Scientific
Research

Identification of HCV Inhibitors from a Cell-Based Sub-Genomic Replicon Screen

David C. Pryde[1]*, Thien-Duc Tran[1], Mark Gardner[1], Chris Pickford[2], Stephen M. Shaw[2], Mike Westby[2], Tanya Parkinson[2], Caroline Smith-Burchnell[2], Malcolm Macartney[2], John H. Tatlock[3], Rohit Duggal[4], Leena B. Patel[3], Tanya M. Jewell[3], Scott C. Sutton[3], Tin H. Tran[3], Weidong Hao[3], Rob Webster[5], Satish Dayal[5]

[1]Worldwide Medicinal Chemistry, Pfizer Global Research and Development, Sandwich, UK
[2]Anti-Infectives Research Unit, Pfizer Global Research and Development, Sandwich, UK
[3]Worldwide Medicinal Chemistry, Pfizer Global Research and Development, San Diego, USA
[4]Anti-Infectives Research Unit, Pfizer Global Research and Development, San Diego, USA
[5]Pharmacokinetics, Dynamics and Metabolism, Pfizer Global Research and Development, Sandwich, UK
Email: *David.Pryde@pfizer.com

ABSTRACT

A high throughput screen of the Pfizer compound collection was carried out using a hepatitis C virus (HCV) genotype 1b sub-genomic replicon cell line. Those confirmed hits that demonstrated broad spectrum activity without overt cyto-toxicity were further evaluated, leading to the identification of a series of pyrrolopyridines with excellent antiviral activity in a fully infectious HCV cell-based assay and pharmacokinetic properties.

Keywords: HCV; Replicon; Antiviral; Cell-Based Screen

1. Introduction

It is estimated that some 3% of the world's population is currently infected with hepatitis C [1] virus (HCV). Approximately 70% of infected individuals develop a chronic infection a portion of whom go on to develop chronic liver disease [2]. While the rate of new infections has declined sharply in the last two decades, the need for broadly efficacious and well-tolerated therapies remains high. Current standard of care therapy for HCV is a combination of injected pegylated interferon and ribavirin [3], which can eradicate the virus with varying degress of efficacy depending on the genotype of virus the patient is infected with, and other genetic factors. Genotype 1 (gt1), the most prevalent genotype in Europe and the US, responds only modestly to interferon therapy with a sustained virologic response in approximately half of the treated population and the treatment is associated with severe side effects and significant discontinuation rates [4].

HCV is also a highly mutable virus, and as resistance mutations emerge, new and effective antiviral mechanisms are needed. Thus, there remains a high need for new HCV therapies with improved efficacy and toleration compared to the current standard of care particularly against the more difficult to treat gt1 viral population.

The most common therapeutic strategy being pursued within the pharmaceutical industry is to target direct acting antivirals [5]. For example, Vertex's telaprevir 1 [6] and Schering-Plough's boceprevir 2 [7] (**Figure 1**) were both approved in the US in 2011 and target the NS3 protease, while filibuvir 3 [8] from Pfizer is currently in Phase II trials and targets the NS5B polymerase. Daclatasvir 4 [9] from Bristol-Myers Squibb is also in Phase II and targets the NS5A protein. The pharmacological targets of these antiviral agents represent the most common under clinical investigation, beyond which there are relatively few compounds in clinical trials against alternative targets, either host or viral in natur [10]. In our internal programme, we sought medicinal chemistry starting points against new HCV viral targets that could offer a differentiated efficacy and/or resistance profile compared to standard of care or agents undergoing current clinical testing.

2. Identifying Novel Antiviral Agents for Treating HCV

In order to identify novel chemistry starting points for broad-spectrum anti-HCV treatments, we initially ran a full-file high throughput screen of the Pfizer corporate compound file, against a genotype 1b HCV sub-genomic replicon screen using a fluorescent read-out in 384-well format [11].

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*Corresponding author.

