Discovery of 3,3'-Spiro[Azetidine]-2-oxo-indoline Derivatives as Fusion Inhibitors for Treatment of RSV Infection

Weihua Shi,[†] Zhigan Jiang,[†] Haiying He,^{*,†} Fubiao Xiao,[†] Fusen Lin,[†] Ya Sun,[†] Lijuan Hou,[†] Liang Shen,[†] Lixia Han,[†] Minggao Zeng,[†] Kunmin Lai,[†] Zhengxian Gu,[†] Xinsheng Chen,[†] Tao Zhao,[‡] Li Guo,[‡] Chun Yang,[‡] Jian Li,[†] and Shuhui Chen[†]

[†]WuXi AppTec (Shanghai) Co., Ltd, 288 FuTe Zhong Road, Shanghai 200131, P. R. China

[‡]Buchang Pharmaceutical, 50 Gaoxin Road, Gaoxin District, Xi'an 710075, Shanxi Province, P. R. China

Supporting Information

ABSTRACT: A new series of 3,3'-spirocyclic-2-oxo-indoline derivatives was synthesized and evaluated against respiratory syncytial virus (RSV) in a cell-based assay and animal model. Extensive structure–activity relationship study led to a lead compound **14h**, which exhibited excellent *in vitro* potency with an EC₅₀ value of 0.8 nM and demonstrated 71% oral bioavailability in mice. In a mouse challenge model of RSV infection, **14h** demonstrated superior efficacy with a 3.9log RSV virus load reduction in the lung following an oral dose of 50 mg/kg.

KEYWORDS: RSV, fusion inhibitor, benzimidazole

Respiratory syncytial virus (RSV) is a major cause of pneumonia and bronchiolitis in young children, immunocompromised adults, and the elderly, resulting in ~125,000 hospitalizations annually in the United States.^{1,2} However, the treatment of RSV lower respiratory tract infection (LRTI) is limited.³ Synagis (Palivizumab), a marketed humanized monoclonal antibody targeting fusion glycoprotein (F protein) of RSV, was licensed for passive prophylaxis intervention in high-risk infants. However, its high cost of treatment and relatively low protection to high-risk children limit its usage. An aerosol formulation of Ribavirin (Virazole) is the only chemotherapeutic agent on the market. Virazole is not a specific protection for young children and is rarely used due to its variable efficacy and toxicity.

Clearly, there is an unmet need for a new drug to prevent and treat RSV infection. In the past two decades, several chemical series of RSV fusion protein inhibitors have been reported (Figure 1), including the most advanced compound GS-5806,

Figure 1. RSV inhibitors explored in early stage clinical studies.

which is currently in Phase 2 study.^{4,5} Another Phase 2 compound is MDT-637 (formerly VP-14637) and is being developed as a dry powder inhaled product due to its low oral bioavailability. Both BMS-433771⁶ and TMC-353121⁷ were progressed into late preclinical/early clinical development but discontinued, mainly

due to the unfavorable pharmaceutical properties or early safety findings. The two RSV F protein inhibitors contain the same benzimidazole heterocycle. Therefore, we intend to address the drug-like properties of benzimidazole RSV fusion inhibitors especially improving activity *in vitro*, pharmacokinetics, and pharmacodynamics. Herein, we describe the identification of a promising candidate isopropyl 1'-((5-bromo-1-(4-hydroxybutyl)-1H-benzo[d]imidazol-2-yl)methyl)-2'-oxospiro[azetidine-3,3'-indoline]-1-carboxylate (**14h**) that was effective in a mouse challenge model of RSV infection. Meanwhile, the extensive

Scheme 1. General Synthesis of Pyridinoimidazolone Replacements Analogs

^aReagents and conditions: (i) 4-aminobutan-1-ol, Et₃N, dioxane, 90%; (ii) Pd/C, H₂, MeOH, 95%; (iii) 2-chloroacetic acid, 4 N HCl, 80 °C, –60%; (iv) for **5a–g**, **5j–p**, K₂CO₃, MeCN, 70 °C, or NaH, DMF, 20 °C; for **5h/i**, K₂CO₃, then Pd(OH)₂; for **5q–s**, K₂CO₃, then TFA.

