

Summary

A novel series of compounds have been identified with potent anti-picornaviral activity, acting through a host cell mechanism; these compounds have good drug-like properties. Optimised compounds within the series have the potential to be effective treatments of the infections that are responsible for a large proportion of the exacerbations in COPD and asthma.

Introduction

Picornavirus infections are responsible for a variety of human diseases, some of which are serious or life-threatening. Notable picornavirus species include:

Rhinovirus

- Major cause of upper respiratory tract infections
- Major cause of exacerbations in asthma, COPD, cystic fibrosis
- Two groups of rhinovirus
 - Major group (90%) use ICAM-1 for entry into cells
 - Minor group use LDL receptor family

Poliovirus

- Largely eradicated by vaccination
- Not effectively implemented in some areas
- Need for specific antivirals post-eradication

Coxsackievirus

- Causes aseptic meningitis, serious neonatal disease, myocarditis, CNS infections, hand, foot & mouth disease

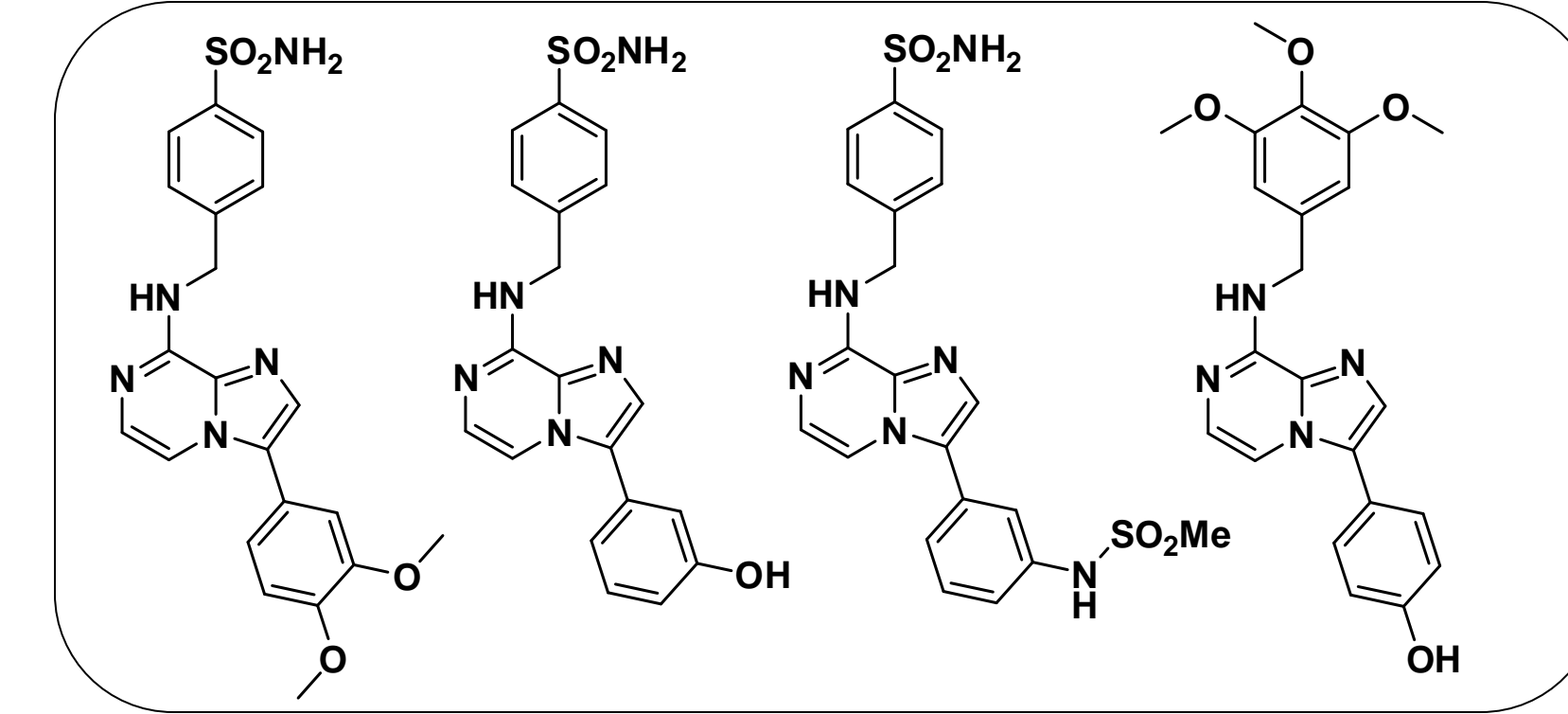
There are currently no approved drug treatments for picornavirus infections

