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Effect of lipophilicity modulation on inhibition of human rhinovirus capsid binders

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ABSTRACT

To try and generate broad spectrum human rhinovirus VP1 inhibitors with more attractive physicochemical, DMPK and safety profiles, we explored the current SAR of known VP1 compounds. This lead to the identification of specific structural regions where reduction in polarity can be achieved, so guiding chemistry to analogues with significantly superior profiles to previously reported inhibitors.

Human rhinovirus (HRV) is a sub-class of the picornaviridae family and is associated with several respiratory diseases such as the common cold, chronic obstructive pulmonary disease (COPD) and asthma.[1] Although HRV infections are mostly mild and self-limiting they represent a significant economic burden, especially in loss of working hours to society and commerce.[2] This global financial burden is increased further by recent findings that HRV is a common pathogen associated with acute exacerbations in asthma and chronic obstructive pulmonary disease.[3–7] The capsid shell of human rhinovirus consists of 60 units, each comprised of four proteins (VP1–4). VP1, 2 and 3 form a canyon-like cleft, which the virus uses to attach to host cells as the first stage towards entry.[8] The HRV family has been sub-divided into two groups, depending on which protein is utilised for viral attachment to the host cell. The major group (~90% of serotypes) binds intercellular adhesion molecule 1 (ICAM-1), whereas the minor group utilise the low-density lipoprotein receptor (LDLr). An alternative classification of HRV serotypes also exists, based on sequence homology (HRV-A, -B and -C).[9,10] The ICAM-1-utilising major group is represented in both A and B homology groups. All minor group serotypes fall into the HRV-A group.[9] The third group, HRV-C, has only been recently identified.[10] The receptor specificity and inhibitor sensitivity of serotypes within this group is as yet unknown.

At the base of the capsid canyon, VP1 (along with components of VP3) forms a conserved hydrophobic pocket. Virus binding to ICAM-1 or LDLr destabilises the capsid unit, resulting in viral uncoating, an essential early step in the viral replication cycle.[1,8] In the late 70's, a series of compounds was discovered from screening of compound libraries for their ability to inhibit rhinovirus-induced cytopathic effect (CPE) in cell culture.[11] The mechanism of action of these compounds was subsequently found to be via binding to and stabilisation of the hydrophobic pocket of VP1. Subsequently, inhibitors such as Pleconaril (**1**) were identified and demonstrated a significant reduction in viral load[12,13] in clinical trials against the common cold. Pleconaril induced CYP3A4 expression in human studies and was subsequently not approved by the FDA.[14,15] Subsequent in vitro studies showed **1** induced CYP3A4 in human hepatocytes with an pEC_{50} of 4.8.[16] This effect can potentially be attributed to the lipophilic nature of **1**, which is a common feature of the VP1 inhibitors that have been published.[1,8] Related second generation inhibitors include the pyridazine analogues **2** and **3**.[17,18]

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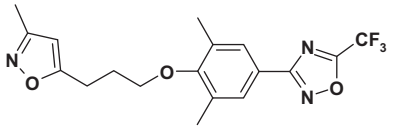
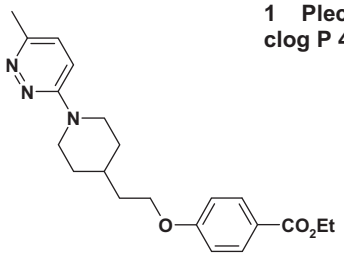
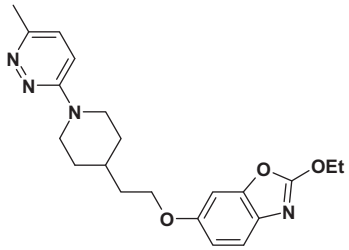


1 Pleconaril
clog P 4.0 logD >4.5



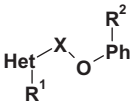
2 Pirodavir
clog P 4.2 logD 4.4



3
clog P 4.4

Like **1**, this series of compounds also have undesirably high calculated lipophilicity values ($c\text{Log}P$[19]) > 4. Following the seminal work of Lipinski, there is a growing body of evidence that molecules which are hydrophobic tend to show greater levels of promiscuity and subsequently have higher rates of attrition due to safety in oral drug development.[20–22]

We envisaged that if a broad serotype inhibitor of VP1 could be identified with an acceptable lipophilicity profile (ideally $c\text{Log}P < 3$) then it could be a more useful therapy for several respiratory indications. Most VP1 inhibitors consist of the general pharmacophore, shown below.