Received: October 14, 2017

Accepted: January 10, 2018

Published: January 10, 2018

Table 1. Antiviral Activity of a Small Library of Pyridinoimidazolone Replacements

cpd.	R	EC ₅₀ (μM) ^a	CC ₅₀ (μM) ^b	cpd.	R	EC ₅₀ (μM)	CC ₅₀ (μM)
BMS-433771		0.020	>100	5j		1.8	>100
5a		0.015	>100	5k		12	>100
5b		0.016	>100	5l		26	>100
5c		0.037	>100	5m		1.7	>100
5d		0.13	>100	5n		0.47	>100
5e		0.20	>100	5o		0.11	>100
5f		0.18	>100	5p		0.11	>100
5g		0.35	>100	5q		5.0	>100
5h		1.0	>100	5r		0.48	>100
5i		2.2	>100	5s		>50	>100

^aRSV CPE assay in Hep-2 cells; data was generated from two or more determinations. ^bCC₅₀ was performed on the same cells and with the same method.

structure–activity relationship (SAR) of this 3,3'-spirocyclic-2-oxo-indoline derivative was revealed herein.

Results and Discussion. In order to investigate a strategy of pyridinoimidazolone replacements on BMS-433771, a small library containing 19 scaffold hopping derivatives were designed and synthesized (Scheme 1). The key intermediate **4** was prepared from starting material **1** via displacement reaction and hydrogenation reaction followed by cyclization reaction with excellent overall yields (68.4%). The target compounds **5a–s** were obtained by alkylation reaction of intermediate **4** in the presence of K₂CO₃ in CH₃CN or NaH in DMF.

Cyclopropyl indolin-2-one **5a** was first recorded in ReViral Ltd.'s patent for RSV treatment, but no antiviral activity was reported.⁸ Hoffmann-La Roche disclosed spiro azaindolin-2-one analogs; however, only spirocyclicpropanes were introduced.^{9–11} In order to perform pyridinoimidazolone replacement strategy, **5a–c** were synthesized and evaluated by the CPE assay. The antiviral activities were listed in Table 1. The importance of the carbonyl group for the activities against RSV was demonstrated when its replacement with a hydrogen (**5e** and **5f**, with EC₅₀ of 0.20 and 0.18 μM, respectively) resulted in a moderate activity in comparison to BMS-433771. The presence of a small aliphatic substituent at the ortho-position of carbonyl was also found to be desirable (**5g–j**). Attempt to replace the fused phenyl ring with saturated ring resulted in a 100-fold less active compound **5k** with an EC₅₀ of 12 μM versus **5f**. Other pyridinoimidazolone replacements (**5m–s**) showed negative impact on activities. In summary, though the SAR for pyridinoimidazolone replacement strategy is kind of rigid, the ring system 3,3'-spiro[cyclopropane]-2-oxo-indoline (**5a–c**) was identified to gain a superior level of activity.

As a new chemical starting point, chloride (**6a**) and methyl group (**6b**) were introduced at the 5-position of the corresponding benzimidazole moiety following Feng's work on imidazolepyrimidines.^{12,13} Unfortunately, both of these two compounds did not boost the antiviral activities, demonstrating 10 and 16 nM, respectively, versus 15 nM of **5a** (Table 2). In the meantime, an entire attrition was observed on 5-cyclohexyl substituent (**6c**) possibly due to increased steric bulk, electronic withdrawing groups 5-CN (**6e**) and 5-CF₃ (**6f**) attenuated the activities by more than 10-fold. Single-digit nanomolar activity was obtained when bromine was introduced, and **6h** demonstrated 7-fold potency improvement in comparison to **5a**. Given that the introduction of 5-bromo had a significant impact on anti-RSV activity, we decided to pursue 5-bromobenzimidazole moiety for further SAR optimizations.

