Bioorganic & Medicinal Chemistry Letters xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Pharmacokinetic optimitzation of CCG-203971: Novel inhibitors of the Rho/MRTF/SRF transcriptional pathway as potential antifibrotic therapeutics for systemic scleroderma

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ARTICLE INFO

Article history:
Received 10 February 2017
Revised 24 February 2017
Accepted 26 February 2017
Available online xxxx

Keywords:
Scleroderma
Fibrosis
Antifibrotic
Myofibroblast
Rho
MRTF

ABSTRACT

We recently reported the development of a novel inhibitor of Rho-mediated gene transcription (**1**, CCG-203971) that is efficacious in multiple animal models of acute fibrosis, including scleroderma, when given intraperitoneally. The modest in vivo potency and poor pharmacokinetics (PK) of this lead, however, make it unsuitable for long term efficacy studies. We therefore undertook a systematic medicinal chemistry effort to improve both the metabolic stability and the solubility of **1**, resulting in the identification of two analogs achieving over 10-fold increases in plasma exposures in mice. We subsequently showed that one of these analogs (**8f**, CCG-232601) could inhibit the development of bleomycin-induced dermal fibrosis in mice when administered orally at 50 mg/kg, an effect that was comparable to what we had observed earlier with **1** at a 4-fold higher IP dose.

It has been estimated that fibrotic diseases are responsible for up to 40% of all deaths worldwide.[1] Although the progression and pathology of fibrosis are well described (relentless and excessive accumulation of collagen-rich extracellular matrix (ECM)), the precise etiology of the disease remains obscure. Systemic sclerosis (scleroderma, SSc) is a fibrotic disease of the connective tissues that involves both the vascular and immune systems. The hallmark of SSc, and in fact of all fibrotic disease, is the transition of normal fibroblasts into myofibroblasts, which are characterized by the expression of alpha smooth muscle actin (α-SMA) and the production of ECM.[2] SSc-associated fibrosis is currently treated with immunosuppressants, which are of limited efficacy, have untoward side effects, and do not address the underlying pathology.[3] Recent research suggests that targeting fibroblast activation is likely to be a more effective strategy.[4]

Evidence is growing that fibroblast activation to myofibroblasts results from gene transcription stimulated by a common Rho-mediated signaling pathway that originates from divergent extracellular pro-fibrotic stimuli.[5,6] Specifically, Rho mediates the conversion of G-actin to F-actin, which releases G-actin-bound myocardin-related transcription factor (MRTF), resulting in accumulation of MRTF in the nucleus, where it binds to serum response factor (SRF) on the serum response element (SRE) promoter. This Rho/actin/MRTF/SRF pathway may in fact serve as a regulator of the fibrotic process used in wound healing,[7] but dysregulation and/or overstimulation can lead to fibrosis. Recently, MRTF-A expression in the nucleus of dermal fibroblasts from SSc patients was shown to be increased relative to normal.[8]

We have discovered and developed a novel class of inhibitors of the Rho/MRTF/SRF pathway.[9–11] A lead compound from this effort, CCG-203971 (**1**, Table 1), has exhibited promising anti-fibrotic activity both in vitro and in vivo in several animal models of disease, including intestinal fibrosis,[12] pulmonary fibrosis[13] and dermal fibrosis.[14] Despite these encouraging preliminary results,

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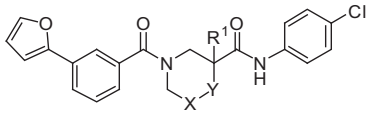
http://dx.doi.org/10.1016/j.bmcl.2017.02.070
0960-894X/

Table 1
Piperidine analogs.



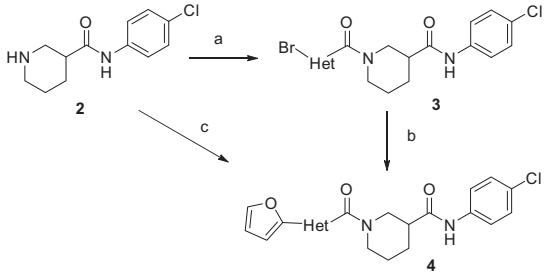
Cmpd	R^1	X	Y	SRE.L IC_{50} (μM)[a]	MLM $T_{1/2}$ (min)[b]	Sol (μg/mL)[c]	ACTA2 (%ctrl)[d]	ClogP[e]	TPSA[f]
1	H	CH_2	CH_2	0.64	1.6	13.4	37	4.25	62.6
8a	H	CF_2	CH_2	0.60	4.9	2.3	9.3	4.27	62.6
11a	H	CH_2	O	3.1				3.49	71.8
11b	Me	CH_2	CH_2	0.55	0.84			4.80	62.6
11c	F	CH_2	CH_2	0.80	2.8			4.39	62.6
12d	H	CH_2	NAc	21				2.78	82.9
13d	H	CH_2	NCO_2Me	2.7				3.40	92.1
22a	H	CHNHAc	CH_2	5.0				2.88	91.7
22b	H	$CHNHCO_2Me$	CH_2	5.0				3.50	100.9
23	H	$C{=}CH_2$	CH_2	0.098	1.4		9.5	4.34	62.6
24	H	$CHNMe_2$	CH_2	5.8	3.3			3.88	65.8
25	H	C=O	CH_2	3.9				3.43	79.6

