SUPPLEMENT MATERIAL AND METHODS

Cell culture

The human lung fibroblasts (HFL-1) [German Collection of Microorganisms and Cell Cultures (DSMZ)] were maintained in DMEM containing 10% FCS and cultured in a humidified atmosphere of 5% CO_2 at 37 °C. For stimulation experiments cell were serum-starved for 24 h before treatment. Cells were treated with TGF-β1 (2 ng/ml) or WNT3a (100ng/ml) for 12 h or 24 h as indicated, and collagen content was determined by qRT-PCR and Sircol collagen assay.

Reverse transcription and quantitative real-time PCR

Total RNA extraction, cDNA synthesis, and quantitative (q)RT-PCR were performed using the primers listed in Table S2. Under identical cycling conditions, all primer sets worked with similar efficiencies to obtain simultaneous amplification in the same run, as described before[24]. Sequences were taken from GeneBank, all accession numbers are denoted. Hydroxymethylbilane synthase (HMBS) and hypoxanthine phosphoribosyltransferase 1 (HPRT1) for mouse and human, respectively and both ubiquitously and equally expressed genes that are free of pseudogenes, were used as reference genes in all qRT-PCR reactions. Relative transcript abundance is expressed as ΔCt value ($\Delta Ct = Ct^{reference} - Ct^{target}$), where higher ΔCt values indicate higher transcript abundances, and negative ΔCt values represent genes that are less expressed compared with the reference gene. The fold-change of the transcript levels in IPF/NSIP *versus* control can be estimated by $2^{\Delta\Delta Ct}$, where ΔΔCt values are calculated as $\Delta Ct^{IPF/NISP} - \Delta Ct^{control}$. Positive $2^{\Delta\Delta Ct}$ values indicate upregulation, negative values indicate downregulation of a target gene.

Collagen assay

Total collagen content was determined using the Sircol Collagen Assay kit (Biocolor). Equal amounts of protein lysates were added to 1 ml of Sircol dye reagent, followed by 30 min of mixing. After centrifugation at 10,000 × g for 10 min, the supernatant was carefully aspirated and 1 ml of alkali reagent was added. Samples and collagen standards were then read at 540 nm in a spectrophotometer (Bio-Rad). Collagen concentrations were calculated using a standard curve with acid-soluble type 1 collagen.

Determination of terguride levels in the mouse

Terguride levels were determined in mouse plasma and lung tissue samples by liquid chromatography-mass spectrometry (LC-MS). In brief, two lung samples each were pooled and homogenised in phosphate buffer (100 mmol/L). A 200µl sample of lung homogenate or plasma was then precipitated with acetonitrile (400µl) and centrifuged. The supernatant was dried in a vacuum centrifuge, resuspended in phosphate buffer (200µl), and quantitated by LC-MS using terguride standards, which were prepared on the respective matrices used fro LC-MS. Proterguride was applied as an internal standard. LC-MS was carried out on a Waters 2795 Alliance Quattro Micro (Micromass/Waters) using a Luna 3µC18 Phenomenex column with a column temperature of 30°C. A gradient with a trinary mobile phase system consisting of water and 0.1% formic acid (mobile phase A), acetonitrile (mobile phase B), and 2% formic acid in water (mobile phase C) at a flow rate of 0.4 ml/min was applied.

Lung function

Compliance was measured invasively in anaesthetized animals with the use of a commercially available system (Hugo Sachs Elektronik-Harvard apparatus, March-Hugstetten, Germany). Briefly, animals were anaesthetized by intraperitoneal injection with ketamine/xylazine (20 µl ketamine/20 µl xylazine/40 µl NaCl). After deep anaesthesia was achieved, animals were

tracheotomised, intubated and artificially ventilated with room air using the above mentioned system. Lung compliance was measured for the next at least ten minutes, after which animals were euthanized by a dose of pentobarbital (100-150 mg/kg body weight). All lung tissues were excised and snap frozen, or placed in 4% (w/v) paraformaldehyde and processed for paraffin embedding.

Assessment of lung fibrosis

Lung paraffin sections were stained with hematoxylin and eosin. For the quantitative histological analysis, a numeric fibrotic scale was used (Ashcroft score)[26]. Briefly, the grade of lung fibrosis was scored on a scale from 0 to 8 by examining more than 25 successive fields at a magnification of ×100 in a blinded fashion. The mean of all scores obtained from each field was employed as the fibrotic score of the specimen. To avoid observer bias, two experienced observers interpreted the images independently in a blinded fashion, and the mean of the observers' findings was considered to be the fibrotic score of the specimen.