Furthermore, 3,3'-spirocyclic-2-oxo-indolines are found in compounds that exert a range of pharmacological activities. Analog 3,3'-spiro[succinimide]-2-oxo-indoline antagonized chemoattractant receptor-homologs expressed on Th2 lymphocytes (CRTH2 or DP2),¹⁴ and 3,3'-spiro[piperidine]-2-oxo-indolines were in development for cathepsin K inhibitors.¹⁵ Therefore, the following study focused on optimizing the 3,3'-spirocyclic-2-oxo-indoline moiety of **6h**.

In order to explore more potent 3,3'-spirocyclic-2-oxo-indoline derivatives, a small library of spiroheterocyclic analogs was successfully designed and synthesized. The synthesis of intermediate **12**, as shown in Scheme 2, was generally commenced with commercially available 2-bromo aniline, which was condensed with

Table 2. Antiviral Activity of Compounds **6a–i**

cpd.	R	EC ₅₀ (nM) ^a	CC ₅₀ (μM) ^b	cpd.	R	EC ₅₀ (nM)	CC ₅₀ (μM)
5a	5-H	15	>100	6e	5-CN	190	>100
6a	5-Cl	10	>100	6f	5-CF ₃	210	>100
6b	5-Me	16	>100	6g	5-CH ₂ NH ₂	7.0	>100
6c	5-cyclohexyl	>10000	>100	6h	5-Br	2.3	>100
6d	5-F	13	>100	6i	5-I	21	>100

^aRSV CPE assay in Hep-2 cells; data was generated from two or more determinations. ^bCC₅₀ was performed on the same cells and with the same method.

Scheme 2. Synthetic Route To Produce Key Intermediate 12

^aReagents and conditions: (i) T_3P , ethyl acetate, 30 °C, 90%; (ii) PMB-Cl, DMF, NaH 30 °C, or CS_2CO_3 , 90 °C, 85%; (iii) *i*-Pr-PEPPSI, *t*BuONa, 1,4-dioxane, 90 °C, 65%; (iv) CAN, acetonitrile, 0 °C, 30% or CF_3SO_3H , TFA, 30 °C, 75%; (v) K_2CO_3 , MeCN, 70 °C, or NaH, DMF, 20 °C, 70–90%.

the acid **8** via T_3P to generate the amide **9**, followed by alkylation with PMB-Cl to yield intermediates **10**. This intermediate was then subjected to an intramolecular palladium-catalyzed α -arylation reaction.¹⁶ After removal of PMB via in CAN or TFOH acid conditions, intermediate **12** was generated in moderate

Table 3. Antiviral Activity of 3,3-Spirocyclic-2-oxo-indoline Derivatives 14a–p

cpd.	R	EC ₅₀ (nM) ^a	CC ₅₀ (μ M) ^b	cpd.	R	EC ₅₀ (nM)	CC ₅₀ (μ M)
6h		2.3	>100	14i		3	>100
14a		6	>100	14j		3	>100
14b		8	>100	14k		19	>100
14c		5	>100	14l		43	>100
14d		21	>100	14m		307	>100
14e		11	>100	14n		42	>100
14f		10	>100	14o		55	>100
14g		8	>100	14p		76	>100
14h		0.8	>100				

^aRSV CPE assay in Hep-2 cells; data was generated from two or more determinations. ^bCC₅₀ was performed on the same cells and with the same method.

