A Breath of Fresh Air: Delivering Novel Respiratory Syncytial Virus (RSV) Inhibitors

Dr John Murray & Dr Euan Fordyce

SMR Meeting

26th September 2018

Introducing Pulmocide: *Dramatis personæ*

- A biotech company formed in Nov 2013 at the Imperial BioIncubator, SW7
- Co-founded by Garth Rapeport, CEO; Pete Strong, Chief Scientific Officer; Kaz Ito, Director of Biology, and John Murray, Director of Medicinal Chemistry
- Management Team previously co-founded RespiVert Ltd., acquired in May 2010 by Centocor Ortho Biotech Inc. (became Janssen Biotech Inc.), part of J & J Inc.
- Series A funding of £17 million (US$27.5 million) raised
- Syndicate of four VC investors: Imperial Innovations Group plc; SV Life Sciences; Fidelity Life Sciences, (previously investors in RespiVert); and the J & J Development Corp.
- Purpose: the discovery of a new generation of inhaled treatments for serious viral and fungal infections of the respiratory tract

Introducing Pulmocide: *The Stage*

- No compounds and no *ab initio* lead discovery capabilities (or intentions)
- Two indications, both serious lung infections: respiratory syncytial virus (RSV) and invasive aspergillosis
- Scope for new more effective /safer agents to meet acute unmet medical needs
- Leadership team with decades of relevant experience and a proven track-record of identifying small molecules suitable for inhaled administration
- A clear understanding of the deficiencies of current treatments and how to surpass them
- A pre-existing and highly effective partnership with Sygnature Discovery Services shown to be capable of delivering clinical candidates

Why Sygnature Discovery?

- Formerly known as Sygnature Chemical Services: one of three medicinal chemistry providers retained by RespiVert during its discovery programme
- By the time of RespiVert's acquisition had become the sole partner for the supply of chemistry-related discovery activities, based upon performance
- Critical mass achieved: a process of well managed growth and expansion of technical competencies provided all the necessary elements of a small molecule discovery programme
- Synthetic expertise continuously enhanced by adoption of state-of-the-art capabilities
- Key appointments made to IT/computational chemistry/molecular modelling
- Exceptional problem solving ability, strong team-work ethic, culture of openness and innovation
- Ready (off-site) access to ELN's and primary data, good communication skills
- Intimate knowledge of the prior-art, including the patent literature; essential for the transformation of known entities into a novel, patentable medicines

The ‘Pulmocide Treatment Paradigm’

- The advantages of inhaled pulmonary medicines:
  - Rapid and direct delivery of drug to the diseased organ
  - No requirement for oral absorption, no barriers to distribution
  - Minimal systemic exposure, typical doses measured in hundreds of micrograms
  - Optimizes efficacy whilst improving safety profile
- Key compound attributes for success:
  - High potency; low nanomolar activities required to overcome dose limitations
  - Low oral absorption/rapid metabolism in plasma: non-target organs spared
  - Strong avidity of drug for the lung to provide a long duration-of-action
  - Appropriate physicochemical characteristics: suitable, stable crystalline form required for micronisation, sterilisation and dispersion in an aq. delivery medium
- No unique set of properties identified that reliably delivers the desired PK/PD profile:
  - Sophisticated cellular and tissue-based phenotypic screens required to identify candidates

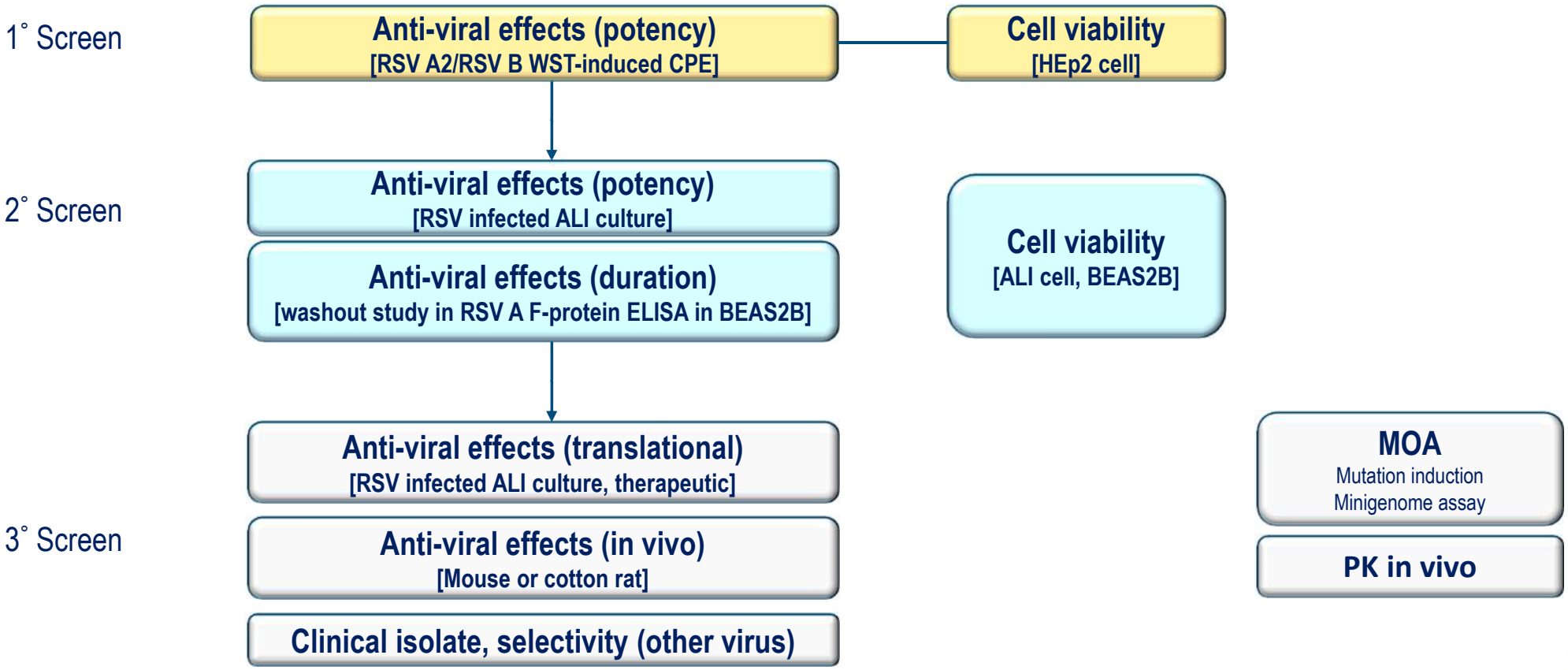
Strong *et al. Drug Discov Today* **2018**, in press; Ritchie *et al. J. Chem. Inf. Model.* **2009**, *49*, 1025-1032; Tronde *et al. J. Pharm. Sci.* **2003**, *92*, 1216-1233; Edwards *et al. Pharm. Res.* **2016**, *33*, 2604-2616.