Several candidate drugs have been assessed in the clinic:

- Pirodivir (Janssen) - Phase II, intranasal, insufficient efficacy
- Rupintrivir (Agouron/Pfizer) - Phase III, inhaled, insufficient efficacy
- Pleconaril (Sanofi) Phase III, insufficient efficacy, and (Schering-Plough) Phase II inhaled
- BTA798 (Biota) currently in phase II clinical trials (oral)

Identification of novel antiviral compounds

- Antiviral screening was carried out using the BioFocus SoftFocus® collection
- Small clusters of hits were identified
- One series based around the SFK28 library scaffold returned 25 structurally related hits with evidence of SAR



- Broad antiviral profiling revealed activity against a range of + ssRNA viruses:

Poliovirus Rhinovirus Coxsackievirus Enterovirus Hepatitis C

Mechanism of action

- Pleconaril and BTA798 bind to the viral capsid - prevent entry into cells

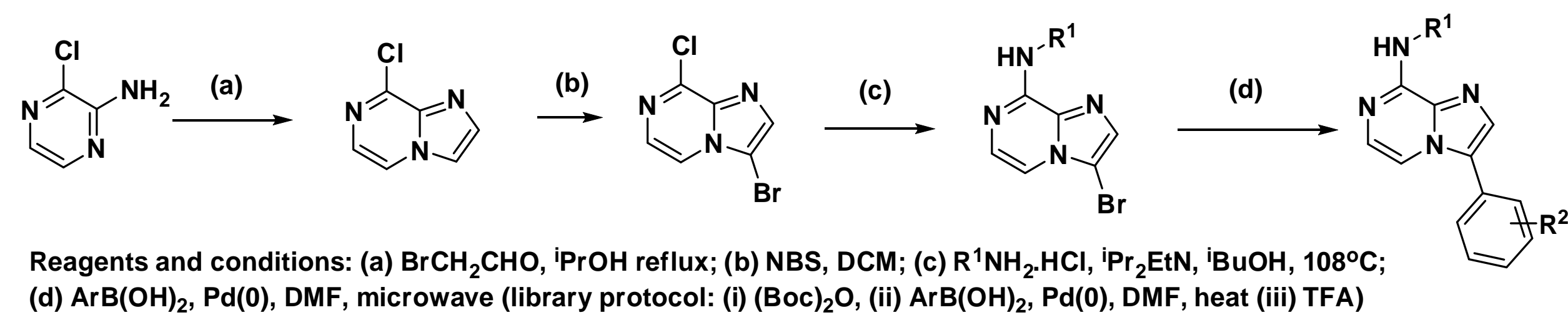
Time of addition studies – variation of compound treatment

	Pleconaril	Compound 1
Untreated control	No inhibition	No inhibition
Pre-treatment (-1 h)	Inhibition	Inhibition
Post-treatment (+1 h)	No inhibition	Inhibition
Post-treatment (+2 h)	No inhibition	Inhibition

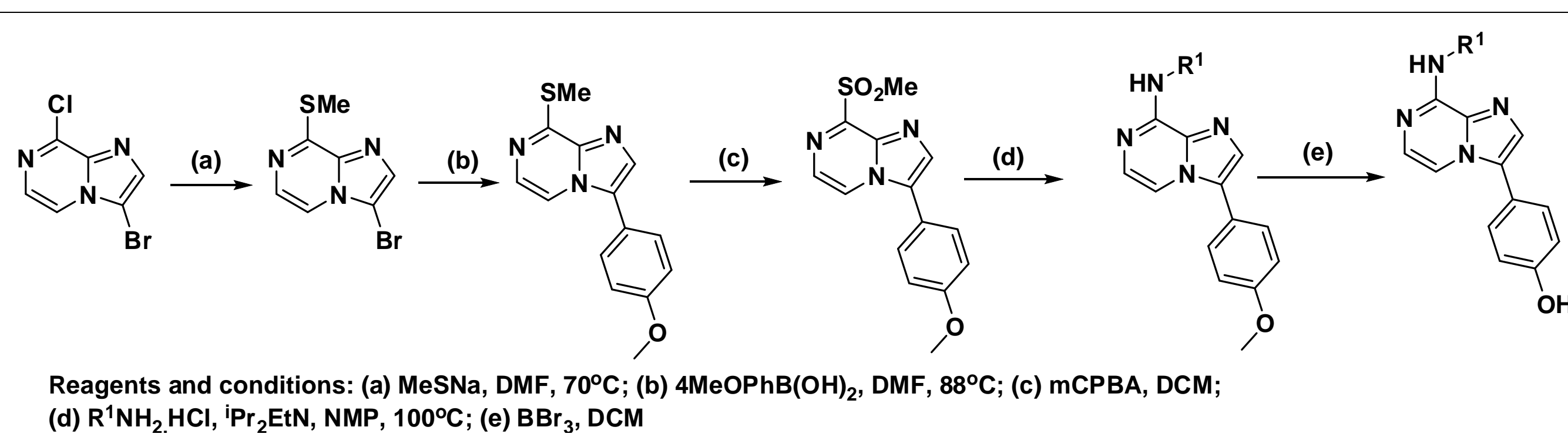
- Novel compounds distinguished from capsid binders