to good yield (30–75%). As shown in Table 3, **14a** and **14b** displayed good potency, with EC₅₀ of 6 and 8 nM. Sulfide **14c** and sulfone **14d** exhibited 5 and 21 nM antiviral activities, respectively. Encouraged by the tolerances of heteroatoms, nitrogen was introduced. Five-membered analogs **14e**, **14f**, and **14g** attenuated 4–5-fold anti-RSV activities compared with **6h**. However, four-membered azetidine spiro analogs, isopropyl carbamate **14h**, improved the antiviral activity into subnanomolar level of 0.8 nM, 3-fold more potent compared to that of **6h**, and isobutyl amide **14i** and methanesulfonyl amide **14j** exhibited comparable activities of 3 nM. Other approaches of aliphatic amine (**14k–l**) and steric bulky six-membered spiro rings (**14m–p**) attenuated the activities.

PK Results and Discussion. Plasma pharmacokinetics after 1 mg/kg intravenous and 10 mg/kg oral administration of compound **14h** and BMS433771⁶ to female balb/c mice were characterized. PK parameters were listed in Table 4, indicating

Table 4. Pharmacokinetic Parameters after Single Intravenous and Oral Administration of 14h and BMS433771 to Female Balb/c Mice^a

cpd.	14h	BMS-433771
	<i>i.v.</i> @ 1 mpk	
CL (mL/min/kg)	28	67
<i>T</i> _{1/2} (h)	1.4	1.7
Vd (L/kg)	1.6	2.4
AUC _{0–inf} (nM·h)	1130	920
	<i>p.o.</i> @ 10 mpk	
<i>C</i> _{max} (nM)	3319	2098
<i>T</i> _{max} (h)	0.25	0.25
AUC _{0–inf} (nM·h)	8076	1170
%F	71	13

^aData reported are mean values from the dosing cohorts (Female Balb/c-Mouse, fasted, *n* = 3/dose). Dosages are 1 mg/kg *i.v.* (1 mg/mL in DMSO/PEG400/water = 5:40:55, clear solution) and 10 mg/kg *p.o.* (2 mg/mL in DMSO/PEG400/water = 5:40:55, clear solution). CL stands for plasma clearance. *T*_{1/2} is the plasma half-life of the compounds. Vd means volume of distribution. *C*_{max} represents the highest observed plasma concentration, and *T*_{max} is time to reach *C*_{max}. AUC is the area under the plasma concentration–time curve. %F represents absolute oral bioavailability.

that compound **14h** showed lower clearance and volume of distribution (Vd), but higher AUC with similar half-life (*T*_{1/2}). However, compound **14h** had much better oral profile than that of BMS433771, as evidenced by oral exposure (8076 versus 1170 nM·h) and absolute oral bioavailability (71% versus 13%).

Pharmacology Results and Discussion. The *in vivo* efficacy was evaluated in the Balb/c mouse RSV infection model. As shown in Table 5, compound **14h** showed a clear dose-dependent

Table 5. *In Vivo* Antiviral Activity in a Mouse Model of RSV Infection

group (<i>n</i> = 7)	viral load in lung (Log10 pfu/g lung)	viral load reduction in lung (Δ log10)
Vehicle	3.91 ± 0.10	
BMS433771-50 mpk	2.75 ± 0.20	1.16 ± 0.24
14h -5 mpk	2.27 ± 0.24	1.64 ± 0.26
14h -15 mpk	1.86 ± 0.31	2.05 ± 0.31
14h -50 mpk	0.00 ± 0.00	3.91

effect on virus load reduction from 5 to 50 mg/kg. In addition, the 5 mg/kg group of **14h** reduced the lung tissue virus load

$\Delta\log_{10}$ to 1.6, which was superior to BMS-433771, 1.2 $\Delta\log_{10}$ at 50 mg/kg dose. Moreover, the 50 mg/kg group of **14h** reduced the virus load of all the experimental animals to below the detection limit, which demonstrated that compound **14h** exhibited excellent anti-RSV efficacy and was significantly superior to BMS433771 in this RSV mouse challenge model.

