Bioorganic & Medicinal Chemistry Letters 23 (2013) 6789–6793

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of a potent respiratory syncytial virus RNA polymerase inhibitor

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ARTICLE INFO

Article history:
Received 30 July 2013
Revised 6 October 2013
Accepted 8 October 2013
Available online 16 October 2013

Keywords:
Respiratory syncytial virus
Antiviral
Polymerase inhibitor

ABSTRACT

Targeting viral polymerases has been a proven and attractive strategy for antiviral drug discovery. Herein we describe our effort in improving the antiviral activity and physical properties of a series of benzothienoazepine compounds as respiratory syncytial virus (RSV) RNA polymerase inhibitors. The antiviral activity and spectrum of this class was significantly improved by exploring the amino substitution of the pyridine ring, resulting in the discovery of the most potent RSV A polymerase inhibitors reported to date.

Respiratory syncytial virus (RSV) is the leading cause of infant hospitalization and acute lower respiratory tract infection worldwide.[1] Despite decades of research and development, effective therapy options for RSV infection remain limited.[2] Palivizumab,[3] a monoclonal antibody targeting the RSV fusion (F) protein, significantly reduced RSV-related hospitalizations among high-risk infants when used as prophylaxis but was not an effective treatment. Ribavirin, the only approved small molecule therapy against RSV, has restricted utility due to its controversial efficacy and toxicity.[4] Several potent, small-molecule fusion inhibitors have been discontinued at preclinical or Phase 1–2 stages,[5] with the exception of inhalable MDT-637[6] and GS-5806[7] advancing to Phase 2 clinical trials. A siRNA agent (ALN-RSV01)[8] was reported to have missed its primary endpoint in a Phase 2b clinical trial. The development of RSV604,[9] a novel benzodiazepine targeting viral N-protein, has been stopped in Phase 2 trials.

In 2005, it was reported that YM-53403, identified from random screening, potently inhibited the replication of RSV in plaque reduction assays[10] (Fig. 1). Resistance studies showed that it targets the viral RNA polymerase L protein, which catalyzes the transcription and replication of the viral genome. A recent patent disclosure[11] revealed that modification of either the biphenyl or the cyclopropyl region of YM-53403 was tolerated for antiviral activity. We confirmed the antiviral activity of key reference compound **1**, and uncovered the intrinsic lack of RSV B activity and suboptimal drug-like properties (e.g., low aqueous solubility and low metabolic stability, presumably due to its high lipophilicity and high molecular weight.).

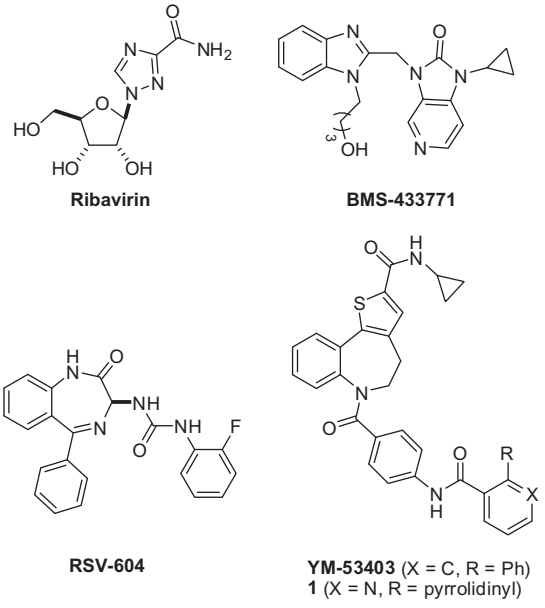

Figure 1. Chemical structure of selective examples of RSV inhibitors.

In this letter, we report the improvement of antiviral potency, spectrum coverage, as well as the drug-like properties of this chemical series.