Given the multifactorial nature of any activity emerging from such a cell-based screen, a detailed screening sequence (**Figure 2**) was put in place to triage the hits, to eliminate cytotoxic compounds and those with limited or no spectrum across the two most prevalent genotypes gt1a and gt1b. The intention was to funnel hits down to a small number of well-characterised, structurally attractive hits. At this stage, hits were categorised according to measurable biochemical activity against NS3 and NS5B, and the remaining actives badged as having "unknown mechanism of action (MOA)". The ultimate goal of the screening campaign was to identify the novel discrete molecular targets of these orphan hits through a combination of virology and chemical biology methods.
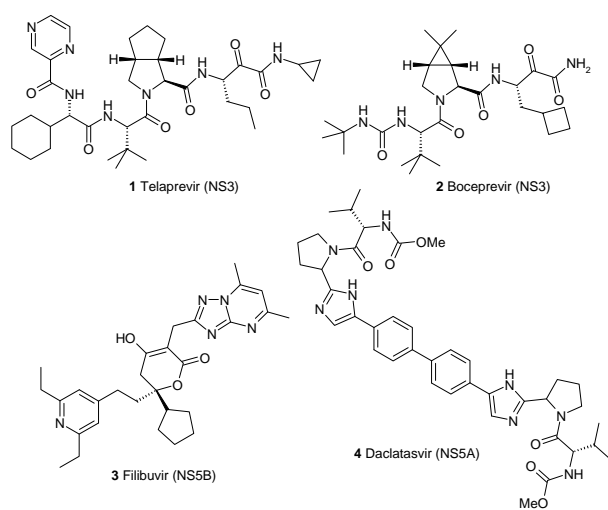

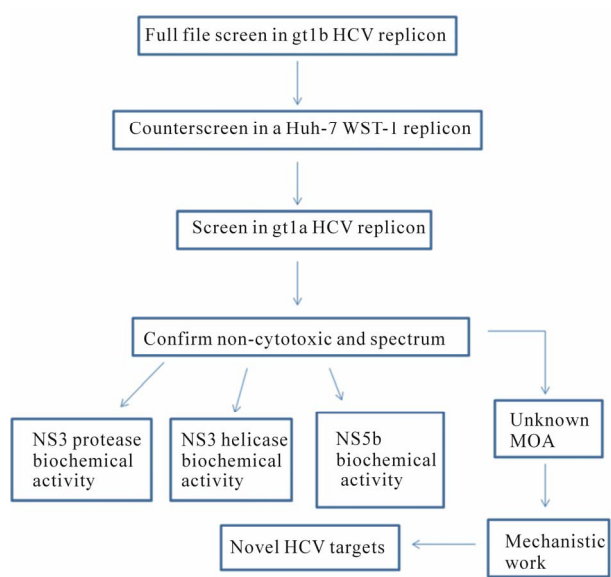**Figure 1. Selected clinically precedented HCV antiviral agents, with their viral target indicated in parentheses.**

Figure 2. The screening cascade followed to identify novel anti-HCV agents.

3. Results

3.1. Output from a Full File High Throughput Screen

The initial gt1b screen was carried out using fresh Huh-7 cells hosting the HCV replicon recombined with the luciferase reporter gene [11]. Active compounds in this assay inhibited firefly luciferase expression, a read-out that has been shown to be directly proportional to the level of viral RNA present in a cell. Hits were defined as displaying a greater than 50% inhibition of luciferase activity at a single test concentration of 10 μM.

Compounds that passed this criterion had their activity confirmed in a rescreen, and then a cytotoxicity check in a replicon-containing Huh-7 cell line using the WST-1 [12] cell viability reagent to determine viable cell number in response to compound incubation [13]. Activity was then determined against a gt1a replicon screen [14]. Further extensive profiling including QC analysis of purity and identity, off-target pharmacology demonstrated by the compounds as well as measured and predicted ADME, toxicity and physicochemical properties was carried out. Following this, three main series were selected as sufficiently interesting for further work, as shown in **Figure 3**.

The series identified were structurally distinct, possessed low micromolar potency, and had reasonable

5 (Series 1)
gt1b EC_{50} 1410nM
gt1a EC_{50} 90nM
CC50 >40μM
cLogP 4.4
MWt. 386
MDCK 18/24 10^{-6} cm/s
NS3 IC_{50} >25μM
NS5B IC_{50} >25μM

6 (Series 2)
gt1b EC_{50} 2720nM
gt1a EC_{50} 7900 nM
CC50 >40μM
cLogP 2.8
MWt. 309
MDCK 5/1 10^{-6} cm/s
NS3 IC50 >25μM
NS5B IC50 >25μM

