, *J* = 12.1, 6.2 Hz, 1H), 2.38 – 2.19 (m, 2H), 2.00 – 1.83 (m, 2H), 1.77 – 1.59 (m, 3H), 1.54 – 1.31 (m, 3H), 0.88 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 169.5, 163.8, 150.5, 146.6, 144.4, 140.8, 124.2, 119.4, 111.9, 108.4, 107.0, 100.8, 75.6, 74.7, 40.7, 40.0, 39.3, 35.7, 32.9, 26.2, 25.9, 16.1. HR-MS (ESI) m/z: calcd for $C_{23}H_{24}O_6$ Na^+ $[M + Na]^+$ 419.1465, found 419.1464.

*(3aR,4aR,6R,8aR,9aR)-8a-Methyl-3,5-dimethylene-2-oxododecahydronaphtho[2,3-b]furan-6-yl 2-(4-fluorophenyl)acetate (**21**).* Following the procedure described for preparation of compound **13**, compound **21** was prepared from compound **4** as a colorless oil. (Yield 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.25 – 7.13 (m, 2H), 6.95 (t, *J* = 8.6 Hz, 2H), 6.08 (s, 1H), 5.53 (s, 1H), 5.27 (d, *J* = 2.9 Hz, 1H), 5.05 (s, 1H), 4.43 (td, *J* = 5.0, 1.6 Hz, 1H), 3.52 (s, 2H), 2.86 (dt, *J* = 12.1, 6.1 Hz, 1H), 2.12 (dd, *J* = 15.6, 1.7 Hz, 1H), 1.91 (dd, *J* = 12.8, 2.4 Hz, 1H), 1.70 (dq, *J* = 6.9, 3.6 Hz, 2H), 1.58 – 1.51 (m, 1H), 1.39 – 1.19 (m, 5H), 0.73 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.5, 170.3, 163.2, 160.7, 145.2, 141.9, 130.9, 130.8, 120.4, 115.4, 115.2, 112.8, 76.5, 75.9, 41.3, 41.0, 40.4, 36.3, 33.8, 26.9, 26.9, 17.0. HR-MS (ESI) m/z: calcd for $C_{23}H_{25}FO_4Na^+$ $[M + Na]^+$ 407.1629, found 407.1630.

*(3aR,4aR,6R,8aR,9aR)-8a-Methyl-3,5-dimethylene-2-oxododecahydronaphtho[2,3-b]furan-6-yl benzoate (**22**).* Following the procedure described for preparation of compound **13**, compound **22** was prepared from compound **4** as a colorless oil. (Yield 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.03 (d, *J* = 7.7 Hz, 2H), 7.57 (t, *J* = 7.6 Hz, 1H), 7.45 (t, *J* = 7.7 Hz, 2H), 6.14 (s, 1H), 5.66 – 5.55 (m, 2H), 5.25 (s, 1H), 4.77 (s, 1H), 4.55 (d, *J* = 5.0 Hz, 1H), 3.04 (dt, *J* = 11.7, 5.8 Hz, 1H), 2.30 (dd, *J* = 24.9, 14.0 Hz, 2H), 2.03 – 1.85 (m, 2H), 1.79 – 1.69 (m, 2H), 1.62 (dd, *J* = 15.0, 3.9 Hz, 1H), 1.56 – 1.46 (m, 1H), 1.41 – 1.32 (m, 1H), 0.89 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.5, 165.5, 145.4, 141. 9, 132.9, 130.8, 129.5, 128.4, 120.5, 113.0, 76.6, 75.8, 41.8, 41.1, 40.4,

36.7, 34.0, 27.2, 27.0, 17.1. HR-MS (ESI) m/z: calcd for $C_{22}H_{24}O_4Na^+$ $[M + Na]^+$ 375.1567, found 375.1568.

*(3aR,4aR,6R,8aR,9aR)-8a-Methyl-3,5-dimethylene-2-oxododecahydronaphtho[2,3-b]furan-6-yl (tert-butoxycarbonyl)-L-valinate (**23**).* Following the procedure described for preparation of compound **13**, compound **23** was prepared from compound **4** as a colorless oil. (Yield 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.14 (d, J = 3.3 Hz, 1H), 5.60 (d, J = 3.3 Hz, 1H), 5.40 (s, 1H), 5.21 – 5.04 (m, 2H), 4.72 (s, 1H), 4.52 (d, J = 4.5 Hz, 1H), 4.16 (ddt, J = 41.1, 7.3, 3.9 Hz, 1H), 3.04 (dt, J = 11.0, 5.9 Hz, 1H), 2.22 (dd, J = 12.4, 6.7 Hz, 2H), 2.13 (d, J = 9.6 Hz, 1H), 2.03 (d, J = 3.4 Hz, 1H), 1.84 – 1.66 (m, 3H), 1.63 – 1.52 (m, 2H), 1.44 (s, 9H), 1.34 (d, J = 13.2 Hz, 1H), 1.24 (d, J = 4.4 Hz, 1H), 0.96 (dd, J = 6.7, 3.5 Hz, 3H), 0.93 – 0.87 (m, 2H), 0.83 (d, J = 3.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 171.5, 170.5, 155.7, 145.0, 141.9, 120.5, 113.1, 79.7,76.6, 76.3, 58.4, 41.4, 41.0, 40.3, 36.4, 33.8, 31.7, 28.3, 27.2, 26.9, 19.0, 17.4, 17.1. HR-MS (ESI) m/z: calcd for $C_{25}H_{37}O_6NNa^+$ $[M + Na]^+$ 470.2513, found 470.2514.

*Ethyl((3aR,4aR,6R,8aR,9aR)-8a-methyl-3,5-dimethylene-2-oxododecahydronaphtho[2,3-b]furan-6-yl) fumarate (**24**).* Following the procedure described for preparation of compound **13**, compound **24** was prepared from compound **4** as a colorless oil. (Yield 38%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.81 (d, J = 3.3 Hz, 2H), 6.10 (d, J = 1.1 Hz, 1H), 5.57 (d, J = 1.1 Hz, 1H), 5.43 (t, J = 2.8 Hz, 1H), 5.15 (t, J = 1.1 Hz, 1H), 4.72 (dd, J = 1.9, 0.9 Hz, 1H), 4.50 (td, J = 4.9, 1.6 Hz, 1H), 4.23 (q, J = 7.2 Hz, 2H), 3.00 (ddd, J = 12.0, 6.9, 5.1 Hz, 1H), 2.25 – 2.16 (m, 2H), 1.86 – 1.78 (m, 2H), 1.73 – 1.66 (m, 1H), 1.61 – 1.52 (m, 2H), 1.43 – 1.33 (m, 2H), 1.29 (t, J = 7.1 Hz, 3H), 0.82 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.5, 165.0, 163.9, 145.0, 142.0, 134.0, 133.6, 120.5, 113.3, 76.6, 76.4, 61.4, 41.5, 40.9, 40.3, 36.4, 33.9, 27.0, 26.9, 17.1, 14.2. HR-MS (ESI) m/z: calcd for $C_{21}H_{26}O_6Na^+$ $[M + Na]^+$ 397.1622, found 397.1622.