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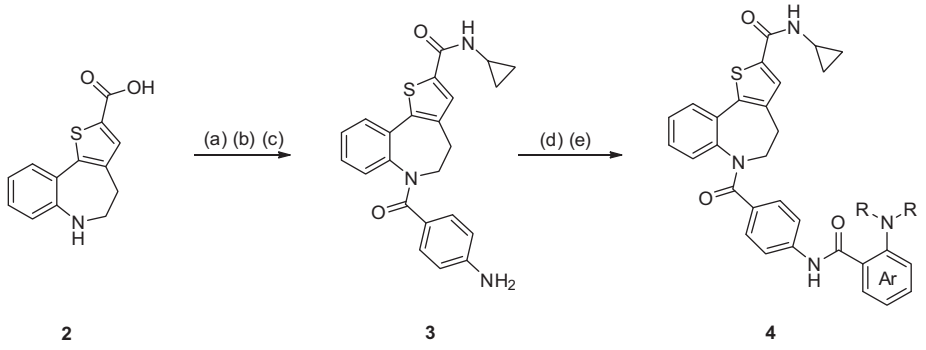
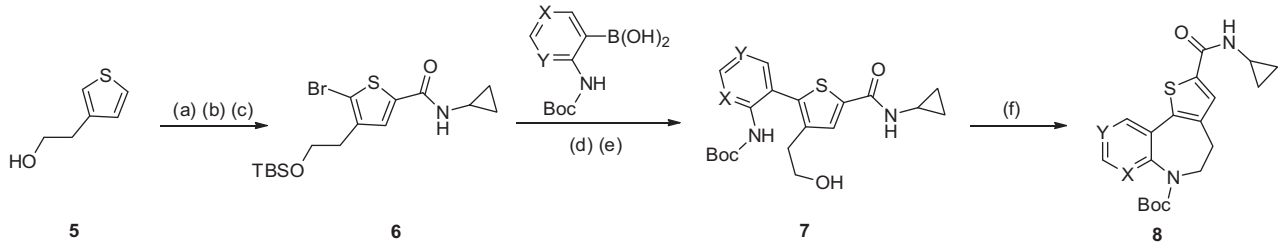
0960-894X/$ - see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.bmcl.2013.10.018



Scheme 1. Synthesis of benzo[*b*]thieno[2,3-*d*]azepine compounds. Reagents and conditions: (a) HATU, cyclopropylamine, DIPEA, DMF; (b) 4-nitro-benzoyl chloride, DIPEA, pyridine; (c) Pd/C, H_2, 1N HCl, EtOH; (d) 2-Halogen-ArCOCl, DIPEA, pyridine; (e) amine, DIPEA, NMP, reflux.



Scheme 2. Synthesis of pyrido[2,3-*b*]thieno[2,3-*d*]azepines. Reagents and conditions: (a) TBSCl, imidazole, DMF; (b) NBS, AcOH/$CHCl_3$; (c) LDA, THF, −78 °C; CO_2 (g); HATU, cyclopropylamine, DIPEA, DMF; (d) Pd-118, K_2CO_3, EtOH/H_2O, reflux; (e) TBAF, THF, 0 °C; (f) DIAD, PPh_3, toluene, reflux.

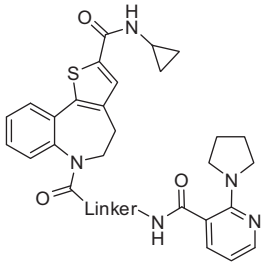
As shown in Scheme 1, the synthesis of the benzothienoazepine series started from commercially available benzothienoazepine carboxylic acid **2**. Installation of the cyclopropyl amide, acylation with 4-nitro-benzoyl chloride, and catalytic hydrogenation of the nitro group provided aniline **3**, which underwent amide coupling and subsequent aromatic substitution with a variety of secondary amines to afford the final compounds.