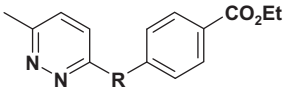


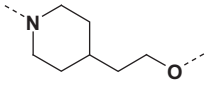
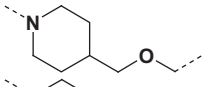
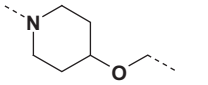
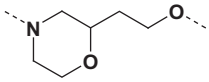
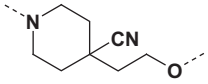
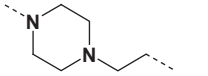
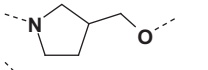
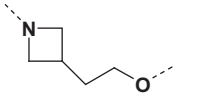
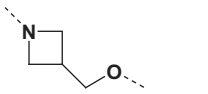
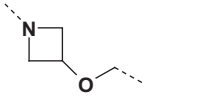
Our strategy was to probe the SAR of the linker (X–O) and the right hand aromatic (Ph–R^2) to see where reductions in lipophilicity could be achieved without impacting on VP1 inhibition. The pyridazine motif, present in **2** and **3** was considered a useful pharmacophore that brought a good balance of VP1 potency and cross serotype activity whilst being adequately polar ($c\text{Log}P = -0.083$[19]) and was thus kept constant during our early assessment. HRV serotype 16 was selected for initial VP1 inhibitory evaluation, as it is a member of the largest HRV cluster (major class A serotype). Compounds were tested for their ability to inhibit HRV16 induced CPE in HeLa OHIO cells.[23] Pirodavir (**2**) was chosen as a reference and initial SAR exploration around the linker unit (X–O) was undertaken. Several cyclic amines possessing different polarities, substitution vectors and linker lengths were evaluated as part of this process, together with varying the position of the ether motif. These changes generated quite significant differences in lipophilicity values and data for key analogues is highlighted in Table 1.

Several modifications are tolerated for inhibiting HRV16 induced CPE, especially where lipophilicity is significantly (>1 unit) reduced, compared to **2**. Some of the key variations include moving the ether away from conjugation with the aromatic ring, which produces a 1 unit reduction in lipophilicity. Various heterocyclic ring systems also provide good inhibition, in some cases showing profound changes in $c\text{Log}P$, LE[24] and LLE.[21] Attempts to add polar substituents at the bridgehead of the piperidine (e.g., **7**) did reduce $c\text{Log}P$, but were less effective at inhibiting HRV16 effects. Additionally it's well known that increasing structural complexity through introduction of chiral centres can have a beneficial effect on parameters like solubility.[25] Compounds such as **6** and **10** showed good inhibition of HRV16 induced CPE. Evaluation of the enantiomeric preferences weren't carried out as part of the initial evaluation.

Attention then focused on the right hand aromatic ring and substituents (O–Ph–R^2) primarily looking to increase polarity

Table 1
Linker SAR



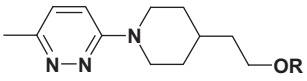
Compd	R	HRV16 pIC_{50}[a]	$c\text{Log}P$	LE[b]	LLE[c]
2		7.2	4.2	0.27	3
4		7.0	3.2	0.26	3.8
5		7.6	2.6	0.29	5
6		7.2	3.2	0.27	4
7		6.8	2.9	0.23	3.9
8		7.1	3.4	0.27	3.7
9		7.7	3.1	0.31	4.6
10		7.5	3.1	0.3	4.4
11		7.2	2.6	0.3	4.2
12		7.2	2.8	0.3	4.4

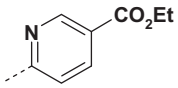
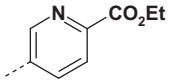
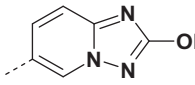
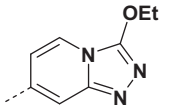
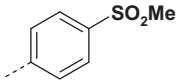
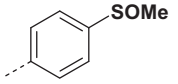
[a] Values mean of $n = 2$ experiments.
[b] LE (Ligand efficiency)* Values are calculated as pIC_{50}/heavy atom count.
[c] LLE (Lipophilic ligand efficiency)* Values are calculated as $pIC_{50} - c\text{Log}P$.

through either the addition or repositioning of heteroatoms. This is summarised in Table 2.

