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Non-benzimidazole containing inhibitors of respiratory syncytial virus

David C. Pryde ^{a,*}, Thien-Duc Tran ^a, Iain Gardner ^b, Helen Bright ^c, Paul Stupple ^a, Sebastien Galan ^a, Liam Alsop ^a, Lesa Watson ^a, Donald S. Middleton ^a, Satish Dayal ^b, Michelle Platts ^a, Edward J. Murray ^c, Tanya Parkinson ^c, Robert Webster ^b

^a Worldwide Medicinal Chemistry, Pfizer Global R and D, Ramsgate Road, Sandwich, Kent CT13 9NJ, UK ^b Pharmcokinetics, Dynamics and Metabolism, Pfizer Global R and D, Ramsgate Road, Sandwich, Kent CT13 9NJ, UK ^c Antiinfectives Biology, Pfizer Global R and D, Ramsgate Road, Sandwich, Kent CT13 9NJ, UK

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ABSTRACT

Several non-benzimidazole containing inhibitors of respiratory syncytial virus are described. Core template modification, analysis of antiviral activity, physicochemistry and optimisation of properties led to the thiazole-imidazole **13**, that showed a good potency and pharmacokinetic profile in the rat. © 2012 Elsevier Ltd. All rights reserved.

Human respiratory syncytial virus (RSV) is an important pathogen that affects the respiratory system.¹ It is a negative sense RNA virus of the *Paramyxoviridae* family responsible for a range of respiratory tract diseases in people of all ages and geographical locations.² RSV infection is usually restricted to being an infection of the upper respiratory tract that causes generally mild symptoms but in the immunocompromised and other susceptible populations such as newborn babies and the elderly, can progress to infect the lower respiratory tract and lead to serious secondary infections such as bronchiolitis and pneumonia.³ The first step in the infection of host cells by RSV involves fusion via the RSV F protein, which initiates a series of conformational changes that ultimately allow fusion of the viral and cellular membranes to take place.⁴

The literature describes a number of small molecule inhibitors of the RSV F protein.⁵ Most small molecule F protein inhibitors, for example compounds **1–3** (Fig. 1),⁶ are based on a common benzimidazole core template, and were generally identified from viral replication assays and subsequently found to generate resistance mutations mapping to the F protein. The most advanced of these, **1**, demonstrates excellent antiviral potency in a moderately lipophilic structure, consistent with a high ligand efficiency (LE) of 0.38 and lipophilic efficiency (LipE) of 5.5.

In some cases, direct binding to the F protein has also been demonstrated using radiolabelled compounds.⁷ Several of these

* Corresponding author. Tel.: +44 1304 643687. *E-mail address:* David.Pryde@pfizer.com (D.C. Pryde). compounds have been shown to be efficacious in rodent RSV infection models when administered up to 24 h post-infection.^{8,6a} While no small molecule F-protein inhibitors have progressed to late stage clinical studies, there is a marketed humanised monoclonal antibody (Palivizumab/Synagis) which targets the F protein and is effective in reducing viral load and subsequent morbidity and mortality associated with infection,⁹ validating this mechanistic approach for RSV therapy.

In this manuscript, we describe structure–activity relationships that allowed us to identify several novel, non-benzimidazoles based on the structures contained in Figure 1 that retained good levels of RSV antiviral activity and for some examples, an encouraging pharmacokinetic profile.

The substituted benzimidazole analogues described in several papers and reviews by the Bristol-Myers Squibb group⁵ and exemplified by the alcohol **1** showed good antiviral potency. In in vitro metabolism studies the compound was shown to be metabolized in liver microsomes from mouse and rat (Clint_{app} 53 and 66 μ l/min/mg protein, respectively). In incubations with rat liver microsomes three mono-hydroxylated oxidative metabolites of **1** were identified. In human liver microsomes turnover of **1** could not be observed under standard assay conditions (Clint_{app} < 8 μ l/min/mg protein) but in human hepatocytes measurable clearance was observed (Clint_{app} 11 μ l/min/10⁶ cells) indicating that the compound was also potentially metabolized by non-P450 drug metabolizing enzymes in human. In addition compound **1** had a short half-life following intravenous dosing to the rat ($T_{1/2}$ 0.4 h). We were

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Figure 1. Some literature RSV fusion inhibitors.

interested in exploring novel structural modifications that would allow for enhanced antiviral potency and/or pharmacokinetic improvements over **1**. In particular, we aimed to improve lipophilic efficiency (LipE)¹⁰ to maximise our chances of combining high potency with a low LogD at which we could achieve appropriate metabolic stability. We aimed to optimise substituents in the lipophilic regions of **1** (i.e., the benzo ring of the benzimidazole and the cyclopropyl region of the imidazolone), by either changing the nature of the lipophilicity, or by adding a polar group, without compromising ligand efficiency (LE)¹¹ or greatly increasing molecular size.¹²