RSV: Disease and Current Treatments

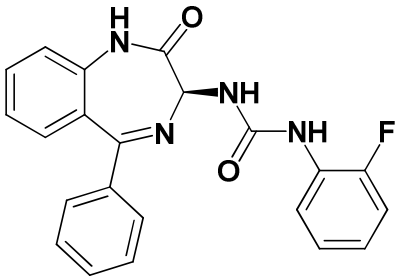
- Leading cause of lower respiratory tract infection (LRTI) in infants and young children
- High risk groups include premature infants and those with a very low birthweight, HIV-infected children and those with compromised immune systems
- Strong correlation between severe RSV infection and the development of asthma
- There are only two licensed drugs for the treatment of RSV:
  - Virazole® (aerosol formulation comprising ribavirin) – use limited by low potency and toxicity
  - Synagis® (humanised monoclonal antibody palivizumab) – approved for immunoprophylaxis in high-risk paediatric patients
- Equates to a big opportunity

Mazur *et al. Lancet Respir. Med.* **2015**, *3,* 888-900; Holt *et al. J. Exp. Med.* **2002**, 196, 1271-1275

RSV Screening Cascade

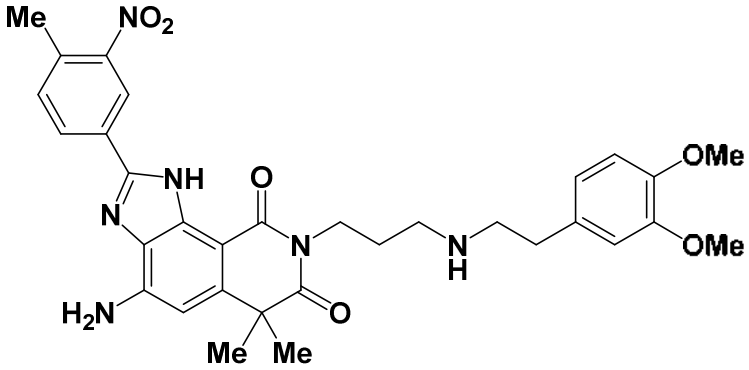


Potential Chemical Starting Points



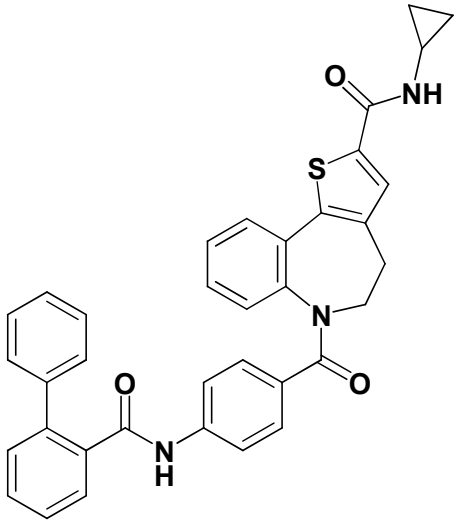
RSV-604

- Discontinued due to poor results in human PoC study



BI compd D

- Toxicity issues
- Synthetic tractability

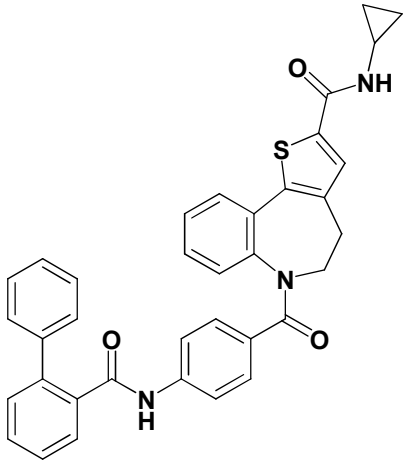


YM-53403

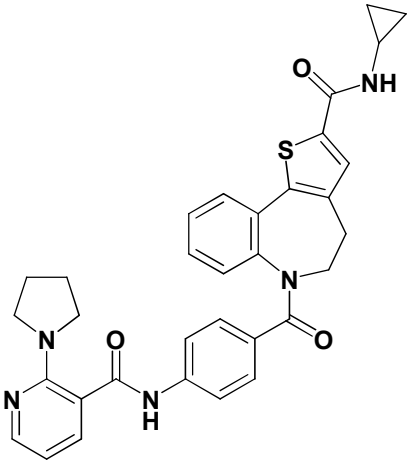
- Modest potency (submicromolar)
- Reasonable physicochemical properties

RSV-604: *Chapman et al. Antimicrob. Agents Chemother*. **2007**, *51*, 3346-3353; **BI**: Liuzzi *et al. J. Virol.* **2005**, *79*, 13105-13115; **YM-53403:** Sudo *et al. Antiviral Research* **2005**, *65*, 124-131.