Changes in this region were not as successful as in the linker unit and indeed conversion of the phenyl ring to pyridine was the only modification that maintained potency whilst reducing lipophilicity. However the more basic *ortho* pyridyl ester (**13**) was ~1.5 log units less active, though LLE, compared to **2**, is the same. Further attempts to modify the bi-cycles by introduction of heteroatoms at the bridgehead were unsuccessful as was attempts to replace the ester with more polar isosteres.

Table 2
Capping motif SAR



Compd	R	HRV16 pIC_{50}	*c*Log*P*	LE	LLE
13		7.2	3.5	0.27	3.7
14		5.6	2.7	0.21	2.9
15		5.8	3.4	0.21	2.4
16		5.8	3.2	0.21	2.6
17		5.7	2.8	0.21	2.9
18		<4.5	2.8	—	—

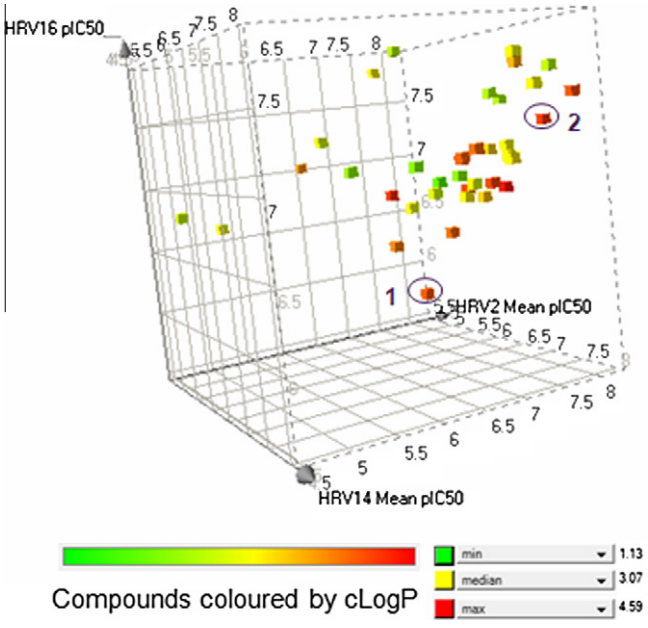


Figure 1. A plot of pIC_{50} values for HRV serotypes 16, 14 and 2. Each point is coloured according to its *c*Log*P* value. Compounds **1** and **2** are highlighted as references.

Following this preliminary SAR assessment, more detailed analysis was undertaken across the series to evaluate other VP1 serotype inhibition. In addition to screening HRV16, active compounds were also tested against HRV14 (major class B serotype) and HRV2 (minor class A serotype). Although this only represents 3% of the HRV serotype family, sequence analysis suggests these are the best combination to efficiently ascertain the spectrum of activity.[26] Selectivity across these three serotypes is shown in Figure 1. Each point is coloured according to its *c*Log*P*. There are examples of compounds with pIC_{50} values >7 against all three serotypes (located in the top right hand corner of Fig. 1) and in most of these cases the LLE[21] are >4, demonstrating broad serotype coverage can be achieved with compounds that are not highly lipophilic. Templates from Table 1 with broad spectrum CPE inhibition include **2**, **4**, **8**, **10**, and **11**. The other templates showed at least a log unit reduction in potency against either or both HRV 14 or 2 CPE.