*(3aR,4aR,6R,8aR,9aR)-8a-Eethyl-3,5-dimethylene-2-oxododecahydronaphtho[2,3-b]furan-6-yl (E)-3-(3-fluorophenyl)acrylate (**25**).* Following the procedure described for preparation of compound **13**, compound **25** was prepared from compound **4** as a colorless oil. (Yield 29%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.62 (d, J = 15.9 Hz, 1H), 7.36 (td, J = 7.9, 5.8 Hz, 1H), 7.29 (dt, J = 7.7, 1.2 Hz, 1H), 7.23 (dt, J = 9.6, 2.1 Hz, 1H),

7.09 (tdd, *J* = 8.3, 2.6, 1.0 Hz, 1H), 6.44 (d, *J* = 16.0 Hz, 1H), 6.15 (d, *J* = 1.1 Hz, 1H), 5.60 (d, *J* = 1.0 Hz, 1H), 5.49 (t, *J* = 2.9 Hz, 1H), 5.21 (t, *J* = 1.2 Hz, 1H), 4.76 (dd, *J* = 1.9, 1.0 Hz, 1H), 4.54 (td, *J* = 4.9, 1.7 Hz, 1H), 3.03 (ddd, *J* = 12.0, 6.9, 5.2 Hz, 1H), 2.33 – 2.23 (m, 2H), 1.92 – 1.88 (m, 1H), 1.75 (ddd, *J* = 14.0, 7.0, 2.7 Hz, 1H), 1.69 – 1.58 (m, 2H), 1.48 – 1.43 (m, 1H), 1.38 (dt, *J* = 13.9, 12.3 Hz, 1H), 1.29 – 1.22 (m, 1H), 0.87 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.4, 165.6, 145.4, 143.3, 142.0, 130.5, 124.1, 120.4, 120.0, 117.2, 117.1, 114.3, 114.1, 112.9, 76.6, 75.6, 41.6, 41.0, 40.4, 36.5, 33.9, 27.1, 27.0, 17.1. HR-MS (ESI) m/z: calcd for $C_{24}H_{25}FO_4Na^+$ $[M + Na]^+$ 419.1629, found 419.1630.

*(3aR,4aR,6R,8aR,9aR)-8a-Methyl-3,5-dimethylene-2-oxododecahydronaphtho[2,3-b]furan-6-yl (E)-hex-2-enoate (**26**).* Following the procedure described for preparation of compound **13**, compound **26** was prepared from compound **4** as a colorless oil. (Yield 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.94 (dt, *J* = 15.6, 6.9 Hz, 1H), 6.12 (d, *J* = 1.2 Hz, 1H), 5.81 (dt, *J* = 15.7, 1.6 Hz, 1H), 5.58 (d, *J* = 1.0 Hz, 1H), 5.40 (t, *J* = 2.9 Hz, 1H), 5.14 (t, *J* = 1.2 Hz, 1H), 4.77 – 4.68 (m, 1H), 4.51 (td, *J* = 4.9, 1.6 Hz, 1H), 3.05 – 2.96 (m, 1H), 2.20 (dddd, *J* = 21.9, 14.6, 8.1, 1.9 Hz, 4H), 1.76 (dddd, *J* = 39.1, 14.3, 6.2, 2.6 Hz, 3H), 1.57 (dd, *J* = 16.3, 4.9 Hz, 2H), 1.48 (q, *J* = 7.4 Hz, 2H), 1.43 – 1.33 (m, 2H), 0.92 (t, *J* = 7.4 Hz, 3H), 0.83 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.5, 165.8, 149.4, 145.6, 142.0, 121.6, 120.4, 112.5, 76.6, 75.0, 41.5, 41.0, 40.4, 36.5, 34.2, 33.9, 27.1, 27.0, 21.3, 17.1, 13.7. HR-MS (ESI) m/z: calcd for $C_{21}H_{28}O_4Na^+$ $[M + Na]^+$ 367.1880, found 367.1879.

*(3aR,4aR,6R,8aR,9aR)-8a-Methyl-3,5-dimethylene-2-oxododecahydronaphtho[2,3-b]furan-6-yl (E)-2-methylpent-2-enoate (**27**).* Following the procedure described for preparation of compound **13**, compound **27** was prepared from compound **4** as a colorless oil. (Yield 39%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.72 (tq, *J* = 7.4, 1.5 Hz, 1H), 6.13 (d, *J* = 1.2 Hz, 1H), 5.58 (d, *J* = 1.1 Hz, 1H), 5.40 (t, *J* = 2.9 Hz, 1H), 5.16 (t, *J* = 1.2 Hz, 1H), 4.71 (dd, *J* = 1.9, 1.0 Hz, 1H), 4.52 (td, *J* = 4.9, 1.7 Hz, 1H), 3.03 (ddd, *J* = 12.0, 7.0, 5.2 Hz, 1H), 2.28 – 2.11 (m, 4H), 1.86 – 1.79 (m, 5H), 1.73 (ddd, *J* = 14.0, 7.1, 2.7 Hz, 1H), 1.65 – 1.53 (m, 2H), 1.46 – 1.30 (m, 2H), 1.04 (t, *J* = 7.6 Hz, 3H), 0.85 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.5, 167.2, 145.6, 143.8, 142.0, 127.6,

120.4, 112.5, 76.6, 75.1, 41.7, 41.1, 40.4, 36.7, 33.9, 27.2, 27.0, 22.0, 17.1, 13.1, 12.3. HR-MS (ESI) m/z: calcd for $C_{21}H_{28}O_4Na^+$ $[M + Na]^+$ 367.1880, found 367.1880.