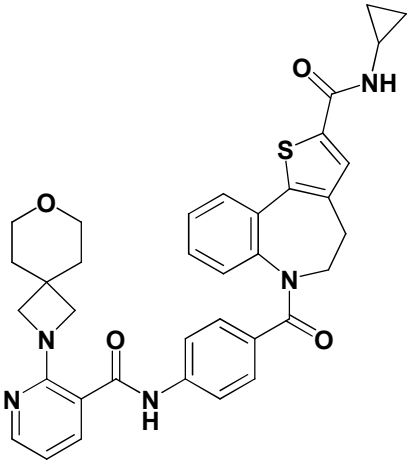
Profile of Published Thienobenzazepines



PC1



PC29

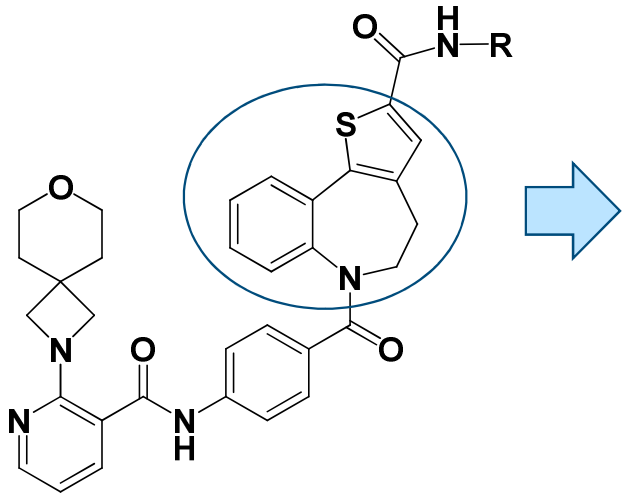


PC36

PC Number	RSV CPE (HEp-2) A2 (nM)		RSV CPE (HEp-2) B-WST (nM)		Cell toxicity (HEp-2)	
	IC_{50}	IC_{90}	IC_{50}	IC_{90}	CC_{50} (μM)	SI [1]
1[2]	68	259	>1713	>1713	>17	$>10^2$
29[3]	49	93	572	>1731	>17	$>10^2$
36[4]	2.1	5.7	280	660	6.4	10^3

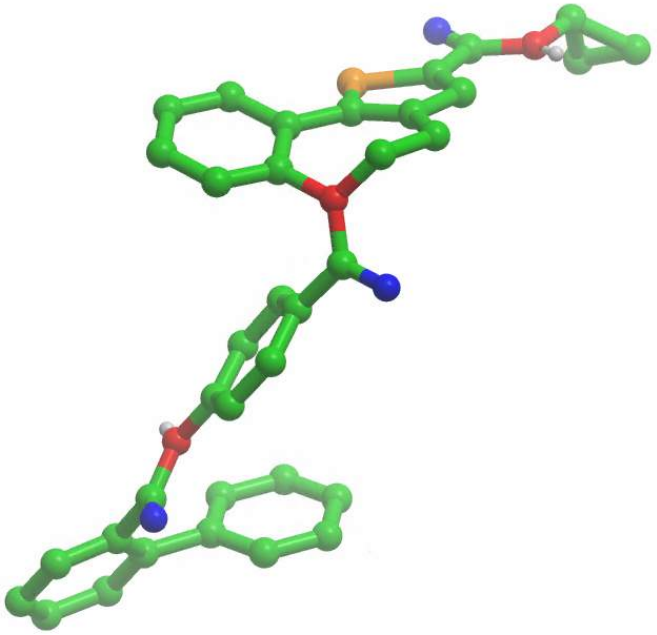
1. Safety index: Ratio of IC_{50}/CC_{50} values; **2.** Sudo *et al. Antiviral Research* **2005**, *65,* 124-131; **3.** Mackman *et al* **WO 2011/005842**; **4.** Yu *et al. Bioorg. Med. Chem. Lett.* **2013**, *23*, 6789-6793

Exploration of the Molecular Scaffold and SAR: The Tricyclic Core



Benzazepine nucleus has a puckered seven membered ring which is crucial in orienting the pharmacophoric domains in a low energy, bio-active conformation

Exploration of the Molecular Scaffold and SAR: The Tricyclic Core

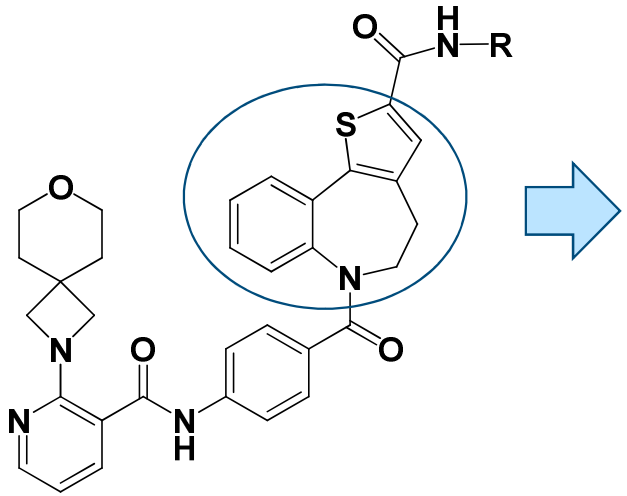


Benzazepine nucleus has a puckered seven membered ring which is crucial in orienting the pharmacophoric domains in a low energy, bio-active conformation

'Southern' region disposed almost orthogonal to the plane of the tricyclic core

Crystal structure of **YM-53403** solved by Pharmorphix (now part of Johnson Matthey)

Exploration of the Molecular Scaffold and SAR: The Tricyclic Core



Benzazepine nucleus has a puckered seven membered ring which is crucial in orienting the pharmacophoric domains in a low energy, bio-active conformation

An early target for modification because alternative cores are (would have been) an expedient route to novelty

Substitution of phenyl ring not tolerated

Replacement of this nucleus with thiophene (but not pyridine) is tolerated and novel

All heterocyclic replacements for the fused thiophene nucleus resulted in loss of activity

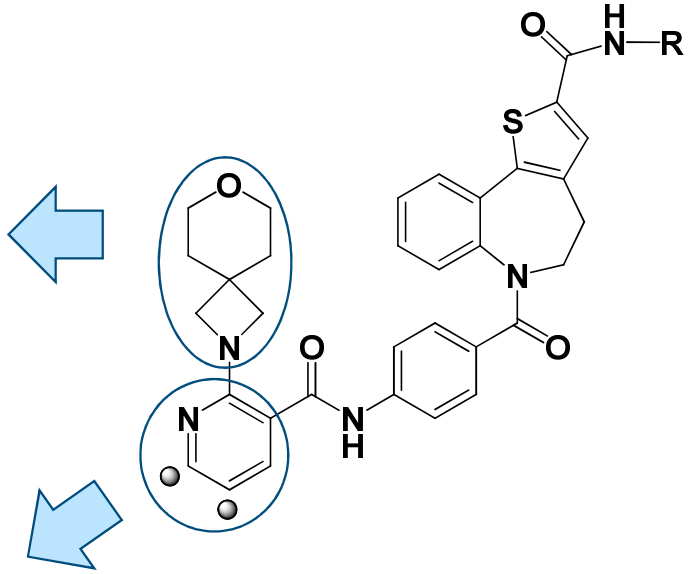
A lesson in frustration and humility

Hunt *et al.* **WO 2017/175000;** Fordyce *et al. Med. Chem. Commun.* **2018**, *9*, 583-589.