Conclusion. In summary, we have explored a series of 3,3'-spiro[azetidine]-2-oxo-indoline derivatives proved to be potent inhibitors of RSV in the CPE assay. The investigation led to the identification of **14h** as a compound demonstrating additional efficacy in the Balb/c mouse model of RSV infection after oral dosing. Further safety assessment will be conducted in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.7b00418.

Synthetic procedures, analytical data, and assay protocol (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: he_haiying@wuxiapptec.com.

ORCID

Weihua Shi: 0000-0003-3746-1583

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

PK, pharmacokinetics; PO, per oral; C_{max} , peak concentration; T_{max} , time to peak; CL, clearance; $T_{1/2}$, half-life; Vd, volume of distribution; AUC, area under curve; mpk, mg/kg; %F, oral bioavailability

■ REFERENCES

- (1) Storey, S. Respiratory syncytial virus market. *Nat. Rev. Drug Discovery* **2010**, 9 (1), 15–16.
- (2) Hall, C. B.; Weinberg, G. A.; Iwane, M. K.; Blumkin, A. K.; Edwards, K. M.; Staat, M. A.; Auinger, P.; Griffin, M. R.; Poehling, K. A.; Erdman, D.; Grijalva, C. G.; Zhu, Y.; Szilagyi, P. The Burden of Respiratory Syncytial Virus Infection in Young Children. *New. N. Engl. J. Med.* **2009**, 360 (6), 588–598.
- (3) Nair, H.; Nokes, D. J.; Gessner, B. D.; Dherani, M.; Madhi, S. A.; Singleton, R. J.; O'Brien, K. L.; Roca, A.; Wright, P. F.; Bruce, N.; Chandran, A.; Theodoratou, E.; Sutanto, A.; Sedyaniingsih, E. R.; Ngama, M.; Munywoki, P. K.; Kartasasmita, C.; Simões, E. A.; Rudan, I.; Weber, M. W.; Campbell, H. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. *Lancet* **2010**, 375 (9725), 1545–1555.
- (4) Mackman, R. L.; Sangi, M.; Sperandio, D.; Parrish, J. P.; Eisenberg, E.; Perron, M.; Hui, H.; Zhang, L.; Siegel, D.; Yang, H.; Saunders, O.; Booram, C.; Lee, G.; Samuel, D.; Babaoglu, K.; Carey, A.; Gilbert, B. E.; Piedra, P. A.; Strickley, R.; Iwata, Q.; Hayes, J.; Stray, K.; Kinkade, A.; Theodore, D.; Jordan, R.; Desai, M.; Cihlar, T. Discovery of an oral respiratory syncytial virus (RSV) fusion inhibitor (GS-5806) and clinical proof of concept in a human RSV challenge study. *J. Med. Chem.* **2015**, 58 (4), 1630–1643.
- (5) DeVincenzo, J. P.; Whitley, R. J.; Machman, R. L.; Weinlich, C. S.; Harrison, L.; Farrell, E.; McBride, S.; Williams, R. L.; Jordan, R.; Xin, Y.; Ramanathan, S.; O'Riordan, T.; Lewis, S. A.; Li, X.; Toback, S. L.; Lin, S. L.; Chien, J. W. Oral GS-5806 activity in a respiratory

syncytial virus challenge study. *New. N. Engl. J. Med.* **2014**, 371 (8), 711–722.

(6) Yu, K. L.; Sin, N.; Civiello, R. L.; Wang, X. A.; Combrink, K. D.; Gulgeze, H. B.; Venables, B. L.; Wright, J. J.; Dalterio, R. A.; Zadjura, L.; Marino, A.; Dando, S.; D'Arienzo, C.; Kadow, K. F.; Cianci, C. W.; Li, Z.; Clarke, J.; Genovesi, E. V.; Medina, I.; Lamb, L.; Colonno, R. J.; Yang, Z.; Krystal, M.; Meanwell, N. A. Respiratory syncytial virus fusion inhibitors. Part 4: optimization for oral bioavailability. *Bioorg. Med. Chem. Lett.* **2007**, 17 (4), 895–901.