In order to evaluate physicochemical and DMPK parameters of the series, the ester motifs of analogues in Table 1 with CPE $pIC_{50} > 6$ against HRV serotypes 16, 14 and 2 were replaced with more stable H bond acceptor analogues, based on literature SAR.[1,8,11,17,18] Compounds were evaluated in a range of physicochemical and in vitro DMPK assays to understand the effect of reducing lipophilicity on these parameters. Figure 2 highlights the effect of *c*Log*P* on two key parameters, solubility (Fig. 2a, weak linear trend, R^2 value of 0.3, *p* value?) and human plasma protein binding (Figure 2b, good correlation, R^2 value of 0.95, *p* value?). Protein binding is not a key parameter in its own right for compound progression, but does have strong contribution to defining whole blood potencies and in vivo pharmacokinetic profiles. The ability to accurately predict this value would be beneficial for future compound prioritisation.

Metabolic stability, measured in human microsomes and rat hepatocytes did not correlate with lipophilicity, though there are weak trends associated with CYP inhibition and *c*log*P*.

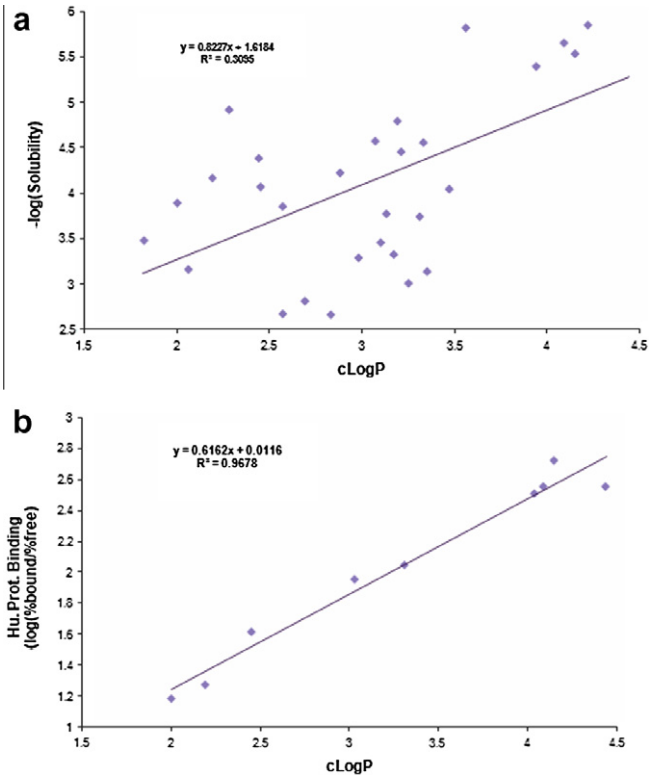


Figure 2. Correlation plots.

As Pleconaril (**1**) has been shown to induce CYP3A4[14] it was important to assess compounds in a suitable in vitro screen to gauge the effects of lipophilicity on this parameter. The pregnane X receptor (PXR) is activated by a range of compounds that induce CYP3A4, including dexamethasone and rifampicin.[27] Subsequently, compounds were tested in a PXR reporter gene assay, as a surrogate marker for possible CYP3A4 induction liabilities.[28] Compounds **1** and **3** did show maximal effects in this assay, exhibiting free absence of protein binding? pEC_{50} of 6.6 and 7.8, respectively, (absolute maximal effect?). Broader testing showed a trend with lipophilicity across the series (Fig. 3, R^2 value of 0.6). Extrapolating from this small data set reinforces the hypothesis that targeting a lower lipophilicity range is likely to reduce CYP induction risks.

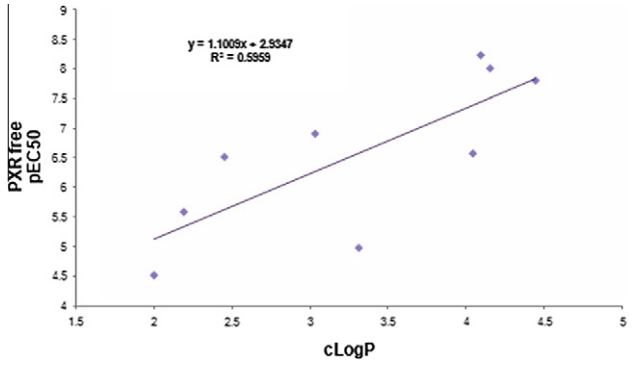


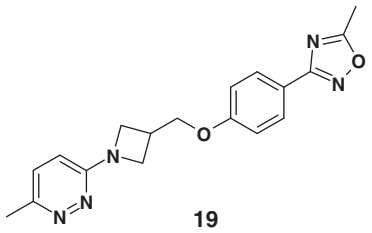
Figure 3. Plot of *c*Log*P* versus PXR free pEC_{50}.