SAR: The Spirocycle and Nicotinamide Groups

Screen of different spirocycles and their replacements failed to identify any alternatives with superior activity

Changes to the size of heterocycles, and regioisomeric ethers (location of oxygen) offered no improvement over the parent and this group was retained as a preferred embodiment



Limited but useful changes discovered at this locus

Substitution in 5- and 6- positions preferred (●)

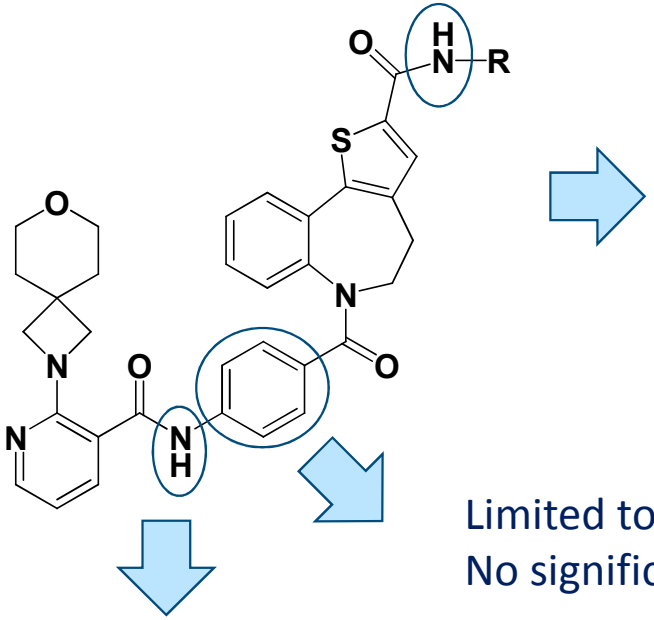
Strongly electron withdrawing groups disfavoured

Fused bicyclic systems also active (optionally substituted with O and S)

Active analogues offer potential for modulation of physicochemical properties, if required, such as log P and solubility profile

A novel, potency enhancing, alkyl pyridine adopted

SAR: The Amidic Linkages



Secondary amide is required for activity

Substitution on nitrogen either:
removes a discrete binding motif (the N-H)

or

changes the energetics of the amidic linkage making the bioactive conformation less readily attainable

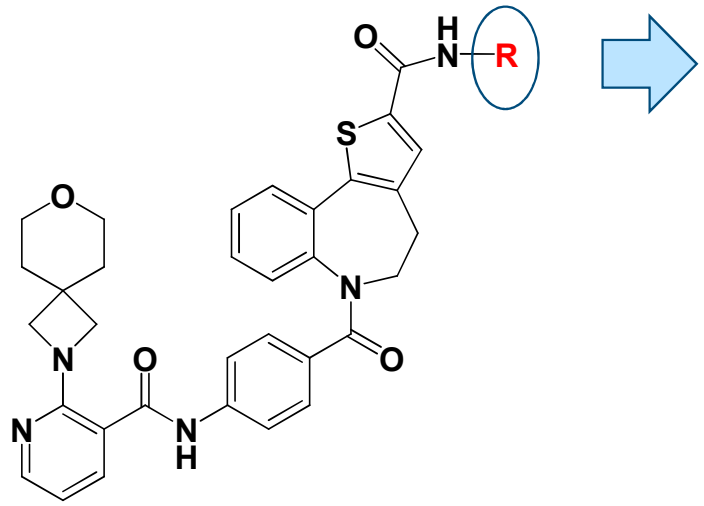
Limited tolerance for modification
No significant enhancement of anti-RSV activity

N-Substitution is detrimental to potency

Unavoidably perturbs space occupancy of the spirocyclic nicotinamide group

Observation is consistent with the importance of this domain for high pathogenicity

SAR: The Thiophene Carboxamide



Conversion of the thiophene carboxamide from an alkyl (R) to an aryl (Ar) derivative gave encouraging biodata

This family was not previously disclosed in the prior art

Exploration of the aniline derivative (Ar = Ph) confirmed that appropriate substitution enhanced the anti-viral activity of the resulting compounds

The 2,6 di-substitution pattern proved especially favourable

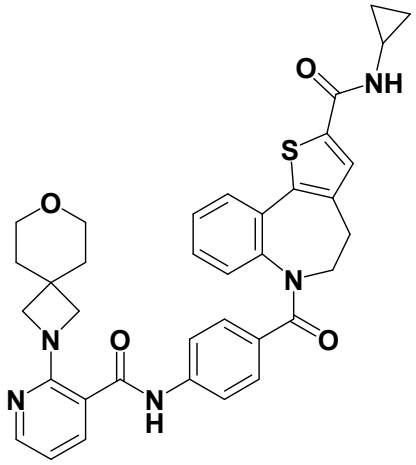
The absence of prior art teaching renders the genus novel and inventive: the principal criteria for patentability

Stage was set to file composition-of-matter patent applications to secure exclusivity and convert an exciting invention into a commercial opportunity