All compounds synthesised through the programme were profiled for antiviral activity, lipophilicity, passive permeability (through MDCK cells grown from clones selected to have low



Scheme 1. Reagents and conditions: (a) *c*-PrNH₂, EtOH, 80 °C; (b) (i) H₂, Pd(OH)₂/C, EtOH; (ii) CDI, THF, 0 °C to rt, 49% over 3 steps; (c) HBr (48% aq), DMSO, 60 °C, 48%; (d) benzyloxyacetaldehyde, NH₄OH, EtOH, rt, 25%; (e) *t*BuCOCI, TEA, DCM, 0 °C-rt, 75%. (f) Cs₂CO₃, DMF, rt, quant; (g) BBr₃, DCM, -50 °C, 57%; (h) **6**, DIAD, PPh₃, THF, 0 °C-rt, 30%; (i) NaOH, H₂O/THF, rt, 64%.



Scheme 2. Reagents and conditions: (a) (i) *t*-BuOK, THF, rt, 1 h; (ii) *i*-PrI, rt, 18 h; (iii) LiAlH₄, THF, 0 °C, 3 h, 76% (over 3 steps); (b) PBr₃, toluene, 120 °C, 1 h, 98%; (c) (i) Et₃N, DCM, rt, 3 h; (ii) NH₂NH₂.H₂O, EtOH, rt, 18 h, 61% (over 2 steps); (d) NaH, DMF, rt, 18 h, 74% (mixture N1/N2); (e) LiAlH₄, THF, 0 °C, 2 h, 82% (mixture N1/N2); (f) DDQ, dioxane, 110 °C, 18 h, 10% (+30% of the N1 diastereoisomer).

expression of efflux transporters) and human liver microsomal stability measurement.

Representative syntheses of the compounds contained within this paper are shown in Schemes 1 and 2. For example, biaryl targets such as the imidazole-thiazole **13** were accessed through building up the imidazole ring as described in Scheme 1.

4-Methoxy-3-nitro-pyridine 4 was subjected to displacement with cyclopropylamine followed by nitro group reduction of 5 and cyclisation with 1,1'-carbonyldiimidazole to give the imidazolone derivative 6. 2-Acetyl-thiazole 7 was oxidised to the dihydroxyketone 8, and then converted to the substituted imidazole 9 using benzyloxyacetaldehyde and ammonium hydroxide. 9 was alkylated with the bromide **11** prepared from 1-bromo-butanol **10** to give the intermediate **12**, which was then taken through to the final target **13** by following the steps outlined in the schemes above. The second scheme, Scheme 2, shows a method of synthesising an indazole template analogue. Commercially available indazole-3-carboxlic acid ethyl ester 14 was alkylated with isopropyliodide and reduced to give the alcohol **15**¹³ which was then brominated to give 16. In parallel, N-morpholino-1-cyclohexene 17 was reacted with hydrazine and methyl-4-(chloroformyl)-butyrate **18** to give the tetrahydro-indazole **19**.¹⁴ This was coupled to the bromide **16** and the ester group reduced to give the alcohol 20. Treatment of 20 with DDQ then afforded the fully aromatic indazole **21**. It should be noted that the alkylation step produced both N1 and N2 substituted indazoles, in favour of the undesired N1 isomer, but that both isomers were readily separable by column chromatography at the final step. Further details of the synthesis of other examples from this paper are contained in the Supplementary data.

Having established robust synthetic routes to access a variety of target structures, our investigations started by expanding the benzimidazolone substituent to incorporate larger groups, or those featuring hydrogen bonding functionality. One of the first



Figure 3. Truncated imidazolone analogues.

compounds synthesised was the piperidine-carbamate **22** (Fig. 2) that displayed a significant jump in antiviral activity, albeit in a more lipophilic template which introduced metabolic liability. By then trimming back the benzimidazole ring to a simple dimethyl imidazole **23**, a much more polar compound was accessed that showed excellent lipoidal lipophilic binding efficiency, and left significant headroom for adjusting polarity to design back in membrane permeability.

By retaining the benzimidazole ring of **22**, but trimming back the benzimidazolone heterocycle, the pyrazole **24** (Fig. 3) was also obtained, which lost all potency. The truncated indazole piperidine **25** was also inactive in the antiviral assay. Taking the inactive **25** as a starting point and building back in larger heterocycle substituents provided access to moderately potent inhibitors exemplified by the bis-indazole **21**.

