Cite this: *Med. Chem. Commun.*,
2019, 10, 970

Received 26th March 2019,
Accepted 30th April 2019

DOI: 10.1039/c9md00178f

rsc.li/medchemcomm

Discovery of (aza)indole derivatives as novel respiratory syncytial virus fusion inhibitors

Xiufang Zheng, Chungun Liang, Lisha Wang, Kun Miao, Baoxia Wang, Weixing Zhang, Dongdong Chen, Guolong Wu, Wei Zhu, Lei Guo, Song Feng, Lu Gao, Hong C. Shen and Hongying Yun *

A new class of indole derivatives (**3**) have been identified as potent RSV fusion inhibitors. SAR exploration revealed that 5-Cl and the sulfonyl side chain of the indole scaffold are crucial for anti-RSV activity. Further optimization led to the discovery of a cyclic sulfone (**8i**) with 2 nM anti-RSV activity and a much improved PK profile compared to the non-cyclic sulfone counterpart.

Respiratory syncytial virus (RSV) is a member of the paramyxovirus family.¹ It is a predominant cause of respiratory tract infections in young children worldwide. Due to incomplete immunity against RSV infection, almost every child will be infected at least once before they turn five. The global mortality rates of RSV infection in children below the age of five have been estimated to be 66 000–199 000.² So far, there is no safe and effective RSV vaccine available.³ Until now, approved antiviral therapies for RSV infection have been limited to humanized monoclonal antibody palivizumab and nucleoside analogue ribavirin.⁴ Unfortunately, palivizumab is only used on high-risk infants for prophylactic purposes, while ribavirin is compromised by its potential toxicity and limited efficacy. The anti-RSV research campaign has focused on viral fusion proteins. The most advanced RSV fusion (RSV F) inhibitors, GS-5806, JNJ-53718678 and RV521 (Fig. 1, **1a–c**), are in phase II clinical trials.⁵ Nevertheless, a safe and effective treatment of the RSV disease remains a high unmet medical need.

In our previous publication, a series of imidazole pyridine derivatives were discovered by an information-driven approach.⁶ The representative compound **2** (Fig. 1) bears a 7-Cl substituent and a sulfonyl side chain with highly potent antiviral activity. Despite its promising antiviral activity and good physicochemical properties, compound **2** displayed unfavorable pharmacokinetic properties which impeded its further development. Starting from compound **2**, we discovered a new chemical series with (aza)indole and spirooxindole moieties. In this article, we will describe the synthesis of (aza)indole derivatives **3**, the SAR exploration, and the improved pharmacokinetics of (aza)indole. Finally, we will describe the mechanism study with a lead compound of the indole series.

The synthesis of (aza)indole derivatives **3** is summarized in Scheme 1.⁷ The synthetic route started with commercially

available (aza)indole-2-carboxylate **4**, followed by alkylation using K₂CO₃ in acetonitrile and then reduction with lithium aluminum hydride to give indol-2-ylmethanol **5**. Side chain R² can also be introduced by using Michael addition with indol-2-ylmethanol derived from indole-2-carboxylate **4**. Activation of alcohol **5** by using methanesulfonyl chloride followed by coupling with (aza)spirooxindole **6** provided the target (aza)indole derivatives **3**. Alternatively, **3** was obtained by the Mitsunobu reaction involving compound **5** and (aza)spirooxindole **6**.

The anti-RSV activity of the prepared indole derivatives **3** was evaluated in the CPE assay.⁸ The SAR of R¹ is summarized in Table 1. A Cl shift revealed that the 5-Cl analogue (**7c**) afforded the best anti-RSV activity (EC₅₀ = 0.018 μM). Replacement of 5-Cl with other halogens led to either lower activity (e.g. **7f**: EC₅₀ = 0.19 μM) or increased cytotoxicity (e.g. **7g**: CC₅₀ = 49.9 μM). The replacement of 5-Cl with methyl can slightly improve the potency (**7h**, EC₅₀ = 0.009 μM).

Fig. 1 Structures of selected RSV inhibitors.