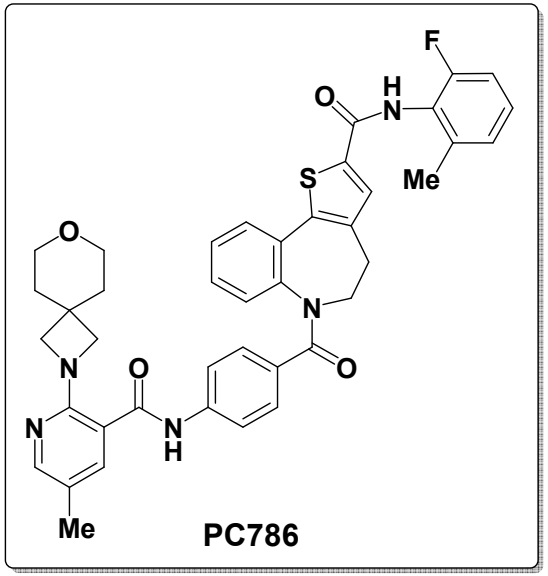
Sygnature's industry standard record keeping facilitated the process of compiling well documented and comprehensive patent applications

Hunt *et al.* **WO 2016/055792**

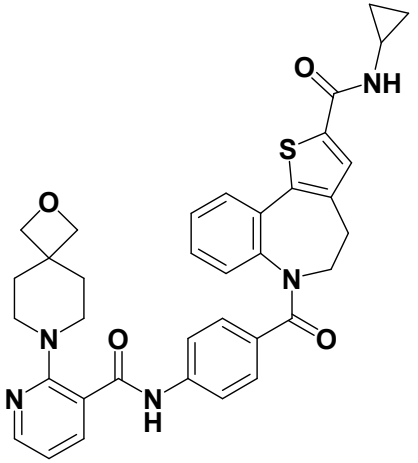
PC786: Profile of the Clinical Candidate



PC36



PC786



AZ-27

Anti-RSV Activity

	RSV CPE /HEp-2 Cells		Cellular Toxicity HEp-2 Cells	
	A2 Strain	B-WST Strain		
	IC_{50} (nM)	IC_{50} (nM)	CC_{50} (μM)	SI
PC36	2.1	280	6.4	10^3
PC786	0.3	6.5	>13	$>10^4$

Persistence of Action

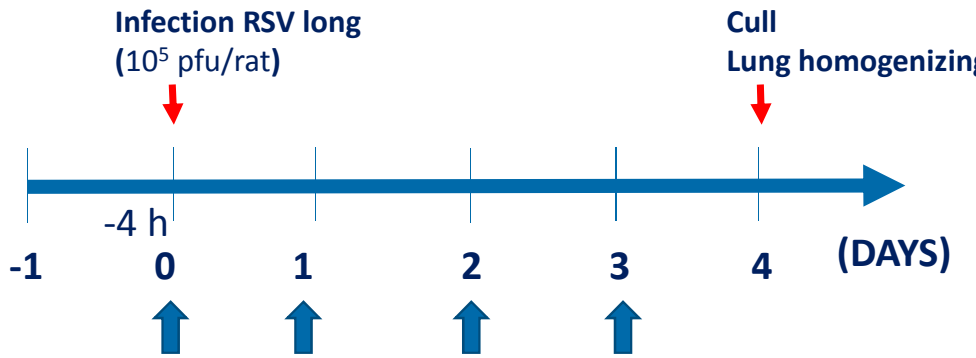
	RSV F-protein ELISA (BEAS-2B)		
	A2 IC_{50} (nM)		Activity Drop off
	No washout	-24 h	
AZ-27	3.17	106	33
PC786	0.13	0.79	6

Coates et al. *Antimicrob. Agents Chemother*. **2017**, *61*, e00737-17

In vivo Studies: The Cotton Rat

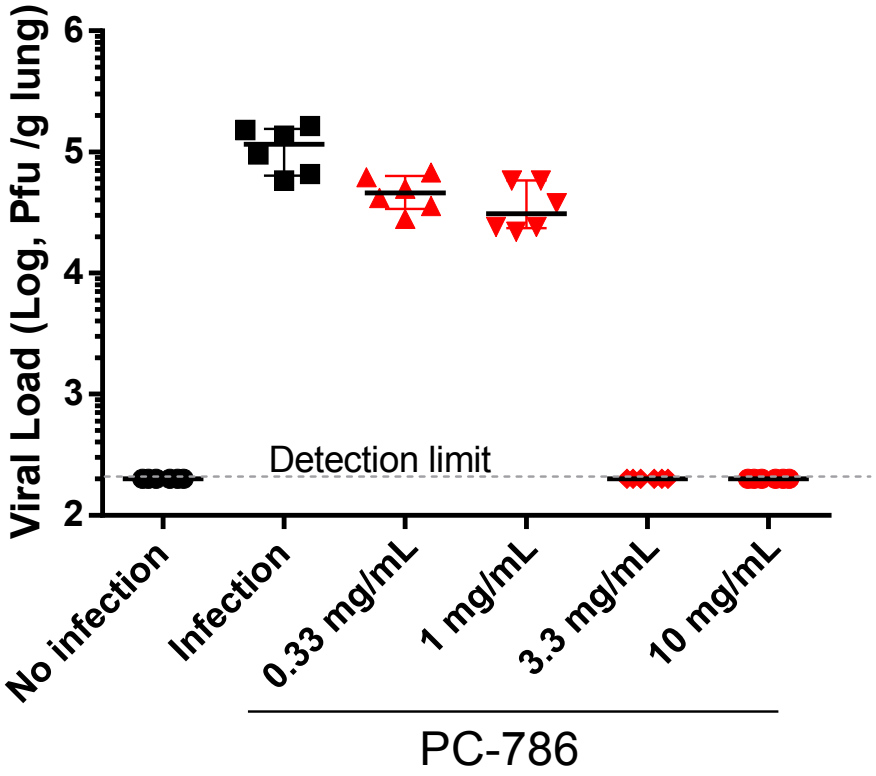
Study Outline:

Cotton rat (6-8 weeks old, male, approx. 100 g)
RSV Long, 10^5 PFU IN



Intranasal treatment at 0.3, 1.0 and 3.3 mg/mL on days 0, 1, 2 and 3

Virus load
(Plaque assay, and RT-PCR)



- **PC786** also showed a dose-dependent inhibition of RSV NS-1 gene transcripts and of RANTES and IP-10 transcripts in lung homogenates

Coates et al. *Antimicrob. Agents Chemother*. **2017**, *61*, e00737-17

Intellectual Property

(12) **United States Patent**
Hunt et al.