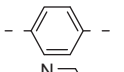
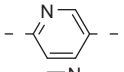
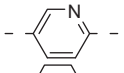
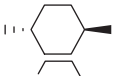
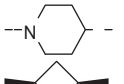
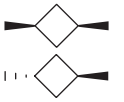
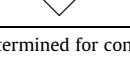
To modify the tricyclic core, we employed an intramolecular Mitsunobu cyclization strategy.[12] Starting from commercially available 2-(thiophen-3-yl) ethanol **5**, silyl ether formation, bromination, and 2-step introduction of the carboxyamide, provided the fully functionalized thiophene **6** (Scheme 2). Suzuki coupling between **6** and appropriate boronic acids formed the desired biaryl C–C bond. Subsequent removal of the TBS ether gave the Boc protected amino alcohol **7**, which was treated with DIAD and PPh_3 in hot toluene to provide the azepine ring. Replacement of the thiophene with either pyrazole or imidazole was accomplished via a similar synthetic strategy.

The RSV subgenomic replicon assay was used as a primary assay to evaluate antiviral activity of the compounds synthesized in this study. The RSV replicon cell line contains a stable and self-replicating RSV A2 subgenomic replicon encoding the viral proteins essential for RSV replication and the Renilla luciferase reporter.[13] Antiviral activity of each compound was measured by inhibition of the Renilla luciferase activity within the replicon-bearing cells in a robust two-day assay.[14]

We also employed a live virus assay in HEp-2 cells using RSV A2 and B Washington (WST) strains to confirm and investigate compound activity against RSV A and B subtypes. This assay uses an ELISA method to measure viral protein expression level after 3 days of infection and treatment with experimental compound. Compound activities in RSV replicon and live virus ELISA assays were presented as 50% effective concentrations (EC_{50}). Compound cytotoxicity was determined similarly as 50% cytotoxicity concentration (CC_{50}). Key physical properties (solubility, lipophilicity) and DMPK characteristics (permeability, metabolic clearance) of the compounds were also measured. Since an RSV L polymerase

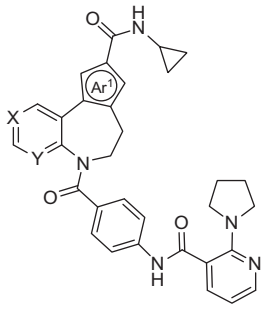
Table 1
Antiviral activity, cytotoxicity and solubility for **1**, **9a**–**9f**



Compd	Linker	Replicon EC_{50}/CC_{50} (μM)	ELISA EC_{50} (μM) A2/B-WST	Sol. (μM)
1		0.31/>50	0.15/>50	<1
9a		3.7/>50	2.4/>50	3
9b		>50/>50	>50/>50	3
9c		>50/>50	nd[a]	11
9d		14/>50	nd[a]	26
9e		>50/>50	nd[a]	2
9f		16/>50	nd[a]	3

[a] nd = not determined for compounds inactive in replicon assay.

Table 2
Antiviral activity, cytotoxicity and solubility for **1**, **10a**–**10d**



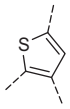
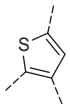
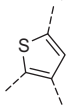
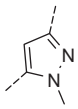
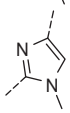
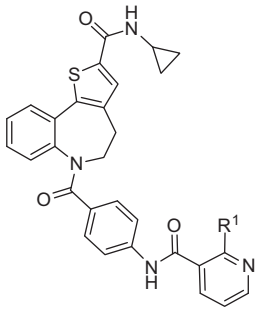
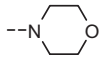
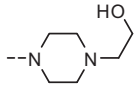
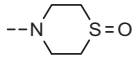
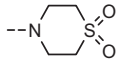
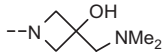
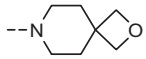
Compd	X/Y	Ar[1]	Replicon EC_{50}/CC_{50} (μM)	ELISA EC_{50} (μM) A2/B-WST	Sol. (μM)
1	C/C		0.31/>50	0.15/>50	<1
10a	N/C		2.2/>50	1.1/>50	1
10b	C/N		8.6/>50	7.8/>50	10
10c	C/C		9/>50	20/50	32
10d	C/C		22/>50	50/50	121