Hit-to-Lead program: synthesis of compounds

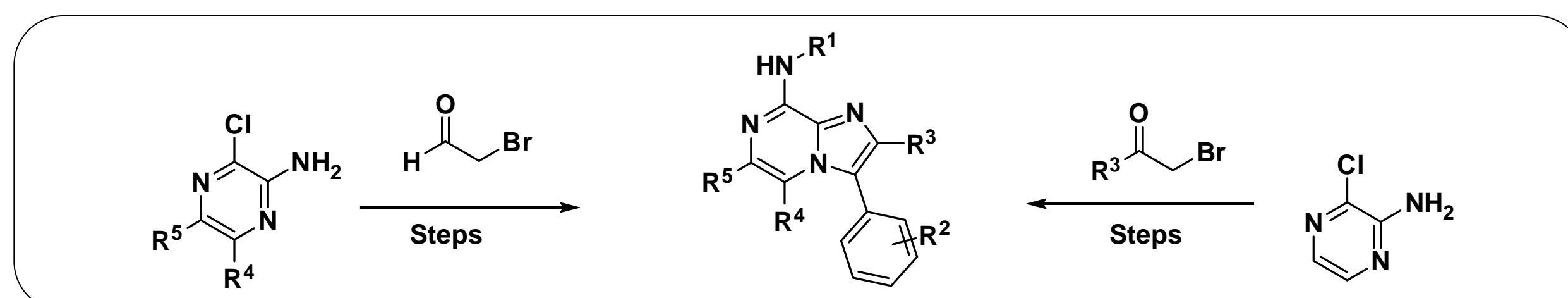
- Synthetic route developed for the SFK28 library was used to prepare an initial sub-library of analogues
- Main improvement to the library protocol was the alteration of the final Suzuki coupling such that a protecting group was no longer required



- Further adaptation was the use of a sulfone intermediate to allow the 8-substituent to be introduced late in the synthetic sequence