(7) Bonfanti, J. F.; Meyer, C.; Doublet, F.; Fortin, J.; Muller, P.; Queguiner, L.; Gevers, T.; Janssens, P.; Szel, H.; Willebrords, R.; Timmerman, P.; Wuyts, K.; van Remoortere, P.; Janssens, F.; Wigerinck, P.; Andries, K. Selection of a respiratory syncytial virus fusion inhibitor clinical candidate. 2. Discovery of a morpholinopropylaminobenzimidazole derivative (TMC353121). *J. Med. Chem.* **2008**, 51 (4), 875–896.

(8) Cockerill, S.; Pilkington, C.; Lumley, J.; Angell, R.; Mathews, N. Pharmaceutical compounds. WO2013/68769.

(9) Feng, S.; Gao, L.; Guo, L.; Huang, M.; Liang, C.; Wang, B.; Wang, L.; Wu, G.; Yun, H.; Zhang, W.; Zheng, X.; Zhu, W. Aza-oxo-indoles for the treatment and prophylaxis of respiratory syncytial virus infection. WO2014/184163.

(10) Gao, L.; Guo, L.; Liang, C.; Wang, B.; Wang, L.; Yun, H.; Zhang, W.; Zheng, X. Novel aza-oxo-indoles for the treatment and prophylaxis of respiratory syncytial virus infection. WO2015/22263.

(11) Wang, L.; Yun, H.; Zhang, W.; Zheng, X. Novel aza-oxo-indoles for the treatment and prophylaxis of respiratory syncytial virus infection. WO2015/22301.

(12) Feng, S.; Hong, D.; Wang, B.; Zheng, X.; Miao, K.; Wang, L.; Yun, H.; Gao, L.; Zhao, S.; Shen, H. C. Discovery of imidazopyridine derivatives as highly potent respiratory syncytial virus fusion inhibitors. *ACS Med. Chem. Lett.* **2015**, 6 (3), 359–362.

(13) Feng, S.; Li, C.; Chen, D.; Zheng, X.; Yun, H.; Gao, L.; Shen, H. C. Discovery of methylsulfonyl indazoles as potent and orally active respiratory syncytial virus (RSV) fusion inhibitors. *Eur. J. Med. Chem.* **2017**, 138, 1147–1157.

(14) Crosignani, S.; Page, P.; Missotten, M.; Colovray, V.; Cleva, C.; Arrighi, J. F.; Atherall, J.; Macritchie, J.; Martin, T.; Humbert, Y.; Gaudet, M.; Pupowicz, D.; Maio, M.; Pittet, P. A.; Golzio, L.; Giachetti, C.; Rocha, C.; Bernardinelli, G.; Filinchuk, Y.; Scheer, A.; Schwarz, M. K.; Chollet, A. Discovery of a New Class of Potent, Selective, and Orally Bioavailable CRTH2 (DP2) Receptor Antagonists for the Treatment of Allergic Inflammatory Diseases. *J. Med. Chem.* **2008**, 51 (7), 2227–2243.

(15) Teno, N.; Masuya, K.; Ehara, T.; Kosaka, T.; Miyake, T.; Irie, O.; Hitomi, Y.; Matsuura, N.; Umemura, I.; Iwasaki, G.; Fukaya, H.; Toriyama, K.; Uchiyama, N.; Nonomura, K.; Sugiyama, I.; Kometani, M. Effect of Cathepsin K Inhibitors on Bone Resorption. *J. Med. Chem.* **2008**, 51 (17), 5459–5462.

(16) Pfefferkorn, J. A.; Choi, C. Convenient synthesis of 1,1'-H-spiro[indoline-3,30-piperidine]. *Tetrahedron Lett.* **2008**, 49 (28), 4372–4373.