Hydroxyproline assay

The whole collagen content of lungs was assessed by determining the hydroxyproline levels using HPLC. Briefly, lungs were homogenized in PBS, dried, and hydrolyzed in 6 N HCl at 110 °C for 24 h. Aliquots were added to 1.4% chloramine T (Sigma), 10% n-propanol, and 0.5 M sodium acetate, pH 6.0. After 20 min of incubation at RT, 1 M p-dimethylaminobenzaldehyde (Sigma) in 70% n-propanol / 20% perchloric acid was added and the sample incubated at 65°C. The absorbance was measured at 550 nm and the amount of hydroxyproline in each sample calculated against a standard curve.

SUPPLEMENT TABLES

Table S1. Characteristics of patients. VC = vital capacity, TLC = total lung capacity, DL_{CO}/VA = diffusing capacity of the lung for CO per unit of alveolar volume (all in % predicted), $Pa_{O2/CO2}$ = partial pressure of O_2 /CO_2 in the arterial blood.

No.	Diagnosis	Gender	Age (yr)	VC (%)	DL_{CO}/VA (%)	TLC (%)	O_2 (l/min)	Pa_{O2} (mmHg)	Pa_{CO2} (mmHg)
1	IPF (UIP)	male	63	56%	33%	48%	3	52	33
2	IPF (UIP)	male	62	50%	26%	52%	3	49	38
3	IPF (UIP)	male	65	59%	20%	42%	3	53	38
4	IPF (UIP)	male	65	59%	20%	42%	4	69	41
5	IPF (UIP)	male	43	48%	27%	51%	na	na	na
6	IPF (UIP)	male	64	59%	22%	52%	2	58	38
7	IPF (UIP)	male	65	51%	20%	66%	2	53	38
8	IPF (UIP)	male	44	47%	25%	55%	2	36	35
9	IPF (UIP)	female	43	40%	na	na	2	54	35
10	IPF (UIP)	female	42	50%	17%	58%	3	52	36
11	IPF (UIP)	female	66	29%	23%	45%	4	56	45
12	IPF (UIP)	female	62	27%	na	48%	4	71	65
1	NSIP	male	60	36%	27%	41%	6	51	45
2	NSIP	male	57	35%	na	43%	8	53	59
3	NSIP	male	35	35%	28%	41%	na	na	na
4	NSIP	male	63	na	na	na	4	56	36
5	NSIP	female	47	41%	36%	50%	na	na	na
6	NSIP	female	66	33%	30%	45%	6	56	43

Table S2. Primer sequences and amplicon sizes for human and mouse tissues.

Gene	Accession		Sequences (5´ → 3´)	Length	Amplicon
5-HTR1A	NM000524	for	AGG CTG GTC CTA CCC CTT GT	20 bp	59bp
		rev	CGG CGT TGC GCT CAT T	16 bp	
5-HTR1B	NM000863	for	GGG CCA GGT GGT CTG TGA	18 bp	58bp
		rev	TGG AGG CAG TGC AAC AAG TG	20 bp	
5-HTR2A	NM000621	for	AGC TGA TAT GCT GCT GGG TTT C	22 bp	69bp
		rev	CCA CCG GTA CCC ATA CAG GAT	21 bp	
5-HTR2B	NM000867	for	CTC ACG GGC TAC AGC ATT CAT	21 bp	98bp
		rev	TCC ACA TCA GTC TCT ATC CCT TTA ATA G	28 bp	
5-HTR2C	NM000868	for	GCC AAC TGA CGC CAT CCT	18 bp	131bp
		rev	ACC GCA TTC CTC AGG TTC AC	20 bp	
5-HTT	NM001045	for	GAA ACC CAA TTG GCA GAA ACT C	22 bp	145bp
		rev	GGG CAT CTT GGT AGC AGT TGT T	22 bp	
HPRT	NM000194	for	AAG GAC CCC ACG AAG TGT TG	20 bp	157bp
		rev	GGC TTT GTA TTT TGC TTT TCC A	22 bp	
5-Htr1a	NM008308	for	CCCAATTCTTCACGATGGAAGT	22bp	91bp
		rev	GGCATGGTAGATGTCCATACAGTTT	25bp	
		for	CACGCTCTCCAACGCCTTT	19 bp	