(10) **Patent No.: US 9,732,098 B2**
(45) **Date of Patent: Aug. 15, 2017**

(54) **5,6-DIHYDRO-4H-BENZO[B]THIENO-[2,3-D]AZEPINE DERIVATIVE**

(71) Applicants: **Simon Fraser Hunt**, Nottingham (GB); **Stuart Thomas Onions**, Nottingham (GB); **Vladimir Sherbukhin**, Nottingham (GB); **Euan Alexander Fordyce**, Nottingham (GB); **Peter John Murray**, London (GB); **Daniel William Brookes**, London (GB); **Kazuhiro Ito**, London (GB); **Peter Strong**, London (GB)

(72) Inventors: **Simon Fraser Hunt**, Nottingham (GB); **Stuart Thomas Onions**, Nottingham (GB); **Vladimir Sherbukhin**, Nottingham (GB); **Euan Alexander Fordyce**, Nottingham (GB); **Peter John Murray**, London (GB); **Daniel William Brookes**, London (GB); **Kazuhiro Ito**, London (GB); **Peter Strong**, London (GB)

(73) Assignee: **PULMOCIDE LIMITED**, London (GB)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **15/305,003**

(22) PCT Filed: **Oct. 8, 2015**

(86) PCT No.: **PCT/GB2015/052944**
§ 371 (c)(1),
(2) Date: **Oct. 18, 2016**

(87) PCT Pub. No.: **WO2016/055791**

(56) **References Cited**

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8,999,969 B2 4/2015 Mackman et al.

FOREIGN PATENT DOCUMENTS

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WO	2011/005842 A1	1/2011
WO	2011/046954 A1	4/2011
WO	2012/016217 A1	2/2012
WO	2012/129562 A2	9/2012
WO	2016/022464 A1	2/2016

OTHER PUBLICATIONS

Yajun Zheng et al.—Bioorganic & Medicinal Chemistry Letters 24 (2014) 3673-3682—"The use of spirocyclic scaffolds in drug discovery".
Sudo, et al.—Antiviral Research (2005) vol. 65: 125-131—"YM-53403, a unique anti-respiratory syncytial virus agent with a novel mechanism of action".
Xiong, et al—Bioorganic & Medicinal Chemistry Letters (2013) vol. 23 No. 24: 6789-6793—"Discovery of a potent respiratory syncytial virus RNA polymerase inhibitor".

Primary Examiner — Michael Barker
(74) *Attorney, Agent, or Firm* — Klauber & Jackson LLC

(57) **ABSTRACT**

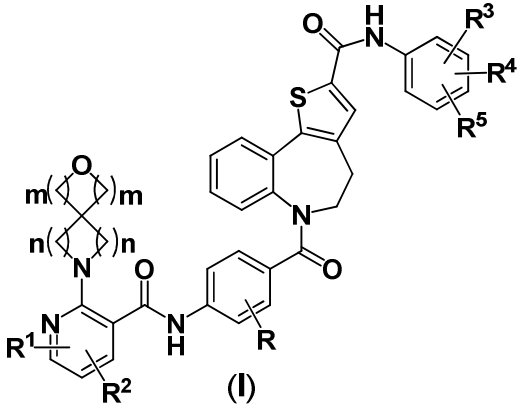
There is provided a 5,6-dihydro-4H-benzo[b]thieno-[2,3-d] azepine derivative which is useful in the treatment of respiratory syncytial virus (RSV) infection and for the prevention of disease associated with RSV infection. (Formula (1)).

(I)

Pulmocide's application in the US, covering **PC786** as a single entity, has already issued as a granted patent: US 9,732098

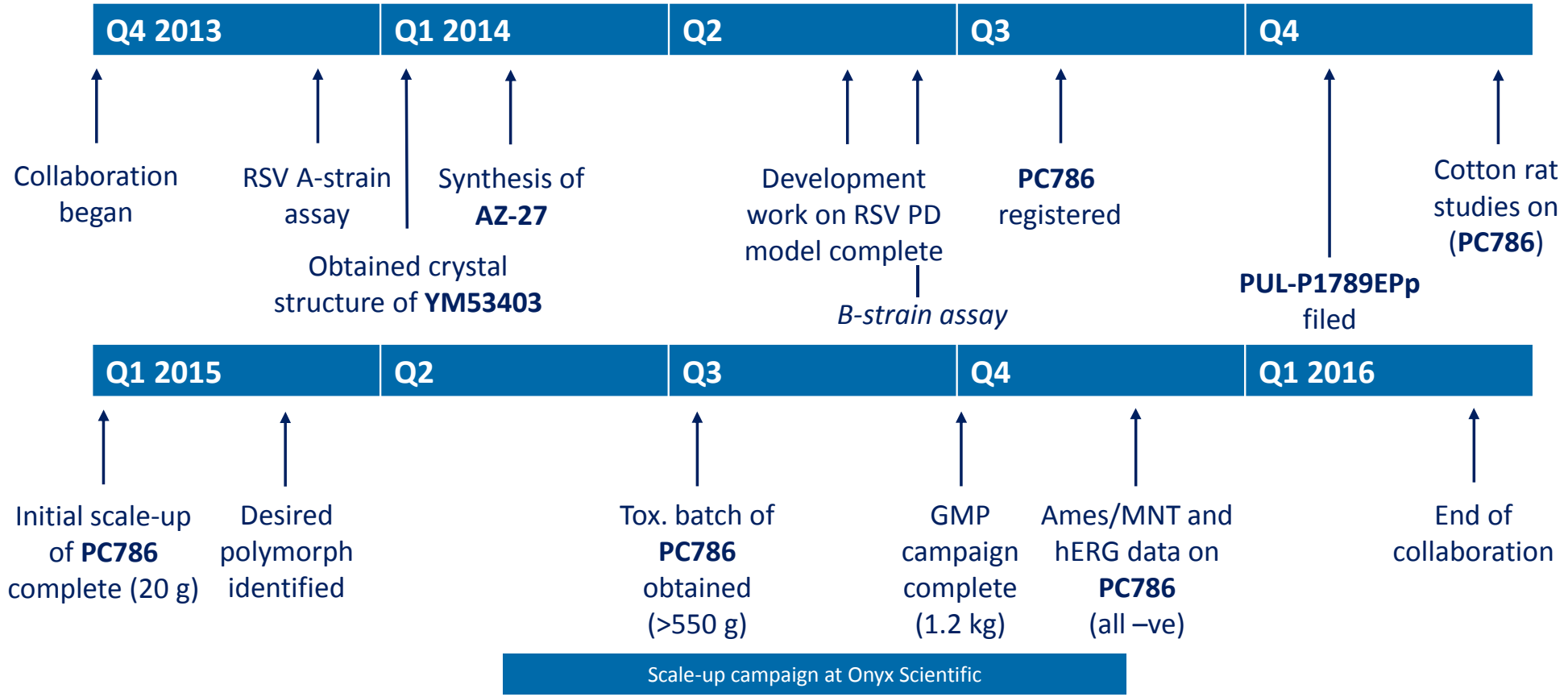
Of the eight co-inventors half are Sygnature scientists