*(3aR,4aR,6R,8aR,9aR)-8a-Methyl-3,5-dimethylene-2-oxododecahydronaphtho[2,3-b]furan-6-yl cyclohex-1-ene-1-carboxylate (**28**).* Following the procedure described for preparation of compound **13**, compound **28** was prepared from compound **4** as a colorless oil. (Yield 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.95 (tt, *J* = 3.8, 1.8 Hz, 1H), 6.12 (d, *J* = 1.0 Hz, 1H), 5.58 (s, 1H), 5.47 – 5.37 (m, 1H), 5.14 (s, 1H), 4.69 (d, *J* = 1.6 Hz, 1H), 4.51 (td, *J* = 5.0, 1.6 Hz, 1H), 3.02 (ddd, *J* = 12.0, 6.9, 5.2 Hz, 1H), 2.27 – 2.15 (m, 6H), 1.81 (ddd, *J* = 11.7, 6.5, 3.2 Hz, 2H), 1.76 – 1.69 (m, 1H), 1.60 (tdd, *J* = 15.8, 8.0, 4.1 Hz, 6H), 1.45 – 1.31 (m, 2H), 0.84 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.5, 166.4, 145.7, 142.0, 139.6, 130.8, 120.4, 112.4, 76.6, 74.8, 41.7, 41.1, 40.4, 36. 7, 33.9, 27.2, 27.0, 25.8, 24.2, 22.1, 21.5, 17.1. HR-MS (ESI) m/z: calcd for $C_{22}H_{28}O_4Na^+$ $[M + Na]^+$ 379.1880, found 379.1881.

*(3aR,5R,6R,8aR,9aR)-8a-Methyl-3-methylene-2-oxodecahydro-2H-spiro[naphtho[2,3-b]furan-5,2'-oxiran]-6-yl octanoate (**29**).* Following the procedure described for preparation of compound **13**, compound **29** was prepared from compound **6** as a colorless oil. (Yield 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.10 (s, 1H), 5.54 (s, 1H), 4.57 – 4.52 (m, 1H), 4.48 (dt, *J* = 5.2, 2.9 Hz, 1H), 2.93 (dt, *J* = 11.9, 6.0 Hz, 1H), 2.73 (d, *J* = 4.3 Hz, 1H), 2.63 (d, *J* = 4.2 Hz, 1H), 2.34 (t, *J* = 7.5 Hz, 2H), 2.18 (ddd, *J* = 23.2, 14.4, 2.1 Hz, 2H), 1.86 (ddd, *J* = 11.5, 5.2, 2.9 Hz, 2H), 1.66 – 1.51 (m, 3H), 1.49 – 1.39 (m, 2H), 1.28 (qd, *J* = 11.5, 9.2, 5.1 Hz, 9H), 0.99 (s, 4H), 0.89 – 0.82 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.8, 170.3, 141.6, 120.7, 76.4, 75.0, 58.4, 49.0, 41.6, 40.3, 38.6, 35.5, 34.5, 33.8, 31.7, 29.0, 29.0, 25.4, 25.0, 22.6, 18.1, 14.1. HR-MS (ESI) m/z: calcd for $C_{23}H_{34}O_5Na^+$ $[M + Na]^+$ 413.2298, found 413.2297.

*(3aR,5R,6R,8aR,9aR)-8a-Methyl-3-methylene-2-oxodecahydro-2H-spiro[naphtho[2,3-b]furan-5,2'-oxiran]-6-yl hex-5-ynoate (**30**).* Following the procedure described for preparation of compound **13**, compound **30** was prepared from compound **6** as a colorless oil. (Yield 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.10 (d, *J* = 1.1 Hz, 1H), 5.55 (d, *J* = 1.0 Hz, 1H), 4.56 (t, *J* = 2.9 Hz, 1H), 4.49 (td, *J* = 4.9, 1.6 Hz, 1H), 2.94 (ddd, *J* = 11.9, 6.8, 5.1 Hz, 1H), 2.74 (d, *J* = 4.2 Hz, 1H), 2.67 – 2.61 (m, 1H), 2.51 (td, *J* = 7.3,

1.3 Hz, 2H), 2.28 (td, *J* = 6.9, 2.6 Hz, 2H), 2.18 (ddd, *J* = 21.4, 14.4, 2.2 Hz, 2H), 1.98 (t, *J* = 2.7 Hz, 1H), 1.87 (qdd, *J* = 8.9, 7.5, 4.1 Hz, 4H), 1.62 – 1.52 (m, 2H), 1.51 – 1.38 (m, 2H), 1.05 – 0.88 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.0, 170.3, 141.5, 120.8, 83.3, 76.4, 75.3, 69.2, 58.4, 49.0, 41.5, 40.3, 38.6, 35.5, 33.8, 33.2, 25.4, 23.7, 22.6, 18.1, 17.8. HR-MS (ESI) m/z: calcd for $C_{21}H_{26}O_5Na^+$ $[M + Na]^+$ 381.1672, found 381.1674.

*(3aR,5R,6R,8aR,9aR)-8a-Methyl-3-methylene-2-oxodecahydro-2H-spiro[naphtho[2,3-b]furan-5,2'-oxiran]-6-yl 2-(3-(trifluoromethyl)phenyl)acetate (**31**).* Following the procedure described for preparation of compound **13**, compound **31** was prepared from compound **6** as a colorless oil. (Yield 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.61 (s, 1H), 7.55 (d, *J* = 7.2 Hz, 1H), 7.47 (d, *J* = 7.8 Hz, 2H), 6.11 (s, 1H), 5.56 (s, 1H), 4.56 (s, 1H), 4.45 (t, *J* = 4.9 Hz, 1H), 3.75 (d, *J* = 3.5 Hz, 2H), 2.87 (dt, *J* = 11.9, 5.9 Hz, 1H), 2.73 (d, *J* = 4.0 Hz, 1H), 2.62 (d, *J* = 4.1 Hz, 1H), 2.15 (d, *J* = 15.6 Hz, 1H), 1.95 (dd, *J* = 13.1, 2.5 Hz, 1H), 1.83 (dd, *J* = 8.2, 4.0 Hz, 2H), 1.53 (ddd, *J* = 13.8, 6.9, 2.4 Hz, 1H), 1.35 (ddd, *J* = 17.5, 11.5, 4.2 Hz, 2H), 1.19 (td, *J* = 16.5, 14.1, 9.7 Hz, 2H), 0.95 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.3, 169.5, 141.5, 135.1, 132.8, 129.1, 126.3, 126.3, 124.0, 124.0, 120.8, 76.3, 76.2, 58.3, 48.9, 41.4, 41.4, 40.2, 38.5, 35.2, 33.7, 25.7, 22.5, 18.0. HR-MS (ESI) m/z: calcd for $C_{24}H_{25}F_3O_5Na^+$ $[M + Na]^+$ 473.1546, found 473.1545.