		rev	ACTGCCAGAGAGGCGATCAG	21 bp	
5-Htr2a	NM172812	for	TTGTCATGCCCGTGTCCAT	19 bp	97bp
		rev	AAGAGCACATCCAGGTAAATCCA	23 bp	
5-Htr2b	NM008312	for	ACAGGACGGCTGGCTTAGG	19 bp	128bp
		rev	TCTCGAAGATGGGACTGTGTACAC	24 bp	
5-Htr2c	NM008312	for	TGCTGATATGCTGGTGGGACTA	22 bp	149bp
		rev	CGCAGAGGTGCATGATGGA	19 bp	
5-Htt	NM010484	for	AGCGACGTGAAGGAAATGCT	20 bp	98bp
		rev	CTGCAAATGATGAACAGGAGAAAC	24 bp	
Hmbs	NM013551	for	ATG TCC GGT AAC GGC GGC	18bp	135bp
		rev	GGT ACA AGG CTT TCA GCA TCG C	22bp	

SUPPLEMENT FIGURE LEGENDS

Figure S1

The mRNA expression profile of 5-HT-receptors (5-HTR) and the serotonin transporter 5-HTT in lung samples from bleomycin- or saline-treated mice 7 or 14 days after injury, as indicated. The mRNA levels of 5-$HTR_{1a,1b}$, 5-HTR_{2a-c} , and the serotonin transporter 5-HTT were assessed by quantitative real-time PCR (qRT-PCR). Results are derived from n = 4 per group and presented as fold change compared with the respective saline control, * $p < 0.05$.

Figure S2

Expression and localization of 5-HTR_{2B} in lung tissues. Additional immunohistochemical stainings of donor and IPF patients using the HPA012867 antibody, as described in detail in Material and Methods. Scale bars indicate 100µm.

Figure S3

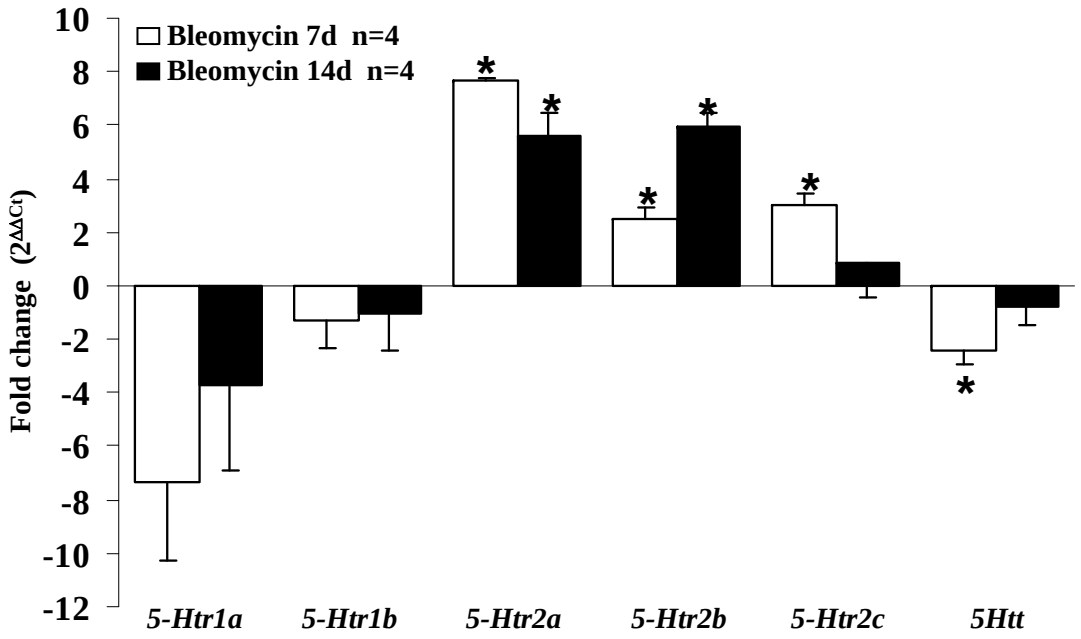
Application of the 5-$HTR_{2A/B}$ antagonist terguride *in vivo*. (A) The chemical structure of terguride. (B) Mice were subjected to a single inhalative instillation of bleomycin. After 7, 14, or 21 days, the 5-$HTR_{2A/B}$ antagonist terguride was applied intraperitoneally (i.p.) and the concentration of terguride in plasma and lung tissues were determined. (C) Body weight was assessed at the indicated time points.