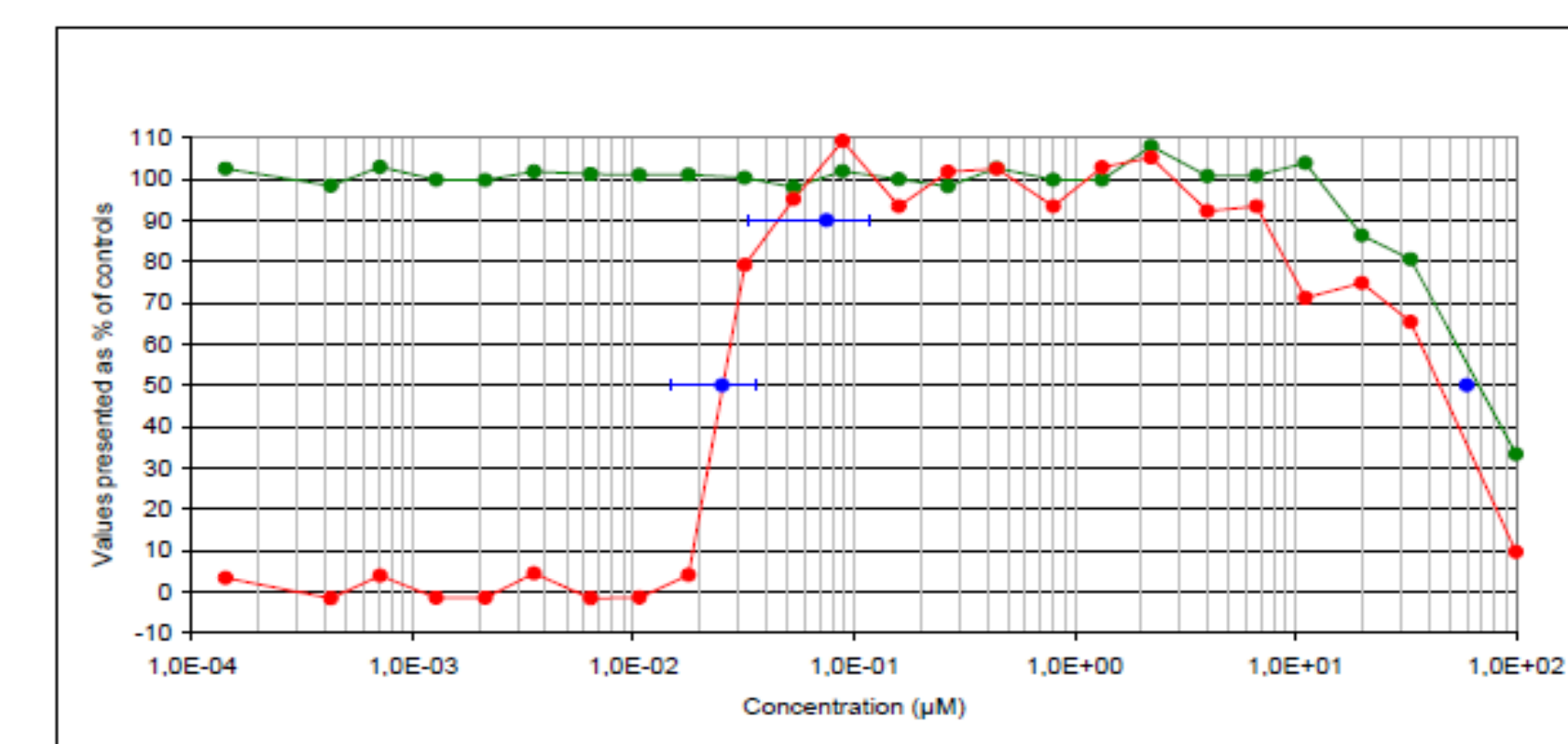


- Further adaptations allowed small alkyl groups to be introduced into the 2-, 5- and 6- positions



Lead optimisation

- Series was optimised primarily against HRV using HRV-14 as the primary screen
- Vero or Hela Rh cells incubated with virus and test compound for 2-3 days; cytopathic effect then evaluated
- Dose response plotted to determine antiviral (EC₅₀) and cytotoxic effects (CC₅₀)