Encouraged by the moderate levels of potency in this compound, we explored the central indazole ring to assess how much more broadly tolerated different bicyclic heterocycles might be, and how physicochemistry and ADME properties were affected. These data are shown in Table 1. Simple insertion of an oxygen atom into the side chain of **21** gave the ether **26** of some fivefold weaker potency than **21**. Subsequent lengthening of the side chain of **26** by a single methylene group to **27** provided a significant increase in potency, while the truncated side chain in **28** was largely equipotent with the starting **21**. An amide linking group in the side chain of **31** was significantly weaker. Inserting an oxo group to give the indazolone **29** was well-tolerated, while reduction of the indazole to the semi-saturated pyrazole bicycle **30** lost potency.

Compounds **32** and **33** finally confirmed that the connectivity of the substituents on the core template was quite specific; the modest structural changes in these analogues were not tolerated. Nonetheless, we had quickly established some novel chemistry starting points, with compounds such as **27** in particular showing good antiviral activity. We remained concerned about the high lipophilicity of **27** and its very modest LipE and the next set of targets sought to address this.

We next returned to analogues based on the imidazole **23**.Simple structural changes to the benzimidazole core generally met with mixed results. Reducing the benzimidazole to the partially saturated analogue **34** (Fig. 4) gave a threefold increase in activity with an accompanying small increase in lipophilicity and an increased metabolic liability. Paring back the benzimidazole ring to a dichloro-imidazole **35** was some eightfold less potent, although ligand binding efficiency was maintained and physico-chemical properties were now much more drug-like than the starting **23**. Smaller imidazole substituents, or the introduction of polar moieties as seen in the methyl ether **36** were generally poorly tolerated, consistent with the imidazole group binding in a lipophilic environment.

Having established that a non-benzimidazole core template was viable, and single heterocycles were suitable scaffolds for new RSV inhibitors, imidazole SAR was developed further. Compounds that were of intermediate lipophilicity between **35** and **36** were proposed, for example the ester **37** (Fig. 5) and the pyridyl-imidazole **38**. This latter compound in particular combined good potency and reasonable permeability in a more polar structure with excellent stability in human liver microsomes and hepatocytes (Clint_{app} <8 μ /min/mg and 2.2 μ /min/10⁶ cells, respectively). Despite the improved metabolic stability in human in vitro systems this compound was still metabolized relatively rapidly by rat liver microsomes (Clint_{app} 44 μ /min/mg) and in an in vivo pharmacokinetic study in the rat showed high plasma clearance (Cl = 95 ml/min/kg) and a comparable half-life to **1** ($T_{1/2}$ = 0.4 h).

Nevertheless, encouraged by the potency and physicochemistry of the pyridyl analogue **38**, we embarked on an SAR investigation around this lead to improve its pharmacokinetic profile. Analogues of the free hydroxyl group of **38** were prepared in an attempt to improve the permeability of the series, and are presented in Table 2. Several heterocycles were inserted in this position, with some subtle SAR observed. While the oxazole **42** lost some 10-fold potency, the isoxazole **39** was some twofold more active, and the isomeric isoxazole **41** was some fourfold more potent. The methyl-pyridyl analogue **40** was of intermediate potency, IC₅₀ 96 nM.

Ultimately, no compounds from this effort showed significant improvements in the combined properties of permeability in the MDCK model, potency and stability in human liver microsomes and no further compounds were made in this avenue of investigation.

We next turned our attention to the pyridyl ring as shown in Table 3. Substituted 5-membered heterocycles, for example, the methyl substituted pyrazole **43**, imidazole **46** and the thiadiazole **48** were all weak in the antiviral assay. Similar 6-membered heterocycles to the starting pyridine showed comparable levels of activity, with the pyrimidine **44** and the 2-pyridyl **45** having an IC₅₀ of some 230 nM. Some of the most interesting compounds came from making simple 5-membered unsubstituted heterocyclic substituents. The oxazole **47** had an IC₅₀ of 95 nM, while the thiazole **13** had an IC₅₀ of 48 nM, some fivefold improvement over the starting pyridine, which took the ligand efficiency of this compound to 0.35 and the LipE to over 5.

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Table 1

Indazole analogues of compound 21



Compd	Structure	AV $IC_{50} (nM)^a$	LE (LipE)	MWt.	Log D ^b	HLM ^c (µl/min/mg)	MDCK (Papp $ imes 10^{-6} \text{ cm/s})^d$
26		992	0.30 (2.1)	364	3.5	246	16
27	HO	65	0.35 (3.3)	378	3.9	ND	ND
28	OH OH	277	0.34 (2.7)	348	3.8	78	14
29		169	0.33 (3.9)	378	2.9	462	32
30		409	0.30 (2.2)	367	3.6	>320	12
31	HN HO	2930	0.26 (3.1)	391	2.4	ND	ND
32		22700	0.23 (1.0)	362	4.4	242	8

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Table 1 (continued)



^a Values are means of at least two experiments. For details of the assay used, see Ref. 15.

^b LogD measured at pH 7.4 in octanol/neutral buffer.