[a] Inhibition of Gα12-stimulated SRE. Luciferase activity (mean of n ≥ 3) in HEK293T cells.
[b] Half-life in mouse liver microsomes.
[c] Thermodynamic solubility in water, determined by Analiza, Inc, using quantitative nitrogen detection.
[d] Inhibition of ACTA2 expression (percent control) in TGF-β-stimulated human dermal fibroblasts (10 μM, 24 h, mean of n = 3).
[e] Calculated log partition coefficient.
[f] Topological polar surface area ($Å^2$).

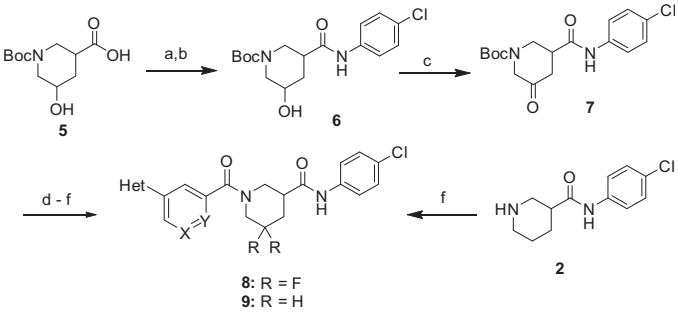
1 has modest potency and poor pharmacokinetic (PK) properties that would make it a poor candidate for further development. We report here our efforts to improve the solubility and metabolic stability of **1** to facilitate additional in vivo studies, and the successful identification of an analog exhibiting *oral* antifibrotic activity in a murine model of bleomycin-induced dermal fibrosis.

The half-life of **1** in mouse liver microsomes (MLM) is only 1.6 min (Table 1), indicating high susceptibility to oxidative metabolism. An analysis by SMARTCyp[15,16] predicted that the primary sites for metabolism are the furan (a known structural alert[17]) and the 5-carbon of the piperidine-3-carboxylic acid. Metabolite id experiments on several analogs indicated oxidation of the piperidine ring and the left-hand aromatic ring as well as hydrolysis of the secondary carboxamide as major pathways (Supplemental). Our medicinal chemistry strategy thus had three major objectives: 1) stabilize the 3-furanylphenyl moiety to oxidation through substitution or replacement of one or both of the rings with heterocycles; 2) block oxidation of the piperidine carbons sterically or through fluorination; and 3) explore alternatives to the labile secondary amide. In most cases these changes would reduce ClogP, which we expected would also improve the modest aqueous solubility of **1**.

Schemes 1 and 2 summarize the preparation of analogs that replace the 3-furanylphenyl of **1** with various heteroaromatics, and the inclusion of geminal difluoro on the piperidine ring. Piperidine carboxamide **2**[11] was coupled with commercially available 3-bromo-heteroaryl carboxylic acids to provide amides **3** which could be reacted with 2-furanylboronic acid under Suzuki conditions to give final amides **4** (Scheme 1). If the requisite furan-2-yl-heteroaryl carboxylic acids were commercially available, they could be coupled directly with **2**. Hydroxyacid **5** could be converted to anilide **6** in two steps (Scheme 2). The initial HATU coupling with 4-chloroaniline gave a mixture of lactone and hydroxyamides **6** that had to be further reacted under Weinreb conditions to drive to completion (Supplemental). Swern oxidation of the alcohol gave ketone **7** that was converted to the geminal difluoride with DAST. *N*-deprotection and amide coupling with diverse aromatic acids under standard conditions provided the difluoro analogs **8**. Coupling of unsubstituted piperidine amide **2** with various aromatic acids gave the simple nipecotic amide derivatives **9**.



Scheme 1. Reagents and Conditions: (a) EDC, DIPEA, DMAP, Br-Het-CO_2H, THF, 63%; (b) 2-furanylboronic acid, $Pd(PPh_3)_4$,Cs_2CO_3, Dioxane:H_2O (4:1), 80 °C, 62%; (c) EDC, DIPEA, DMAP, furan-2-yl-Het-CO_2H, THF, 15–82%.



Scheme 2. Reagents and Conditions: (a) 4-chloroaniline, HATU, Et_3N, DMF; (b) 4-chloroaniline, Me_3Al, CH_2Cl_2, 0 °C to RT, 74% over 2 steps; (c) $(ClCO)_2$, DMSO, TEA, CH_2Cl_2, 69%; (d) DAST, CH_2Cl_2 −78 °C to 0 °C, 23%; (e) TFA, CH_2Cl_2, 84%; (f) Ar-CO_2H, EDC, DIPEA, DMAP, THF, 46–98%.

The route to analogs incorporating heteroatoms into the piperidine ring or bearing substitution α to the carboxamide is