We successfully generated compounds (e.g., **19**) that met our Lead Optimisation criteria by combining our own early SAR findings with that in the literature (Table 3).

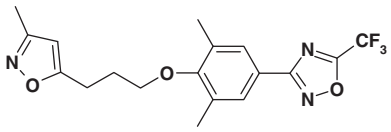
The profile of **19** is encouraging when compared to **1,** with greatly increased solubility and free fraction**,** alongside significantly lower activity in the PXR assay. Human microsomal Cl_{ints} are modest, with metabolite identification studies showing hydroxylation of the methyloxadiazole as the major product. It has previously been shown that replacement of the methyl motifs appended to oxadiazole analogues which are closely related to **1**, results in significant increases in microsomal stability.[12] Reduction in lipophilicity has a benefit on CYP inhibition profiles, with **19** being clean against all 5 isoforms tested.

These studies demonstrate that broad spectrum VP1 inhibition can be achieved with compounds possessing significantly reduced lipophilicity to those reported in the literature. The physicochemical and most importantly predicted in vitro safety profiles of compounds like **19** are superior to compounds that have failed to progress through clinical development. This will increase confidence that VP1 inhibitors can de discovered that possess *c*Log*P* profiles <3 and exhibit a broad HRV serotype activity. Compounds such as **19** can potentially provide a foundation to identify analogues having a better chance of successfully progressing to market for HRV related viral diseases.

Table 3
Profiles of **19** and **1**



19



1

Parameter	Lead criteria[29]	**19**	**1**
HRV16, 2 pIC_{50}	pIC_{50} > 7	7.1, 7.3	6.0, 7.0
*c*Log*P*/Log*D*	<3.0	2/2.6	4/>4.5
Mol wt	<450	337	381
LE (HRV16)		0.29	0.22
LLE (HRV16)		5.1	2
Solubility[30]	>100 μM	125 μM	<2 μM
Hu/rat			
Prot binding	% free	6/5	0.13/<0.2
Hu Mics[a]	<30 μL/min/mg	26	22
Rat Heps[b]	<15 μL/min/10^6cells	5	20
Chem stability [31]	$t_{½}$ > 100 h	>200	ND[c]
PAMPA			
(Papp 10^{-6} cm/s)		47	ND[c]
Cyp Inhib pIC_{50}[d]	pIC_{50} < 5	<5 (5/5)	<5 (3/5) 5.9[e], 5.2[f]
CypTDI[g]	pIC_{50} < 5	<4.3(5/5)	<4.3(5/5)
PXR			
(%E_{max}, pEC_{50})		19%, 4.5	88%, 6.6
Rat PK Cl/$t_{1/2}$/V_{dss}/F[h]	$t_{1/2}$ > 1 h	9/2.2/1.5/60	NT

pIC_{50} values are the means of at least three experiments.
[a] Human microsome metabolism intrinsic clearance (μL/min/mg).[32]
[b] Rat Sprague–Dawley hepatocyte metabolism intrinsic clearance (μL/min/10^6 cells).[33]
[c] ND–not determined.
[d] Inhibition of cytochrome P450 isoforms: 1A2, 2C9, 2C19, 2D6 and 3A4.
[e] CYP 3A4 pIC_{50}.
[f] CYP 2D6 pIC_{50}.
[g] TDI–time dependant inhibition
[h] Compounds dosed at 2 mg/kg iv, n = 2 animals (Cl, mL/min/kg, $t_{1/2}$, h, V_{dss}, L/kg, F,%)

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.08.083.

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