Figure S4

The treatment scheme for the therapeutic approach *in vivo*. Mice were subjected to a single inhalative application of bleomycin, which led to the development of lung fibrosis by day 14 - 21 after an initial inflammatory phase (day 3 - 7). Treatment with terguride was initiated 14 days after bleomycin instillation, using the indicated concentrations, via intraperitonal (i.p.) application twice daily until day 28. As an internal control, a treatment arm with daily

administration of 50 mg/kg BW imatinib was included in the study. Treatment of imatinib was started immediately following instillation of bleomycin (n=10 for each group).

Figure S1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Bleomycin 14d n=4
Fold change (2^ΔΔCt)
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8
6
4
2
0
-2
-4
-6
-8
-10
-12
5-Htr1a
5-Htr1b
5-Htr2a
5-Htr2b
5-Htr2c
5Htt

Figure S2

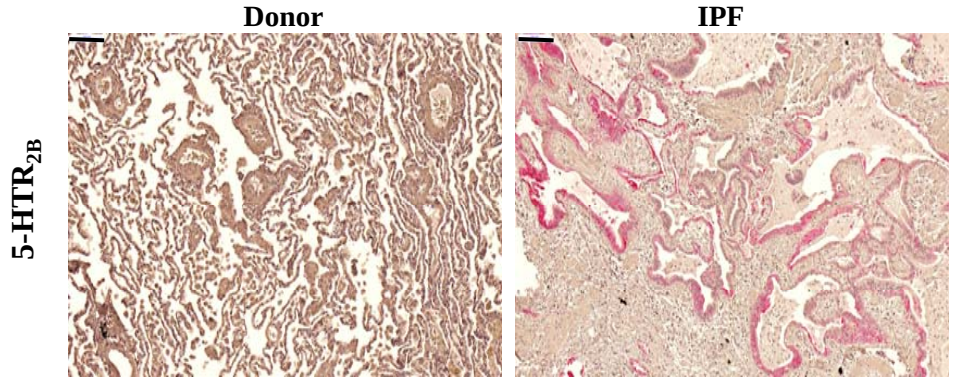
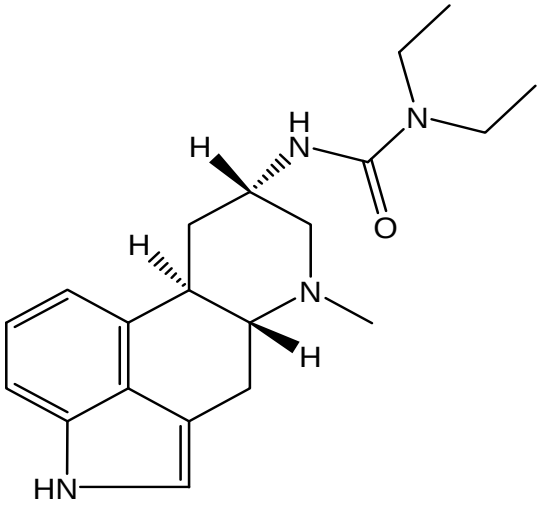


Figure S3

A



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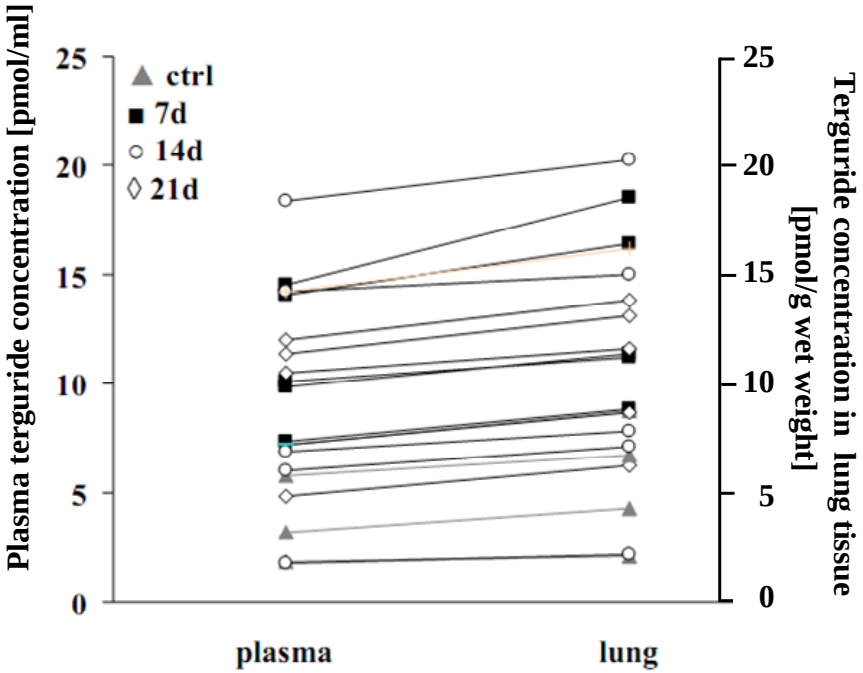