*(3aR,4aR,5R,6R,8aR,9aR)-8a-Methyl-3-methylene-2-oxodecahydro-2H-spiro[naphtho[2,3-b]furan-5,2'-oxiran]-6-yl benzo[d][1,3]dioxole-5-carboxylate (**32**).* Following the procedure described for preparation of compound **13**, compound **32** was prepared from compound **6** as a colorless oil. (Yield 28%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.67 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.48 (d, *J* = 1.7 Hz, 1H), 6.86 (d, *J* = 8.2 Hz, 1H), 6.10 (d, *J* = 28.9 Hz, 3H), 5.58 (s, 1H), 4.77 (d, *J* = 2.8 Hz, 1H), 4.54 (t, *J* = 4.6 Hz, 1H), 3.02 (dt, *J* = 11.9, 6.0 Hz, 1H), 2.80 (d, *J* = 4.2 Hz, 1H), 2.71 (d, *J* = 4.2 Hz, 1H), 2.30 – 2.22 (m, 2H), 2.07 – 1.96 (m, 2H), 1.67 – 1.55 (m, 4H), 1.05 – 0.99 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 169.3, 163.8, 150.6, 146.6, 140.5, 124.5, 119.8, 108.5, 107.0, 100.8, 75.3, 74.8, 57.5, 48.0, 40.6, 39.2, 38.0, 34.7, 32.9, 28.7, 24.5, 21.6, 17.1. HR-MS (ESI) m/z: calcd for $C_{23}H_{24}O_7Na^+$ $[M + Na]^+$ 435.1414, found 435.1415.

*(3aR,4aR,5R,6R,8aR,9aR)-8a-Methyl-3-methylene-2-oxodecahydro-2H-spiro[naphtho[2,3-b]furan-5,2'-oxiran]-6-yl benzoate (**33**).* Following the procedure described for preparation of compound **13**, compound **33** was prepared from compound **6** as a colorless oil. (Yield 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.13 – 8.02 (m, 2H), 7.64 – 7.53 (m, 1H), 7.45 (dd, *J* = 8.4, 7.0 Hz, 2H), 6.12 (d, *J* = 1.1 Hz, 1H), 5.57 (d, *J* = 1.0 Hz, 1H), 4.81 (t, *J* = 2.9 Hz, 1H), 4.58 – 4.49 (m, 1H), 3.01 (ddd, *J* = 11.9, 6.8, 5.1 Hz, 1H), 2.80 (d, *J* = 4.2 Hz, 1H), 2.72 (d, *J* = 4.2 Hz, 1H), 2.35 – 2.21 (m, 2H), 2.10 – 1.92 (m, 2H), 1.69 – 1.57 (m, 3H), 1.52 – 1.46 (m, 1H), 1.04 (s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 169.3, 164.5, 140.5, 132.1, 129.3, 128.6, 127.4, 119.8, 75.4, 74.8, 57.5, 48.0, 40.6, 39.2, 38.0, 34.7, 32. 9, 24.5, 21.6, 17.1. HR-MS (ESI) m/z: calcd for $C_{22}H_{24}O_5Na^+$ $[M + Na]^+$ 391.1516, found 391.1516.

*(3aR,5R,6R,8aR,9aR)-8a-Methyl-3-methylene-2-oxodecahydro-2H-spiro[naphtho[2,3-b]furan-5,2'-oxiran]-6-yl acetate (**34**).* Following the procedure described for preparation of compound **13**, compound **34** was prepared from compound **6** as a colorless oil. (Yield 56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.08 (s, 1H), 5.53 (s, 1H), 4.54 – 4.49 (m, 1H), 4.46 (dt, *J* = 5.0, 2.9 Hz, 1H), 2.92 (dt, *J* = 11.9, 6.0 Hz, 1H), 2.72 (d, *J* = 4.2 Hz, 1H), 2.62 (d, *J* = 4.2 Hz, 1H), 2.22 – 2.12 (m, 2H), 2.08 (d, *J* = 0.9 Hz, 3H), 1.93 – 1.77 (m, 2H), 1.60 – 1.50 (m, 2H), 1.47 (dd, *J* = 12.4, 5.3 Hz, 1H), 1.40 (dt, *J* = 13.4, 3.8 Hz, 1H), 0.99 – 0.85 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.3, 170.0, 141.6, 120.7, 76.4, 75.3, 58.4, 49.0, 41.5, 40.2, 38.5, 35.4, 33.8, 25.4, 22.6, 21.3, 18.1. HR-MS (ESI) m/z: calcd for $C_{17}H_{22}O_5Na^+$ $[M + Na]^+$ 329.1359, found 329.1360.

*(3aR,5R,6R,8aR,9aR)-8a-Methyl-3-methylene-2-oxodecahydro-2H-spiro[naphtho[2,3-b]furan-5,2'-oxiran]-6-yl 2-ethylhexanoate (**35**).* Following the procedure described for preparation of compound **13**, compound **35** was prepared from compound **6** as a colorless oil. (Yield 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.05 (s, 1H), 5.50 (s, 1H), 4.53 (d, *J* = 2.8 Hz, 1H), 4.44 (dt, *J* = 5.5, 2.7 Hz, 1H), 2.89 (dt, *J* = 11.9, 6.0 Hz, 1H), 2.68 (d, *J* = 4.2 Hz, 1H), 2.58 (d, *J* = 4.2 Hz, 1H), 2.25 (td, *J* = 8.9, 4.5 Hz, 1H), 2.21 – 2.14 (m, 1H), 2.08 (dd, *J* = 13.2, 2.5 Hz, 1H), 1.82 (dt, *J* = 7.6, 3.1 Hz, 2H), 1.49 (dddd, *J* = 47.8, 20.6, 11.2, 4.7 Hz, 9H), 1.24 (dq, *J* = 12.6, 5.3, 3.6 Hz, 4H), 0.94 (s, 3H), 0.88 –

0.81 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 174.3, 169.3, 140.6, 119.7, 75.4, 73.7, 57.4, 48.0, 46.4, 40.6, 39.3, 37.8, 34.6, 32.8, 30.8, 30.7, 28.5, 24.6, 21.6, 21.6, 17.1, 12.9, 10.8. HR-MS (ESI) m/z: calcd for $C_{23}H_{34}O_5Na^+$ $[M + Na]^+$ 413.2298, found 413.2297.

*(3aR,4aR,5R,6R,8aR,9aR)-8a-Methyl-3-methylene-2-oxodecahydro-2H-spiro[naphtho[2,3-b]furan-5,2'-oxiran]-6-yl (tert-butoxycarbonyl)-L-valinate (**36**).* Following the procedure described for preparation of compound **13**, compound **36** was prepared from compound **6** as a colorless oil. (Yield 58%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.13 (s, 1H), 5.58 (s, 1H), 5.07 (d, *J* = 8.9 Hz, 1H), 4.63 (d, *J* = 2.9 Hz, 1H), 4.56 – 4.46 (m, 1H), 4.33 (dd, *J* = 8.9, 4.3 Hz, 1H), 2.98 (dt, *J* = 11.9, 5.9 Hz, 1H), 2.75 (d, *J* = 4.2 Hz, 1H), 2.65 (d, *J* = 4.3 Hz, 1H), 2.31 – 2.09 (m, 3H), 1.95 – 1.85 (m, 2H), 1.58 (ddd, *J* = 20.3, 14.8, 6.8 Hz, 3H), 1.48 – 1.42 (m, 10H), 1.03 – 0.93 (m, 7H), 0.90 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.3, 169.3, 154.5, 140.4, 119.9, 78.7, 75.3, 75.2, 57.4, 57.2, 47.8, 40.5, 39.2, 37.6, 34.6, 32.8, 30.6, 27.3, 24.5, 21.5, 18.0, 17.1, 16.3. HR-MS (ESI) m/z: calcd for $C_{25}H_{37}NO_7Na^+$ $[M + Na]^+$ 486.2462, found 486.2460.