 $^{\rm c}\,$ Details of the HLM assay used have been published previously. $^{\rm 16}\,$

^d Methodological details for the MDCK permeability assay have been published previously.¹⁷ ND = not determined.



Figure 5. Substituted imidazole analogues.

The metabolic stability of compounds **38**, **44**, **45** and **13** were further investigated in human hepatocytes. All compounds had low rates of metabolism (Clint_{app} $\leq 2.2 \,\mu$ l/min/million cells) in this in vitro system suggesting that the free hydroxyl group was not extensively glucuronidated.

Permeability in the MDCK assay¹⁷ was uniformly low to moderate in this set of relatively polar structures. The permeability of the compounds contained in Table 1 were notably higher, but also notably more lipophilic and less metabolically stable. It became apparent at this stage of our investigations that balancing permeability and stability in a potent structure would be a significant challenge in the series. Plotting human liver microsomal stability



Analogues of the hydroxyalkyl side chain of 38



Compd	Ar	AV IC ₅₀ (nM) ^a	LE (LipE)	MWt.	Log D ^b	HLM ^c (µl/min/ mg)	$\begin{array}{l} \text{MDCK} \\ (Papp \times \\ 10^{-6} \text{ cm} / \\ \text{s})^{d} \end{array}$
39	N O N	139	0.30 (4.9)	427	1.4	17	4
40		96	0.29 (4.4)	437	1.5	32	2
41		41	0.31 (5.4)	427	1.4	ND	3
42		2230	0.25 (3.6)	413	0.9	10	ND

^a Values are means of at least two experiments. For details of the assay used, see Ref. 15.

^b Log*D* measured at pH 7.4 in octanol/neutral buffer.

^c Details of the HLM assay used have been published previously.¹⁶

^d Methodological details for the MDCK permeability assay have been published previously.¹⁷ ND = not determined.

and MDCK permeability against cLogP is shown in Figure 6 and illustrates that for a cLogP of below 2 permeability is consistently low and metabolic stability is greatest, as expected, but that above 2 being able to retain good microsomal stability and permeability is much less predictable. It is also clear from the plot that there were no examples from within the series that showed high microsomal stability above cLogP of 2. Our attention therefore focussed on the more polar examples from the series, and based on compound potency, moderate permeability and low metabolic rate in rat liver microsomes in vitro (Clint_{app} 21.1 µl/min/mg protein) compound **13** was selected for an in vivo pharmacokinetic study in the rat (Fig. 7).

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Table 3

Imidazole heterocycle analogues



Compd	Ar	$AV^{a} IC_{50} (nM)$	LE (LipE)	MWt.	Log D ^b	HLM ^c (µL/min/mg)	MDCK (Papp $ imes 10^{-6} \text{ cm/s})^{d}$	Hheps (μ L/min/10 ⁶ cells
38	N	265	0.31 (5.2)	390	1.2	<8	4	<2.2
43	N N N	>20000	<0.22 (<3.9)	393	0.9	<8	2	ND
44	N	235	0.30 (5.3)	405	0.6	<8	1	<2.2
45	N	233	0.30 (5.0)	404	1.4	<8	5	<2.2
46	N N	10400	0.23 (4.7)	393	0.8	<8	1	ND
13	S N	58	0.35 (5.3)	396	1.3	<8	4	<2.2
47	N	95	0.34 (5.9)	380	0.6	<8	1	ND
48	N N S Jun	5110	0.25 (4.1)	411	0.9	<8	1	ND

^a Values are means of at least two experiments. For details of the assay used, see Ref. 15.

^b Log*D* measured at pH 7.4 in octanol/neutral buffer.

^c Details of the HLM assay used have been published previously.¹⁶

^d Methodological details for the MDCK permeability assay have been published previously.¹⁷ ND = not determined.





Figure 7. Selected in vitro and in vivo data for compound 13.

oral dosing in the rat the compound had a bioavailability of 34%, showing good absorption (>60%) despite the relatively modest permeability observed in in vitro cell-based experiments. Good exposure was also seen following oral dosing in the mouse. While no data was generated with any of the compounds described in this study to confirm their pharmacological target, it is assumed that they are acting upon the F protein by analogy to the compounds depicted in Figure 1. Further experiments to evaluate the in vivo efficacy of **13** against RSV will be reported in due course.

Figure 6. HLM stability (left *y*-axis) and MDCK permeability (right *y*-axis) plotted against cLogP (*x*-axis) for the compounds described in this Letter.

The compound had a comparable clearance (48 ml/min/kg) but a larger Vd_{ss} (3.5 L/kg) and longer half-life (1.5 h) than compound **1**. About 16% of the dose of compound **13** was recovered unchanged in the urine of the rats showing that in vivo it is cleared by a mixture of metabolic and non-metabolic pathways. Following

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.11. 062.

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