Roche Innovation Center Shanghai, Building 5, Lane 720, Cai Lun Road, Shanghai 201203, China. E-mail: hongying.yun@roche.com

Scheme 1 Synthesis of spirooxindole derivatives **3**. Reagents and conditions: (a) i. R^2-X or R^2-OTs , K_2CO_3 , CH_3CN , heat; ii. $LiAlH_4$, THF, $0^\circ C$; (b) i. $LiAlH_4$, THF, $0^\circ C$; ii. CH_2CHSO_2R , Cs_2CO_3 , DMF, heat; (c) i. $MsCl$, Et_3N , DCM, $0^\circ C$; ii. **6**, NaH , DMF, $0^\circ C$; (d) **6**, PPh_3 , DIAD, THF, rt.

Unfortunately, further elongation of the side chain such as in compound **7i** led to a much reduced potency ($EC_{50} = 0.22 \mu M$). The introduction of a methoxy group led to a reduced activity. Replacement of 5-Cl with an electron withdrawing group such as 5- CF_3 (**7k**) or 5-CN (**7l**) resulted in a significant loss of potency.

Based on the SAR of the substituents in Table 1, the 5-Cl group was retained for further optimization. As previously described in the imidazole pyridine series,⁶ the sulfonyl side chain was favorable for potency improvement. Therefore, the exploration of the sulfonyl side chain was conducted and the SAR is summarized in Table 2. Clearly, the elongation of the

Table 1 The SAR of indole analogs **7a-l** in the CPE assay^a

Cmpd.	R^1	EC_{50} (μM)	CC_{50} (μM)	TI
7a	H	0.22	77.5	352
7b	4-Cl	1.67	>100	>60
7c	5-Cl	0.018	67.4	3744
7d	6-Cl	4.83	59.7	12
7e	7-Cl	0.16	86.9	543
7f	5-F	0.19	>100	>526
7g	5-Br	0.014	49.9	3564
7h	5- CH_3	0.009	61.6	6844
7i	5-Et	0.22	86.8	395
7j	5- OCH_3	0.074	>100	>1351
7k	5- CF_3	0.56	>100	>179
7l	5-CN	0.94	>100	>106

^a EC_{50} : the concentration of the compound that reduced 50% of the cytopathic effect (CPE) of RSV Long strain infected HEP-2 cells. CC_{50} : the concentration of the compound that manifests cytotoxicity towards 50% of uninfected HEP-2 cells. The therapeutic index (TI) is the ratio of CC_{50}/EC_{50} . The values are the means of at least two experiments performed in consecutive weeks.⁹

Table 2 Structures and activities of indole analogs **8a-i**^a

Cmpd.	R^2	EC_{50} (μM)	CC_{50} (μM)	TI
8a		0.008	>100	12 500
8b		0.003	38.6	12 867
8c		0.003	51.2	17 067
8d		0.006	>100	16 667
8e		0.007	78.3	11 186
8f		0.006	68.3	11 383
8g		0.009	92.1	10 233
8h		0.009	42.5	4722
8i		0.002	37.1	18 550

^a EC_{50} : the concentration of the compound that reduced 50% of the cytopathic effect (CPE) of RSV Long strain infected HEP-2 cells. CC_{50} : the concentration of the compound that manifests cytotoxicity towards 50% of uninfected HEP-2 cells. The therapeutic index (TI) is the ratio of CC_{50}/EC_{50} . The values are the means of at least two experiments performed in consecutive weeks.⁹

sulfone chain from both sides can further improve the potency (**8a–8d**). Interestingly, replacement of sulfonyl with sulfoximine (**8e** and **8f**) or sulfinyl (**8g**) still maintained a good potency. These results indicate that the length and functionality of the side chain can be optimized. Unexpectedly, the cyclic sulfonyl side chains found in **8h** and **8i** exhibited the same activity as the linear side chain. This observation, in contrast to the BMS result,¹⁰ has stimulated considerable interest in a new line of further optimization.

After the optimization of R^1 and R^2 , we performed screening of aza-indole in the left indole (**9a–9c**) and an N-shift in the right spirooxindole (**9d** and **9e**). Compared to **7c**, the anti-RSV potencies of **9a** and **9b** were reduced, and a narrow therapeutic window of **9c** was observed (Table 3). The N-shift in spirooxindole also led to reduced potency.