*(3aR,4aR,5R,6R,8aR,9aR)-8a-Methyl-3-methylene-2-oxodecahydro-2H-spiro[naphtho[2,3-b]furan-5,2'-oxiran]-6-yl 2-(diethoxyphosphoryl)acetate (**37**).* Following the procedure described for preparation of compound **13**, compound **37** was prepared from compound **6** as a colorless oil. (Yield 46%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.10 (s, 1H), 5.54 (s, 1H), 4.59 – 4.42 (m, 2H), 4.16 (p, *J* = 7.2 Hz, 5H), 2.74 (d, *J* = 4.2 Hz, 1H), 2.62 (d, *J* = 4.2 Hz, 1H), 2.26 – 2.14 (m, 2H), 1.98 – 1.79 (m, 3H), 1.57 (ddt, *J* = 16.4, 9.7, 4.6 Hz, 3H), 1.32 (dd, *J* = 7.1, 2.0 Hz, 8H), 0.98 – 0.91 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.3, 164.8, 141.5, 120.8, 77.2, 76.9, 76.4, 62.7, 58.2, 48.8, 41.5, 40.2, 38.4, 35.3, 35.2, 34.0, 33.8, 25.3, 22.6, 18.1, 16.4. HR-MS (ESI) m/z: calcd for $C_{21}H_{31}O_8PNa^+$ $[M + Na]^+$ 465.1649, found 465.1650.

*Ethyl((3aR,4aR,5R,6R,8aR,9aR)-8a-methyl-3-methylene-2-oxodecahydro-2H-spiro[naphtho[2,3-b]furan-5,2'-oxiran]-6-yl) fumarate (**38**).* Following the procedure described for preparation of compound **13**, compound **38** was prepared from compound **6** as a colorless oil. (Yield 39%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.95 – 6.82 (m, 2H), 6.12 (d, *J* = 1.1 Hz, 1H), 5.57 (d, *J* = 1.0 Hz, 1H), 4.67 (t, *J* = 2.9 Hz, 1H), 4.51 (td, *J* = 4.9, 1.6

Hz, 1H), 4.27 (q, *J* = 7.1 Hz, 2H), 2.98 – 2.94 (m, 1H), 2.77 (d, *J* = 4.1 Hz, 1H), 2.67 (d, *J* = 4.1 Hz, 1H), 2.21 (td, *J* = 5.9, 2.1 Hz, 1H), 2.17 (d, *J* = 2.9 Hz, 1H), 1.99 – 1.89 (m, 2H), 1.62 – 1.55 (m, 2H), 1.54 – 1.42 (m, 2H), 1.33 (t, *J* = 7.1 Hz, 3H), 1.02 (s, 3H), 0.99 – 0.89 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.3, 164.9, 164.0, 141.5, 134.0, 133.5, 120.9, 76.5, 76.3, 61.5, 58.3, 49.0, 41.5, 40.2, 38.7, 35.4, 33.8, 25.4, 22.6, 18.1, 14.2. HR-MS (ESI) m/z: calcd for $C_{21}H_{26}O_7Na^+$ $[M + Na]^+$ 413.1571, found 413.1570.

*(3aR,4aR,5R,6R,8aR,9aR)-8a-Methyl-3-methylene-2-oxodecahydro-2H-spiro[naphtho[2,3-b]furan-5,2'-oxiran]-6-yl (E)-hex-2-enoate (**39**).* Following the procedure described for preparation of compound **13**, compound **39** was prepared from compound **6** as a colorless oil. (Yield 44%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.98 (dt, *J* = 15.6, 6.9 Hz, 1H), 6.10 (d, *J* = 1.2 Hz, 1H), 5.86 (dt, *J* = 15.6, 1.6 Hz, 1H), 5.54 (d, *J* = 1.0 Hz, 1H), 4.59 (t, *J* = 2.8 Hz, 1H), 4.49 (td, *J* = 4.9, 1.6 Hz, 1H), 2.94 (ddd, *J* = 11.9, 6.8, 5.1 Hz, 1H), 2.75 (d, *J* = 4.2 Hz, 1H), 2.65 (d, *J* = 4.2 Hz, 1H), 2.25 – 2.14 (m, 4H), 2.00 – 1.82 (m, 2H), 1.63 – 1.53 (m, 2H), 1.52 – 1.38 (m, 4H), 1.03 – 0.88 (m, 7H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.3, 165.7, 149.9, 141.6, 121.2, 120.7, 76.4, 75.0, 58.5, 49.0, 41.5, 40.3, 38.7, 35.5, 34.2, 33.8, 25.4, 22.6, 21.2, 18.1, 13.7. HR-MS (ESI) m/z: calcd for $C_{21}H_{28}O_5Na^+$ $[M + Na]^+$ 383.1829, found 383.1828.

*(3aR,4aR,5R,6R,8aR,9aR)-8a-Methyl-3-methylene-2-oxodecahydro-2H-spiro[naphtho[2,3-b]furan-5,2'-oxiran]-6-yl (E)-but-2-enoate (**40**).* Following the procedure described for preparation of compound **13**, compound **40** was prepared from compound **6** as a colorless oil. (Yield 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.11 (d, *J* = 1.2 Hz, 1H), 5.93 (ddt, *J* = 17.0, 9.6, 6.9 Hz, 1H), 5.55 (s, 1H), 5.30 – 5.05 (m, 2H), 4.61 – 4.44 (m, 2H), 3.15 (dt, *J* = 6.9, 1.5 Hz, 2H), 2.95 (dt, *J* = 11.9, 5.9 Hz, 1H), 2.75 (d, *J* = 4.2 Hz, 1H), 2.64 (d, *J* = 4.2 Hz, 1H), 2.18 (ddd, *J* = 24.3, 14.4, 2.1 Hz, 2H), 1.89 (tdt, *J* = 7.9, 4.8, 2.4 Hz, 2H), 1.62 – 1.35 (m, 4H), 1.02 – 0.88 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.5, 170.3, 141.5, 130.5, 120.7, 118.6, 76.3, 75.6, 58.3, 48.9, 41.5, 40.2, 39.2, 38.5, 35.4, 33.8, 25.3, 22.6, 18.1. HR-MS (ESI) m/z: calcd for $C_{19}H_{24}O_5Na^+$ $[M + Na]^+$ 355.1516, found 355.1518.