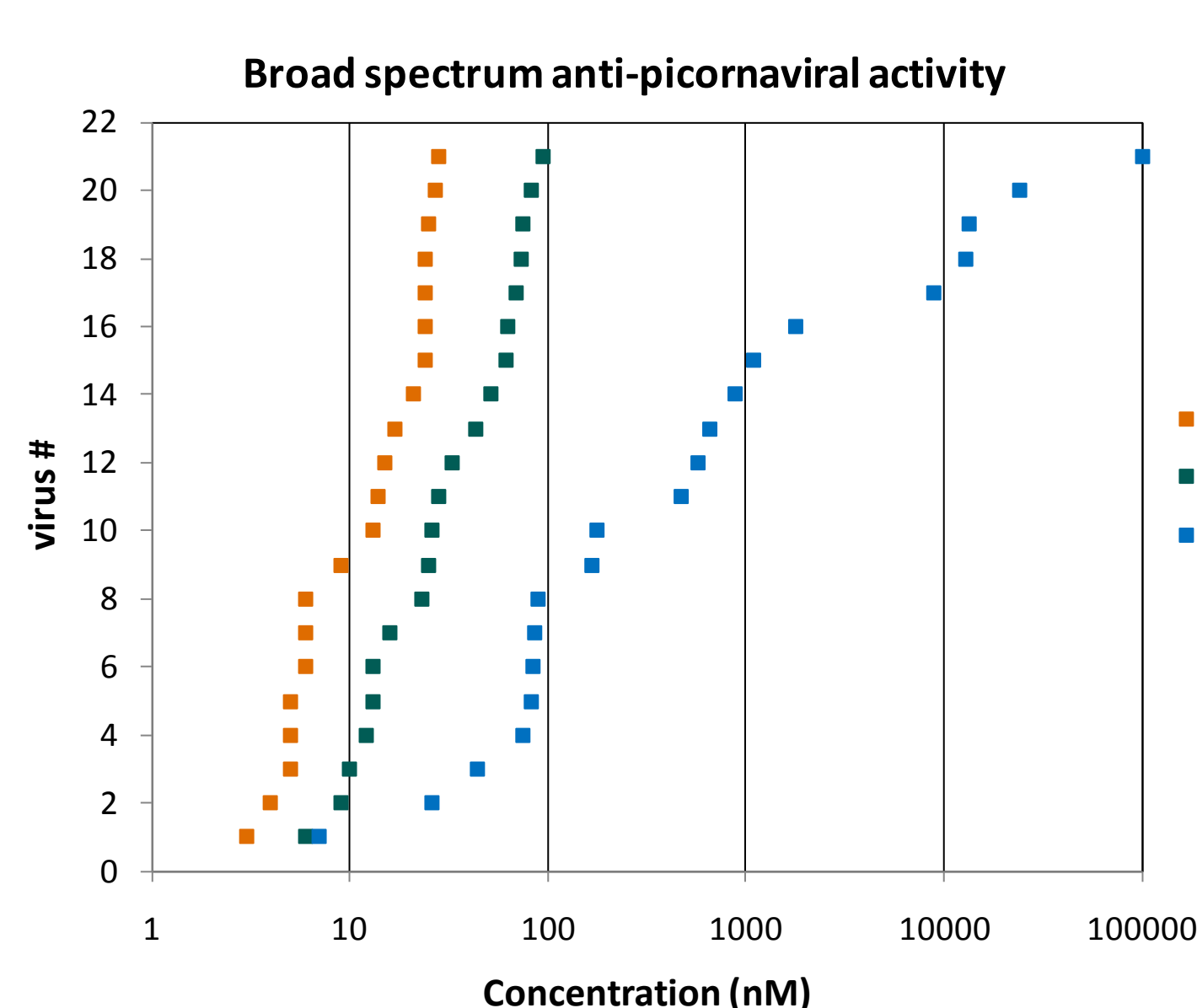
- Compounds of interest tested against additional +ssRNA viruses (activities in µM)

	Compound 1			Compound 2			Compound 3		
	EC ₅₀	CC ₅₀	EC ₉₀	EC ₅₀	CC ₅₀	EC ₉₀	EC ₅₀	CC ₅₀	EC ₉₀
HCV	0.07	41.2	6.2	0.02	>100	11.3	0.1	47.7	4.6
HRV-14	0.04	>100	0.06	1.1	>100	3.7	0.04	>100	0.1
PV-1	0.04	>100	0.17	0.59	>100	1.9	0.04	>100	0.18
CV-B3	0.07	>100	0.14	1.9	>100	3.7	0.07	>100	0.14
EV-71	0.01	>100	0.05	0.37	>100	0.59	0.03	>100	0.09

Broader profiling

- Selected compounds were profiled against a broader panel of picornaviruses
 - 17 serotypes used as representative of HRV species (based on K. Andries et al, *Antiviral Res.* 1991, 16, 213-225)