Table 3
Antiviral activity, cytotoxicity and solubility for **1**, **11a**–**11j**



Compd	R^1	Replicon EC_{50} (μM)	ELISA EC_{50} (μM) A2/B-WST	HEp-2 CC_{50} (μM)	Sol. (μM)
1		0.31	0.15/>50	>50	<1
11a		0.63	0.23/17	>50	36
11b		13	8.0/>50	>50	40
11c		12	9.6/>50	>50	26
11d		3.6	2.2/>50	>50	4
11e		>50	> 50/>50	>50	>100
11f		0.01	0.01/1.0	>50	2
11g		8.5	34/17	34	55
11h		9	5.3/45	39	4
11i		0.04	0.03/2.2	>50	<1
11j		<0.007	0.01/0.5	>50	<1

structure and robust biochemical assay were unavailable, we relied on RSV replicon and live-virus assay readouts to guide the chemistry campaign.

We first explored stepwise truncation of various regions of compound **1**, and found that these changes generally led to the loss of antiviral activity (details not shown). We next attempted replacing the phenyl linker (Table 1), and found that the pyridyl linker in compound **9a** led to ~10-fold loss of antiviral activity in both replicon and ELISA assays. The isomeric pyridyl compound **9b** led to even greater loss of antiviral activity. Insertion of saturated linkers (compounds **9c**–**9f**) resulted in large losses of antiviral activity, while aqueous solubility was improved (compounds **9c**, **9d**).

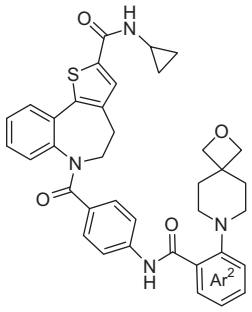
As shown in Table 2, several heterocyclic replacements of the tricyclic benzothienoazepine core were designed to lower the lipophilicity. The pyridyl compound **10a** showed ~7-fold loss of antiviral activity than **1**, and the isomeric pyridyl compound **10b** was even less active in both assays. Replacement of the thiophene ring with either pyrazole (compound **10c**) or imidazole (compound **10d**) gave promising improvements in aqueous solubility, but at the cost of losing antiviral activity.

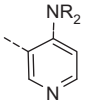
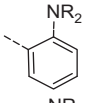
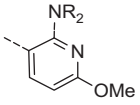
Various secondary amines substituted on the pyridine ring were also examined (Table 3). Compared to pyrrolidine in compound **1**, morpholine (**11a**) led to improved aqueous solubility, comparable antiviral activity in replicon assay, and weak activity towards RSV B in the ELISA assay. Several other attempts at improving physical properties (**11b**–**11e)** led to loss of antiviral activity. Interestingly, introduction of an oxetane spiro-fused piperidine (2-oxa-7-azaspiro[3.5]nonane, **11f**) gave significantly improved antiviral activity of 10 nM against RSV A2, and 1 μM against RSV B-WST, whereas compound **11g** with 2-methyl-2,7-diazaspiro[3.5]nonane showed much weaker antiviral activity, which was thought to be due to the protonation of the basic tertiary amine in **11g** under physiological pH environment. However, removal of the basic azetidine nitrogen through the formation of the corresponding acetamide (**11h**) did not reverse the activity loss. Incorporation of either 3-oxa-9-azaspiro[5.5]undecane (**11i**) or 7-oxa-2-azaspiro[3.5]nonane (**11j**) led to good antiviral activity against both RSV A2 and B strains, with compound **11j** as the most potent RSV A polymerase inhibitor reported to date.