*(3aR,4aR,5R,6R,8aR,9aR)-8a-Methyl-3-methylene-2-oxodecahydro-2H-spiro[naphtho[2,3-b]furan-5,2'-oxiran]-6-yl cyclohex-1-ene-1-carboxylate (**41**).* Following the

procedure described for preparation of compound **13**, compound **41** was prepared from compound **6** as a colorless oil. (Yield 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.97 (td, *J* = 3.9, 1.9 Hz, 1H), 6.05 (d, *J* = 1.1 Hz, 1H), 5.51 (d, *J* = 1.0 Hz, 1H), 4.60 – 4.54 (m, 1H), 4.46 (td, *J* = 4.9, 1.6 Hz, 1H), 2.94 (ddd, *J* = 11.9, 6.8, 5.1 Hz, 1H), 2.71 (d, *J* = 4.2 Hz, 1H), 2.62 (d, *J* = 4.2 Hz, 1H), 2.28 – 2.09 (m, 6H), 1.93 – 1.78 (m, 2H), 1.67 – 1.51 (m, 6H), 1.48 – 1.36 (m, 2H), 0.99 – 0.85 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.3, 166.3, 141.6, 140.1, 130.4, 120.7, 76.4, 74.9, 58.6, 49.0, 41.6, 40.2, 38.9, 35.7, 33.8, 25.8, 25.5, 24.2, 22.6, 22.0, 21.4, 18.1. HR-MS (ESI) m/z: calcd for $C_{22}H_{28}O_5Na^+$ $[M + Na]^+$ 395.1829, found 395.1830.

*(1aR,2S,5aR,6aR,9S,9aR,9bS)-9-((Dimethylamino)methyl)-2,5a-dimethyloctahydro-2H-oxireno[2',3':4,4a]naphtho[2,3-b]furan-8(9H)-one fumarate (**42**).* To a solution of compound **3** (1.20 g, 4.40 mmol) in CH_2Cl_2 (100 mL) was added dimethylamine hydrochloride (5.40 g, 66.0 mmol) and K_2CO_3 (18.0 g, 0.13 mmol). The reaction mixture was stirred at 40 ℃ for 3 h. Then the mixture was filtered off, and the filtrate was washed with H_2O, dried over Na_2SO_4, and evaporated under reduced pressure. The crude product was dissolved in MeOH (10 mL). Then fumarate (500 mg, 4.31 mmol) was added to the solution. The reaction mixture was stirred at room temperature for 30 min. The white solid in the mixture was filtered off, and washed with EtOAc to obtain compound **42** (white solid, 1.45 g, 80%). M.p. 159-162℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 6.55 (s, 2H), 4.59 (dt, *J* = 6.9, 3.0 Hz, 1H), 3.41 (ddd, *J* = 12.2, 10.5, 4.3 Hz, 1H), 3.19 (s, 1H), 3.11 – 3.04 (m, 1H), 2.66 (t, *J* = 12.5 Hz, 1H), 2.51 – 2.49 (m, 2H), 2.25 (s, 6H), 1.87 – 1.55 (m, 3H), 1.51 – 1.17 (m, 7H), 1.09 – 1.08 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 177.1, 167.6, 135.1, 75.5, 67.6, 57.1, 56.3, 45.1, 39.0, 38.0, 37.9, 35.7, 34.5, 32.1, 29.8, 24.2, 17.9, 16.6. HR-MS (ESI) m/z: calcd for $C_{17}H_{28}NO_3^+$ $[M+H]^+$ 294.2064 found 294.2064.

*(3S,3aR,4aR,6R,8aR,9aR)-3-((Dimethylamino)methyl)-6-hydroxy-8a-methyl-5-methylenedecahydronaphtho[2,3-b]furan-2(3H)-one fumarate (**43**).* Following the procedure described for preparation of compound **42**, compound **43** was prepared from compound **4** as a white solid. (Yield 58%). M.p. 168-170 ℃. ^{1}H NMR (400 MHz,

DMSO-d_6) δ (ppm) 6.57 (s, 2H), 4.88 (s, 1H), 4.67 – 4.50 (m, 2H), 4.11 (d, *J* = 3.0 Hz, 1H), 3.32 – 3.19 (m, 1H), 2.94 – 2.74 (m, 1H), 2.71 – 2.60 (m, 1H), 2.59 – 2.48 (m, 2H), 2.42 – 2.28 (m, 7H), 2.07 – 1.87 (m, 1H), 1.76 – 1.36 (m, 5H), 1.30 – 1.07 (m, 1H), 0.95 (d, *J* = 12.9 Hz, 1H), 0.67 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 177.5, 167.2, 152.2, 134.9, 108.2, 78.2, 71.8, 53.8, 44.7, 44.6, 41.0, 40.6, 39.4, 38.9, 35.9, 34.8, 29.9, 20.7, 17.5. HR-MS (ESI) m/z: calcd for $C_{17}H_{28}NO_3^+$ $[M+H]^+$ 294.2064 found 294.2065.

*(3S,3aR,4aR,5R,8aR,9aR)-3-((Dimethylamino)methyl)-4a,8a-dimethyldecahydro-2H-spiro[naphtho[2,3-b]furan-5,2'-oxiran]-2-one fumarate (**44**).* Following the procedure described for preparation of compound **42**, compound **44** was prepared from compound **6** as a white solid. (Yield 72%). M.p. 179-180 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.58 (s, 2H), 4.51 (s, 1H), 3.19 (d, *J* = 2.7 Hz, 2H), 2.80 – 2.61 (m, 2H), 2.60 – 2.39 (m, 6H), 2.30 (m, 6H), 2.09 (d, *J* = 11.4 Hz, 1H), 2.02 – 1.92 (m, 1H), 1.74 (t, *J* = 14.6 Hz, 1H), 1.64 – 1.46 (m, 3H), 1.31 (dd, *J* = 12.3, 4.7 Hz, 1H), 1.19 (dd, *J* = 9.8, 7.1 Hz, 1H), 0.83 (s, 3H), 0.64 (q, *J* = 12.9 Hz, 1H).^{13}C NMR (100 MHz, DMSO-d_6) δ 177.5, 167.1, 134.8, 78.0, 71.5, 61.3, 53.9, 48.5, 44.9, 44.5, 41.7, 38.8, 37.2, 35.1, 34.7, 28.1, 18.4, 16.0. HR-MS (ESI) m/z: calcd for $C_{17}H_{28}NO_4^+$ $[M+H]^+$ 310.2013 found 310.2012.