Comparison with Pleconaril

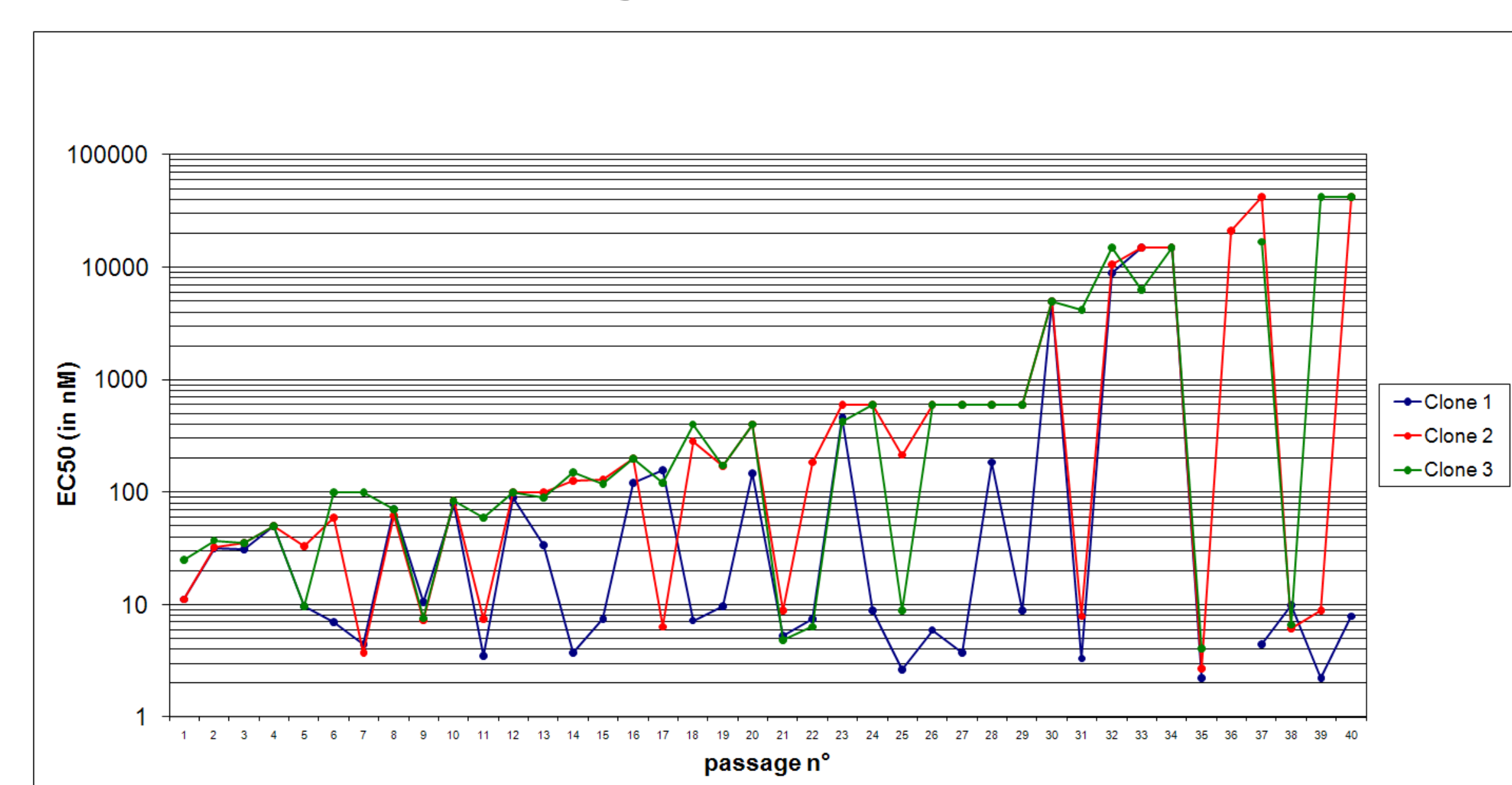


	Compound 4	Compound 5	Pleconaril
HRV2	9	16	7
HRV9	6	13	83
HRV14	14	28	177
HRV15	21	33	166
HRV29	5	9	26
HRV39	4	10	44
HRV41	17	23	655
HRV42	27	61	100000
HRV45	25	83	8900
HRV59	6	12	471
HRV63	6	6	85
HRV70	24	26	578
HRV72	28	95	1800
HRV85	24	75	883
HRV86	24	69	89
HRV89	24	43	75
CVB3	3	63	13000
EV71	5	25	13400
PV1	15	74	24200
PV2	13	52	1084
PV3	5	13	84

Broad kinase and receptor profiling

- Representative compounds profiled against a broad panel of receptors and enzymes
- Very clean profile - no significant activity found (compared to antiviral potencies)
- No activity against panels of up to 402 kinases

Target elucidation



EC₅₀ evolution against CVB3

Resistance mutation study carried out using coxsackievirus CVB3

- Emergence of resistance much slower than for capsid binders
- No significant mutations found in structural protein coding region
- H57Y mutation observed in viral non-structural protein 3A

- 3A interacts with host cell proteins important for viral replication
 - Hsu et al. *Cell* (2010), 141, 799-811

Additional studies indicate compounds selectively inhibit a lipid kinase essential for replication of certain viruses

Further MOA studies will be published in due course