As a next step, we selected representative compounds (**2**, **7c**, **8i** and **9c**) for a PK study in male ICR mice based on their anti-RSV activity, TI and metabolic stability (mouse liver microsomal clearance of **2**, **7c**, **8i** and **9c**: $35 \text{ mL min}^{-1} \text{ kg}^{-1}$, $38 \text{ mL min}^{-1} \text{ kg}^{-1}$, $43 \text{ mL min}^{-1} \text{ kg}^{-1}$ and $20 \text{ mL min}^{-1} \text{ kg}^{-1}$, respectively). Compared to the previous compound **2**, the solubility of the new compounds is less desirable (LYSA of **2**, **7c**,

Table 3 Structures and activities for indole analogs **9a–e**^a

Cmpd.	EC ₅₀ (μM)	CC ₅₀ (μM)	TI
9a	0.063	74.5	1183
9b	0.33	98.9	300
9c	0.026	41.7	1604
9d	0.17	>100	588
9e	0.20	96.8	484

^a EC₅₀: the concentration of the compound that reduced 50% of the cytopathic effect (CPE) of RSV Long strain infected HEP-2 cells. CC₅₀: the concentration of the compound that manifests cytotoxicity towards 50% of uninfected HEP-2 cells. The therapeutic index (TI) is the ratio of CC₅₀/EC₅₀. The values are the means of at least two experiments performed in consecutive weeks.⁹

8i and **9c**: 59 μg mL⁻¹, 17 μg mL⁻¹, 2 μg mL⁻¹ and 5 μg mL⁻¹, respectively), but compounds **7c** and **8i** showed a higher oral exposure and a lower plasma clearance with similar volumes of distribution (*V*_{ss}). Compound **9c** demonstrated the best oral exposure, the lowest *in vivo* clearance, and good oral bioavailability (Table 4).

Finally, the mode of action of **8i** was confirmed in an inhibition assay of the RSV F protein-induced fusion process as shown in Fig. 2. With 1 nM (<EC₅₀) **8i** treatment, the RSV F protein will induce a cell fusion process and generate syncytia as shown in the red dotted circle (Fig. 2, left figure). In the presence of 10 nM (>EC₅₀) **8i**, the syncytia formation process induced by the RSV F protein was completely inhibited (Fig. 2, right figure). These assay results confirmed that **8i** was an RSV F inhibitor.

Table 4 *In vivo* PK profile of selected analogues in mice^a

Cmpd.	2	7c	8i	9c
Dose i.v./p.o. (mg kg ⁻¹)	2/10	5/25	5/25	5/25
AUC (μg L ⁻¹ h ⁻¹)	315	1550	3360	10 200
CL (mL min ⁻¹ kg ⁻¹)	93.3	54.2	30.2	23.7
<i>V</i> _{ss} (L kg ⁻¹)	1.29	1.38	2.35	0.845
<i>T</i> _{1/2} (h)	1.73	0.64	3.04	0.70
<i>F</i> (%)	16.9	NA	24.5	58.3

^a The single-dose pharmacokinetics (SDPK) study in male ICR mice was carried out according to the standard procedures. Major parameters, including AUC (p.o.), plasma clearance (CL), *T*_{1/2} (p.o.), *V*_{ss} (i.v.) and oral bioavailability (*F*), are reported.

Fig. 2 **8i** inhibited RSV F protein-induced fusion process. The F gene of RSV Long strain was codon-optimized and synthesized by GenScript. The full length of the F gene was cloned into the pcDNA5 TOPO TA vector (Invitrogen). Then RSV-F stable cell lines were generated by co-transfection of the RSV-F-pcDNA5 and pOG44 plasmids into the Flp-In T-REx 293 cell line (Invitrogen) and 100 μg mL⁻¹ hygromycin B was used to select stable transfectants. RSV-F expression was induced by 1 μg mL⁻¹ tetracycline. The compound was serially diluted and added into the RSV-F expressing cells. The cell-cell fusion was observed by microscopy three days after the compound addition. The cell fusion induced by the RSV fusion protein was marked with a red circle.

Conclusions

In summary, a new class of indole derivatives **3** have been identified as potent RSV fusion inhibitors. The preliminary SAR study revealed that 5-Cl indoles in combination with the cyclic sulfonyl head portion and 6-aza-spirooxindole moiety led to the discovery of a low nM RSV F inhibitor with promising oral exposure. Further lead optimization to discover a more potent, orally available RSV fusion inhibitor with the potential to treat RSV infection will be reported in due course.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

We are grateful to Qinshan Gao and Peilan Ding for their analytical assistance and purification of the final compounds. We also thank Rong Zhao, Yuxia Zhang, Yi Zhang, Hongxia Qiu, Xiaoyue Wu and Sheng Zhong for carrying out the physico-chemical, metabolic stability and PK studies.

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