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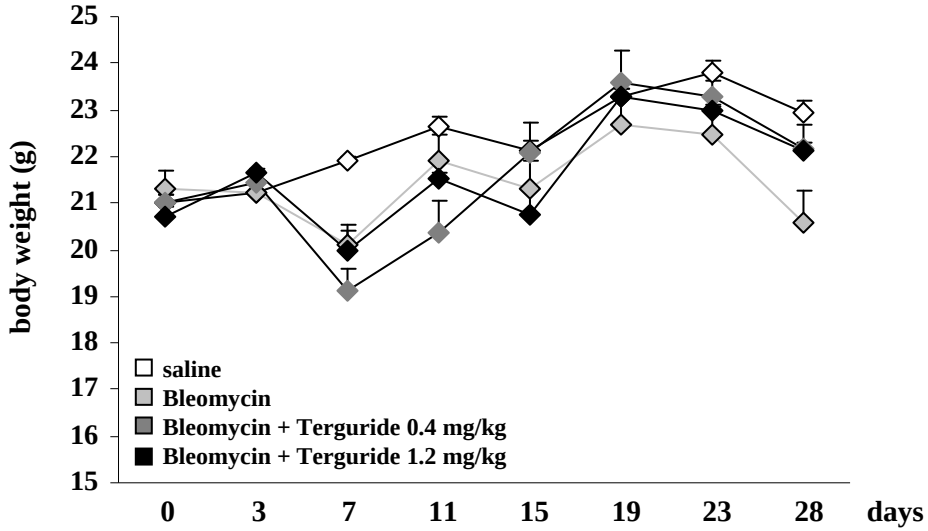
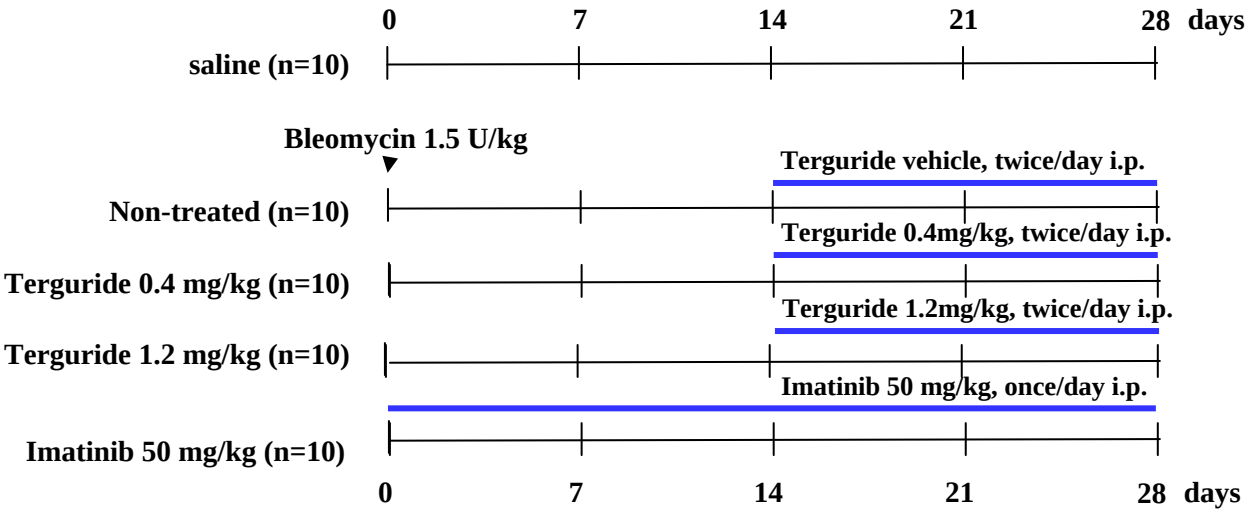


Figure S4


0
7
14
21
28
days
saline (n=10)
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Terguride vehicle, twice/day i.p.
Non-treated (n=10)
Terguride 0.4mg/kg, twice/day i.p.
Terguride 0.4 mg/kg (n=10)
Terguride 1.2mg/kg, twice/day i.p.
Terguride 1.2 mg/kg (n=10)
Imatinib 50 mg/kg, once/day i.p.
Imatinib 50 mg/kg (n=10)
0
7
14
21
28
days