*(3S,3aR,4aR,5R,6R,8aR,9aR)-3-((Dimethylamino)methyl)-8a-methyl-2-oxodecahydro-2H-spiro[naphtho[2,3-b]furan-5,2'-oxiran]-6-yl ethyl fumarate fumarate (**45**).* Following the procedure described for preparation of compound **42**, compound **45** was prepared from compound **38** as a white solid. (Yield 78%). M.p. 159-161 ℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 6.82 (d, *J* = 2.6 Hz, 2H), 6.59 (s, 2H), 4.59 (d, *J* = 2.7 Hz, 1H), 4.54 (d, *J* = 4.4 Hz, 1H), 4.22 (q, *J* = 7.1 Hz, 2H), 3.26 (dt, *J* = 10.3, 5.2 Hz, 1H), 2.90 (d, *J* = 4.4 Hz, 1H), 2.83 – 2.72 (m, 2H), 2.65 (dd, *J* = 13.0, 4.5 Hz, 1H), 2.54 (d, *J* = 5.6 Hz, 1H), 2.37 (s, 6H), 2.17 – 2.10 (m, 1H), 2.03 – 1.89 (m, 2H), 1.84 – 1.72 (m, 1H), 1.62 (dd, *J* = 15.5, 4.3 Hz, 1H), 1.49 (td, *J* = 13.8, 3.9 Hz, 1H), 1.34 (d, *J* = 13.1 Hz, 2H), 1.25 (t, *J* = 7.1 Hz, 3H), 0.88 (s, 3H), 0.70 (q, *J* = 12.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 176.8, 166.4, 164.3, 163.4, 134.2, 133.5, 133.2, 77.4, 76.5,

61.1, 58.3, 53.2, 48.3, 44.1, 43.8, 40.7, 38.1, 38.0, 34.8, 33.9, 24.9, 17.8, 15.6, 14.0. HR-MS (ESI) m/z: calcd for $C_{23}H_{34}NO_7^+$ $[M+H]^+$ 436.2330 found 436.2329.

*(3S,3aR,4aR,5R,6R,8aR,9aR)-3-((Dimethylamino)methyl)-8a-methyl-2-oxodecahydro-2H-spiro[naphtho[2,3-b]furan-5,2'-oxiran]-6-yl cyclohex-1-ene-1-carboxylate fumarate (**46**).* Following the procedure described for preparation of compound **42**, compound **46** was prepared from compound **41** as a white solid. (Yield 89%). M.p. 160-162℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 7.10 – 6.95 (m, 1H), 6.58 (s, 3H), 4.51 (dd, J = 12.6, 3.7 Hz, 2H), 3.24 (dt, J = 10.6, 5.3 Hz, 1H), 2.88 (d, J = 4.4 Hz, 1H), 2.82 – 2.68 (m, 2H), 2.65 – 2.58 (m, 1H), 2.34 (s, 6H), 2.19 (d, J = 4.9 Hz, 4H), 2.11 – 1.97 (m, 2H), 1.89 (t, J = 14.6 Hz, 1H), 1.78 – 1.68 (m, 1H), 1.66 – 1.51 (m, 5H), 1.48 – 1.28 (m, 3H), 0.88 (s, 3H), 0.70 (q, J = 12.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 177.3, 167.0, 165.9, 140.5, 134.8, 130.3, 77.9, 75.3, 59.0, 53.8, 48.7, 44.7, 44.3, 41.4, 38.9, 38.6, 35.6, 34.4, 25.7, 25.6, 24.3, 22.1, 21.4, 18.9, 16.1. HR-MS (ESI) m/z: calcd for $C_{24}H_{36}NO_5^+$ $[M+H]^+$ 418.2588 found 418.2589.

Cell culture

Mouse lung fibroblast cell line Mlg, human lung fibroblast cell line HFL1 and $(CAGA)_{12}$-Lux reporter stable transfected NIH 3T3 cells (kindly supplied by Professor Wen Ning, Nankai University) were cultured in DMEM medium (KeyGEN BioTECH, Nan Jing，China) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and antibiotics (100 μg/ml streptomycin, and 100 U/ml penicillin G) in a 37℃ atmosphere of 95% humidified air and 5% CO_2.

BLM-induced animal model of pulmonary fibrosis

Thirty 7-8 weeks male C57BL/6 mice were purchased from Charles River (Bei Jing, China). All mice were housed and cared for in a pathogen-free facility at Nankai University. The mice were acclimatized in a room with constant temperature(25±2℃) and relative humidity (60±2%) and allowed free access to food and water. All animal experiments were approved by the Animal Care and Use Committee at Nankai University. The mice were randomly divided into 6 groups (n=5 per group): control group, BLM group, Pirfenidone-treated group (100mg/kg), compound 42-treated

group (100mg/kg), compound 44-treated group (100mg/kg), compound 45-treated group (100mg/kg). For BLM administration, mice were anesthetized with 10% chloral hydrate (Sangon) and then intratracheally injected with bleomycin (Medicine co., Tokyo, Japan) at a dose of 2U / kg body weight for analysis of the fibrotic response. The sham-operated group received intratracheal injections of the same amount of saline. The drug administration group began to be administered daily from the seventh day of modeling to 14th day. Mice were sacrificed at Day 14 and lung tissue was harvested for the following experiments to evaluate the degree of pulmonary fibrosis.

Luciferase Assay

The logarithmic growth phase CAGA-NIH 3T3 cell were plated in a 96-well plate at a density of 0.5×10^5cells /ml in a 100μL suspension and cultured overnight. Cells were treated with 5 ng/ml TGF-β1 with/without compounds (10μM or 5μM) in DMEM containing 0.1% FBS. After co-incubated for 18h, cells were harvested and the luciferase activity of cell lysates was determined by a luciferase assay system (Promega) as described by the manufacturer. Total light emission during the initial 20 s of the reaction was measured in a luminometer (Lumat LB 9501; Berthold). All assays were repeated in triplicate.

MTT Assay

The logarithmic growth phase NIH 3T3 cell were plated in a 96-well plate at a density of 0.5×10^5cells /ml in a 100μl suspension and cultured overnight and the experimental group were added different concentrations of drugs with/without TGF-β1 (5ng/ml), the concentration set to 0μM, 5μM, 10μM, 20μM, 40μM, 80μM. After 24h, 20 μl of 5×MTT solution was added to each well and incubated for 4 hours in the incubator. Then each well was added 150μl DMSO. Finally, the absorbance (A value) of each well was measured on a microplate reader. Survival rate =Administration A value - Zero A value) / (Blank A value - Zero A value) ×100%. All assays were repeated in triplicate.

Wound-Healing Assay

Mlg cells were grown on a 35 mm dish to 100% confluence and then scratched to

form a 100-μm wound using sterile pipette tips. The cells were then cultured in the presence or absence of TGF-β1 (5ng/ml) and compounds (10μM) in serum-free media for 24 h. Images of the cells were taken at 12 and 24 h using a light microscope (Nikon, Japan).