A published PCT application (WO 2016/055792) reveals the full scope of the family of compounds that comprise Pulmocide's generic claims in this area



All of Pulmocide's Intellectual Property is filed and managed by Sagittarius IP; UK Chartered and European Patent Attorneys http://www.sagittariusip.com/

Timelines


Q4 2013
Q1 2014
Q2
Q3
Q4
Collaboration began
RSV A-strain assay
Synthesis of AZ-27
Obtained crystal structure of YM53403
Development work on RSV PD model complete
B-strain assay
PC786 registered
PUL-P1789EPp filed
Cotton rat studies on (PC786)
Q1 2015
Q2
Q3
Q4
Q1 2016
Initial scale-up of PC786 complete (20 g)
Desired polymorph identified
Tox. batch of PC786 obtained (>550 g)
GMP campaign complete (1.2 kg)
Ames/MNT and hERG data on PC786 (all –ve)
End of collaboration
Scale-up campaign at Onyx Scientific

Observations on the Collaboration

- The collaboration has delivered a patentable NCE into clinical development
- **PC786** is a potent anti-viral agent that has improved potency and selectivity against the two principal stains of the pathogenic organism and a profile consistent with a long duration-of-action *in vivo*
- The collaboration has created a portfolio of intellectual patent rights, ensuring the invention can be exploited as a commercial opportunity
- Key to success has been the complementary experience and expertise of the Pulmocide and Sygnature Teams and an investment by both parties in building strong interpersonal relationships
- Pulmocide has contributed a world-class awareness of the characteristics required of an inhaled medicine and the advanced biological screening technologies to identify exceptional candidates
- Sygnature have demonstrated ‘intellectual ownership’ of the medicinal chemistry enterprise and brought to bear their cutting edge synthetic expertise, creativity, and problem solving skills to this successful joint enterprise
- It has also been tremendous fun!

Acknowledgements

Pulmocide	Sygnature Discovery	Consultants
Dr Garth Rapeport	Dr Stuart Onions	Dr Alan Naylor[1]
Dr Peter Strong	Fraser Hunt	John King-Underwood[2]
Dr Kaz Ito	Dr Jennifer Stockwell	Dr Harry Finch[1]
Dr Matthew Coates	Dr Jennifer Thomas	
Dr Daniel Brookes	Dr Claire-Lise Ciana	
	Dr Guillaume Parra	
	Vladimir Sherbhukin	

1. Freelance Medicinal Chemistry Consultant
2. Computational Chemistry Resource, john@compchemres.co.uk

imperial innovations

SV Life Sciences

Fidelity Life Sciences

Johnson & Johnson INNOVATION

Post Script

“It doesn’t make sense to hire smart people and then tell them what to do;

we hire smart people so they can tell us what to do’’

Steve Jobs

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Bioorganic & Medicinal Chemistry Letters

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

Discovery of novel benzothienoazepine derivatives as potent inhibitors of respiratory syncytial virus

Euan A.F. Fordyce [a,*], Daniel W. Brookes [b], Claire Lise-Ciana [a], Matthew S. Coates [b], S. Fraser Hunt [a], Kazuhiro Ito [b,*], John King-Underwood [c], Stuart T. Onions [a], Guillaume F. Parra [a], Garth Rapeport [b], Vladimir Sherbukhin [a], Jennifer A. Stockwell [a], Peter Strong [b], Jennifer C. Thomas [a], John Murray [b]

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[b] Pulmocide Ltd., Imperial Biolncubator, Level 1 Bessemer Building (RSM), Imperial College, London SW7 2BP, United Kingdom
[c] CompChem Resource, Old Cottage Hospital, Homend, Ledbury, Herefordshire HR8 1ED, United Kingdom

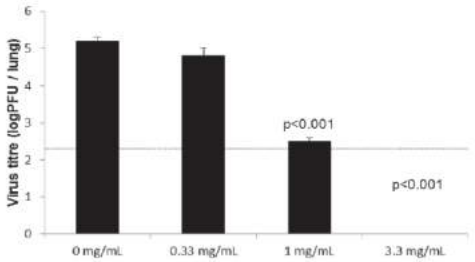
ARTICLE INFO

Article history:
Received 24 January 2017
Revised 15 March 2017
Accepted 20 March 2017
Available online 23 March 2017

Keywords:
RSV
Polymerase inhibitor
Antiviral

ABSTRACT

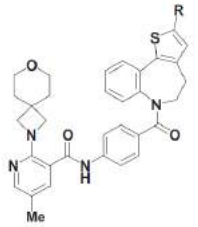
The development of novel non-nucleoside inhibitors of the RSV polymerase complex is of significant clinical interest. Compounds derived from the benzothienoazepine core, such as AZ-27, are potent inhibitors of RSV viruses of the A-subgroup, but are only moderately active against the B serotype and as yet have not demonstrated activity *in vivo*. Herein we report the discovery of several novel families of C-2 arylated benzothienoazepine derivatives that are highly potent RSV polymerase inhibitors and reveal an exemplary structure, compound **4a**, which shows low nanomolar activity against both RSV A and B viral subtypes. Furthermore, this compound is effective at suppressing viral replication, when administered intranasally, in a rodent model of RSV infection. These results suggest that compounds belonging to this chemotypes have the potential to provide superior anti-RSV agents than those currently available for clinical use.