Further modification of the aromatic ring bearing the amine substitution was also explored. As shown in Table 4, among all four possible pyridyl isomers and the phenyl ring, the pyridine ring in compound **11f** was superior. Furthermore, a methoxyl substitution on the pyridine ring (**11o**) was also tolerated, indicating a possible position for further modification.

Considering the physical properties of compound **11f** (molecular weight of 638, low aqueous solubility of 2 μM and low metabolic stability), and the site of RSV infection in respiratory tract, we evaluated the lung retention and distribution properties of

Table 4
Antiviral activity, cytotoxicity and solubility for **11f**, **11k–11o**



Compd	Ar^2	Replicon EC_{50} (μM)	ELISA EC_{50} (μM) A2/B-WST	HEp-2 CC_{50} (μM)	Sol. (μM)
11f		0.01	0.01/1.0	>50	2
11k		0.16	<0.21/5.1	4.8	<1
11l		0.57	0.68/14	34	9
11m		0.35	0.47/49	43	<1
11n		0.19	<0.21/>50	>50	<1
11o		0.02	<0.21/1.8	>50	<1

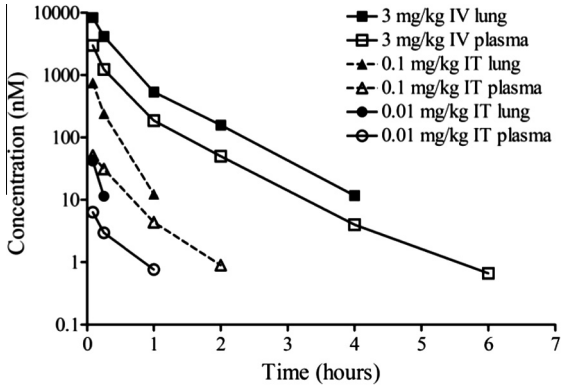


Figure 2. Plasma and lung tissue exposure of **11f** in rat.

11f via intratracheal (IT) and intravenous (IV) dosing.[15] Compound **11f** was dosed to rats as a solution at 10 μg/kg, or a nanoparticle suspension at 100 and 3000 μg/kg doses. Following IT dosing, plasma, lung tissue and bronchoalveolar lavage fluid (BALF) were collected and drug concentrations were determined. In the IV dose group, plasma and lung tissues were collected and analyzed (Fig. 2).

Compound **11f** was absorbed rapidly through lungs into blood circulation in solution form (absorption $t_{1/2}$ <1 min). Only 2.5% of the administered 10 μg/kg IT dose remained in the lung and BALF at 5 min post dosing. When the dose was normalized, the 10 μg/kg IT plasma and the 3 mg/kg IV plasma profiles appeared to be identical, suggesting rapid and complete absorption from the IT dose site to the systemic circulation. The nano-suspension formulation did not show significant delay in absorption from lung. Only 4.5% of the administered dose remained in lung and BALF at 5 min post dosing.

These experiments suggested that compound **11f** was not retained to a significant extent in the lung and BALF, and was instead rapidly absorbed into the systemic circulation following IT dosing. Further improvement of the lung retention properties is needed to obtain an inhalable RSV antiviral from this series.

We have identified novel modifications that delivered potent and broad-spectrum RSV L polymerase inhibitors without cytotoxicity. This provides new knowledge that may pave the way towards effective RSV therapeutics and new tool compounds to interrogate RSV L protein function.

Acknowledgments

The authors would like to thank Camil Joubran, Nancy DeGrace, Natascha Bezdenejnih-Snyder, Ziling Lu, Ying Liu and Bridget Reaney for analytical chemistry support; Steven Hsu, Ken Grime, Britt-Marie Fihn and Anders Lundqvist for in vitro and in vivo DMPK support; Nancy Ulbrandt and Hong Ji for RSV strains and protocols support; Ruben Tommasi, Martin Hentemann, Giuseppe Ciaramella and Scott Butler for scientific discussions.

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