Western Blot Analysis

The samples of lung tissues and cells were homogenized in RIPA lysis (Beyotime Biotechnology, Shanghai, China) buffer with PMSF or NaF (phosphatase inhibitor; need to add when extracting the phosphorylating protein), then centrifuged (10000rpm,10min) to obtain supernatants. The total protein concentration was measured by BCA Protein Assay kit (Beyotime Biotechnology, Shanghai, China). The primary antibodies were as follows: mouse anti-α-SMA antibody (1:1000dilution, Protein-Tech, China), rabbit anti-fibronectin antibody (1:1000 dilution, Affinity, China), rabbit anti-collagen I antibody (1:1000 dilution, Affinity, China), rabbit anti-p-Smad3 (1:1000 dilution, Cell Signaling, China), and mouse anti-β-actin antibody (1:5000 dilution, Cell Signaling, China). HRP-labeled Goat Anti-Rabbit IgG (H+L) (1:5000 dilution) and HRP-labeled Goat Anti-Mouse IgG (H+L) (1:5000 dilution) antibodies were used as the secondary antibodies. Relative density of each band was analyzed by Image J.

Quantitative real-time PCR (qRT-PCR)

The total RNA was extracted using TRIzol Reagent. The cDNA was obtained from total RNA through reverse transcribed. qRT-PCR was performed by using SYBR GreenER qPCR SuperMix Universal (Invitrogen) according to the manufacturer's protocols. The relative quantification of gene expression (*α-SMA*, *collagen I*, *fibronectin*) was measured relative to the endogenous reference gene *β-actin* using the comparative CT method in the experiment. Sequences of the specific primer sets are as follows: *α-SMA* (NM_007392.2), 5-GCTGGTGATGATG- CTCCCA-3 and 5-GCCCATTCCAACCATTACTCC-3; *Col1a1* (NM_007742.3), 5-CCAAGAAGACATCCCTGAAGTCA-3 and 5-TGCACGTCAT- CGCACACA-3; *Fn1* (NM_010233.1), 5-GTGTAGCACAACTTCCAATTACGAA-3 and 5-GGAATTTCCGCCTCGAGTCT-3; *β-actin* (NM_007393.3),

5-AGGCCAACCGTGAAAAGATG-3 and 5-AGAG- CATAGCCCTCGTAGATGG-3;

Hematoxylin-Eosin staining (HE staining)

Left lungs were fixed in 10% formalin for 24 h and embedded in paraffin. Then lung sections (5μm) were prepared and stained with hematoxylin-eosin staining. HE staining images were collected using an upright transmission fluorescence microscope and opened in Image-Pro Plus Version 6.0 (Media Cybernetics, Inc. American). The software selection tool can select the entire lung tissue area and automatically calculate the total pixel (Pw) of the region, and then use the same method to calculate the total pixel (Pf) of the fibrosis region, fibrosis ratio=fibrosis area pixel (Pf) /total lung pixel (Pw).

Immunohistochemistry staining

Paraffin-embedded lung tissue was dewaxed with xylene, and the sections were immersed in a microwave oven with antigen-fixing solution (0.01 M citrate buffer) and repaired for 20 min. After cooling to room temperature, blocking with 5% fetal bovine serum (BSA) for 20min, then incubated with the primary antibody at 4℃ overnight. The primary antibodies were as follows: mouse anti-α-SMA antibody (1:200 dilution Protein-Tech, China), rabbit anti-collagen I antibody (1:100 dilution, Affinity, China), rabbit anti-p-Smad3 (1:50 dilution, Cell Signaling, China). After incubated with primary antibodies overnight, the tissue sections were washed with TBST for three times and incubated with the secondary antibody at room temperature for 1h. Subsequently, tissue sections were stained with hematoxylin to observe histological changes and the target gene expression in lung tissues were analyzed under microscope.

Hydroxyproline Assay

The collagen contents in right lungs of mice were measured with a conventional hydroxyproline method. Briefly, the right lungs were dried and acid hydrolyzed, then the residue was filtered and the PH value was adjusted to 6.5-8.0. The hydroxyproline analysis was performed using chloramine-T spectrophotometric absorbance as previously describe. [26]

Plasma stability studies. A 7.5 μL volume of compound 42 or 44 solution (1 mM) was

placed in 750 µL mouse blank plasma solution. The tubes were then incubated in a bath incubator at 37 °C. 50 µL plasma samples were combined with 150 µL internal standard (buspirone 10 ng/mL) in acetonitrile/MeOH (1:1, v/v), and centrifuged at 15000 rpm for 10 min at 4 °C. The supernatant was collected. The plasma concentration of compound **2**, **6**, **42** and **44** was determined by LC/MS-MS. Summary of LC/MS-MS method was provided in supporting information.

PK Study. Sprague-Dawley male rats (220-350 g) were purchased from Academy of Military Medical Sciences (Beijing, China). For a single dose PK study of compounds **42** and **44**, 12 rats were divided into four groups: (A) IV of compound **42** (30 mg/kg), (B) PO of compound **42** (150 mg/kg); (C) IV of compound **44** (30 mg/kg), (D) PO of compound **44** (150 mg/kg). For IV study, 200 µL blood was collected from different rats at 2 min, 5 min, 10 min, 20 min, 30 min, 1 h, 4 h, 6 h and 8 h. For PO study, 200 uL blood was collected from different rats at 5 min, 15 min, 30 min, 1 h, 4 h, 6 h, 8 h and 12 h. Blood samples were treated with heparin to prevent coagulation. Blood samples were centrifuged for 10 min at 13000 rpm in a desktop centrifuge to collect plasma. 50 µL plasma samples were combined with 200 µL internal standard (buspirone 100 ng/mL) in acetonitrile/MeOH (1:1, v/v), and centrifuged at 15000 rpm for 10 min at 4 °C. The supernatant (100 µL) was collected and mixed with 5 part of 50% MeOH/water. Plasma drug concentration was determined by LC/MS-MS. Summary of LC/MS-MS method was provided in supporting information. The processed plasma concentration-time data were analyzed using non-compartmental analysis.

Toxicity Study. C57BL6 mice of 20-22 grams Academy of Military Medical Sciences (Beijing, China). Mouse were administrated with graded concentrations of aqueous solutions of compound **42** and **44**.

ASSOCIATED CONTENT

Supporting Information

LC/MS-MS method and NMR spectrums of compounds **3-46**. This material is available free of charge

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Notes

The authors declare no competing financial interest.

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HIGHTLIGHTS

41 Sesquiterpene Lactone analogues derived from alantolactone and Isoalantolactone were synthesised.

The analogues were screened by a high-throughput TGF-β1 reporter luciferase assay.

An Anti-IPF drug candidate was discovered.