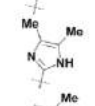


a. Dotted line indicates lower limit of quantitation (LOQ)
b. Statistical analysis: Kruskal Wallis-Dunn's multiple comparison

Fig. 3. The effects of intranasal treatment with Compound **4a** on RSV A Long viral titre in lung from RSV A Long infected cotton rats.[a,b]

Table 4
Antiviral activity and cytotoxicity for compounds **4a–e**.[a]



Entry	Compound	R	IC_{50}/nM		CC_{50}/nM
			A2	B-WST	
1	**3c**		0.8	5.2	>14,600
2	**4a**		1.4	2.1	>14,300
3	**4b**		1.8	14.4	>14,600
4	**4c**		0.7	8.1	>14,600
5	**4d**		1.6	6.2	>15,100
6	**4e**		0.7	3.3	>15,500

[a] IC_{50}: the concentration of compound that reduced by 50% the cytopathic effect (CPE) of RSV infection in HEp-2 cells; CC_{50}: the concentration of compound that is cytotoxic towards 50% of uninfected HEp-2 cells; Data were averaged from ≥2 experiments.

MedChemComm

RESEARCH ARTICLE

Cite this: *Med. Chem. Commun.*, 2018, 9, 583

Conformationally restricted benzothienoazepine respiratory syncytial virus inhibitors: their synthesis, structural analysis and biological activities†

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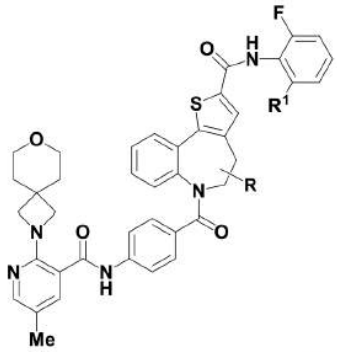
Atropisomeric drug substances are known to have different biological properties. Compounds containing the *N*-benzoylbenzazepine motif have been shown to exhibit energetically restricted rotation around the Ar(CO)N axis. Herein we report, for the first time, the synthesis, physical characterisation and anti-viral profiles of a series of C-4 and C-5 methylated thieno-benzazepines. NMR analysis reveals that incorporation of a single additional substituent at either of these loci influences the conformational dynamics of the azepine ring system. In the case of the C-5 alkyl analogues, the influence of the new stereocentre is so pronounced that its absolute configuration determines which unique atropisomer is obtained following the generation of the benzazepine nucleus. Screening of the alkylated derivatives for their anti-respiratory syncytial virus (RSV) activity indicates that the desired viral pathogenicity is strongly associated with the conformation adopted by the modified tricyclic scaffolds. This is particularly evident in the case of the C-5 homologues in which one atropisomer was found to be potently active and the other essentially inert. These results provide compelling evidence that we have determined the bioactive conformation shared by RSV inhibitors that employ the thienobenazapine nucleus as their core molecular architecture. Furthermore, the understanding obtained from these studies may make it possible to design improved agents against RSV infection in the future.

Received 19th January 2018,
Accepted 16th February 2018

DOI: 10.1039/c8md00033f

rsc.li/medchemcomm

Table 1 Anti-RSV activity and mammalian cytotoxicity for compounds 8a, 14 and 20



Entry	Compound	R	R^1	IC_{50} values[a] (nM) A2[b]	B-WST[c]
1	**8a**	H	Me	0.32	7.2
2	(*rac*)-**14**[d]	4-Me	Me	1.1	17
3	(*rac*)-**20**	5-Me	F	0.45	13
4	(4*R*)-**14**[d,e]	4-Me	Me	0.51	15
5	(4*S*)-**14**[d,e]	4-Me	Me	>14[f]	>1370
6	(5*R*)-**20**	5-Me	F	0.18	9.2
7	(5*S*)-**20**	5-Me	F	>14[g]	>1363[h]

[a] IC_{50}: drug concentration that reduces by 50% the cytopathic effect (CPE) of RSV infected HEp-2 cells (ATCC® CCL-23™); data were averaged from ≥2 experiments. [b] NCPV, Public Health England (Wiltshire, UK); lot number 0709161v. [c] ATCC (Manassas, VA, USA); lot number VR-1580. [d] Present as a 5 : 3 mixture of atropisomeric diastereomers. [e] Compound arbitrarily assigned this absolute stereochemical configuration. [f] 40% inhibition at 0.014 μM. [g] No inhibition observed up to 0.014 μM. [h] No inhibition observed up to 1.4 μM.

Sygnature Group Overview

PULMOCIDE
INHALED RESPIRATORY MEDICINE

SYGNATURE DISCOVERY

SYGNATURE DISCOVERY

- Founded in 2004 in BioCity (Nottingham, UK)
- Provide high quality integrated or single discipline drug discovery support to pharma, biotech, medical charities and academics with funding
- Enviable track record of success in drug discovery
  - 14 compounds into the clinic (Phases I and II) since 2011
  - Additional 14 compounds into pre-clinical development
- 240 staff (as of September 2018)
  - 80% of scientists have PhDs
  - Considerable pharmaceutical industry R&D experience
- Private equity-backed company since September 2017
  - Senior management team are co-investors
  - Financially stable
  - Investment to fund expansion of capabilities & capacity

RenaSci

- Founded in 2001 in BioCity (Nottingham, UK)
- Acquired by Sygnature Discovery in July 2018
- Highly-experienced team (40 staff) provide a blend of consultancy and pre-clinical experimental services
- Provide *in vivo* testing in drug abuse and dependence, CNS, obesity, NASH, and diabetes and its complications
- Collaborate with clients at all stages of the drug discovery and development process - from target identification to post registration
- Have facilitated more than 30 NCEs into clinical development and 10 drugs to the market

PEAK PROTEINS

- Affiliate company and strategic partner for protein production and crystallography
  - Highly experienced team (ex-AZ) with excellent track record in X-ray structure determination and protein biochemistry
  - Protein expression in a range of systems up to 20 litre scale
  - Based in Alderley Park (near Manchester, UK)