7 (Series 3)
gt1b EC_{50} 4700nM
gt1a EC_{50} 5600 nM
CC50 >100μM
cLogP 4.2
MWt. 408
MDCK 8/10 10^{-6} cm/s
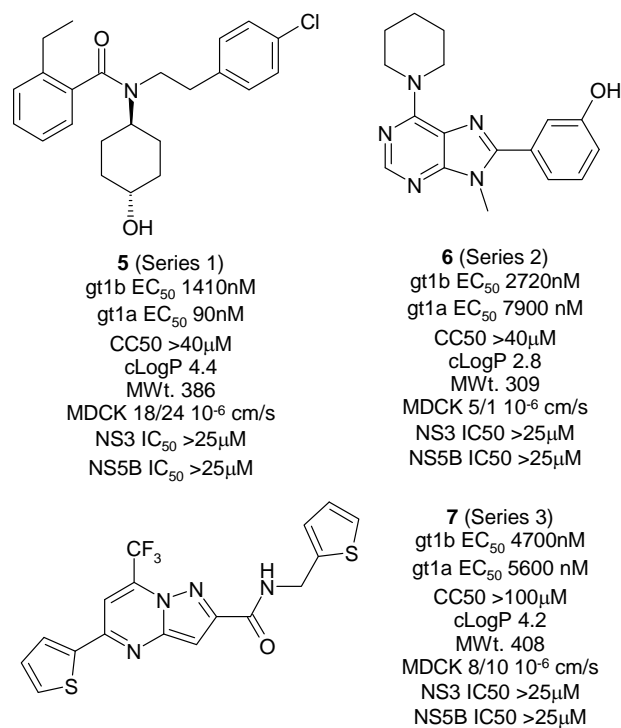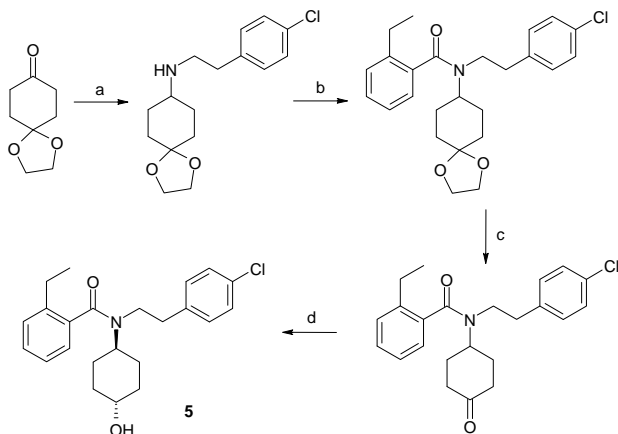NS3 IC50 >25μM
NS5B IC50 >25μM

Figure 3. Primary compound series identified through an HCV replicon high throughput screen.

physicochemical properties with molecular weights in the region 300 - 400 and clogP in the region 3 - 4.5. All compounds showed excellent cell permeability, an essential component for our cell based screening cascade. None of the series seed compounds showed any appreciable activity against isolated NS5B or NS3 proteins [15]. Our efforts focussed on developing SAR within each of these series to identify the most robust series to take forward into a full medicinal chemistry programme. For all compounds, gt1b and gt1a potency was obtained, along with confirmation in the WST-1 assay that activity was not due to cytotoxicity.

3.2. Series 1

Compound 5 emerged as a relatively weak inhibitor of the gt1b replicon, but upon further profiling, a much more potent inhibitor of the gt1a replicon, a mismatch in favour of gt1a of approximately 15 fold that is quite unusual; gt1a activity tends to be more difficult to achieve. When we investigated the screening history of this particular compound, we found that it and close analogues had been pursued as part of a previous programme to find antagonists of the C5a receptor. Our efforts in this area have been published previously [16,17]. This history allowed us to rapidly access several hundred analogues from our compound collection to confirm activity against the HCV replicons. The synthetic methods to add to this existing collection were also well-established and we were able to generate a substantial amount of SAR very quickly. Syntheses of compounds from this series were carried out according to **Scheme 1** and is illustated for compound 5.

One of the first aspects of the series we wished to establish was whether replicon activity tracked with C5a activity in which bound C5a was measured at 4C using dibutyryl cAMP-differentiated U937 cells and a filtration assay using 160 pM ^{125}I-labelled recombinant human C5a to measure bound radioligand. Compound 5 itself has an IC_{50} of 428 nM at the C5a receptor. Using a selection of compounds from the legacy C5a receptor antagonist programme, **Figure 4** demonstrates that there was no correlation between C5a activity and gt1b replicon activity; the line of unity between the two assays is indicated by the solid line on the chart (there was similarly no correlation with gt1a activity—data not shown). Having established that the replicon actvity was not C5a- dependent, we then set out to explore the replicon SAR of the series in more detail.

In **Table 1** below, an initial set of compounds is shown in which the synthetically straightforward amide grouping was modified.

Early results suggested that making significant improvements in gt1b activity could be difficult to achieve. Bicyclic systems featuring a lipophilic substituent (8, 9 and 14) displayed similar gt1b activity, although it is notable that the pyrimidine nucleus in 8 was much weaker against the gt1a replicon than a phenyl nucleus. Heterocyclic systems such as 10 and 16 were also weaker against the gt1a replicon, while the benzothiophene retained the activity of the starting compound 5 agisnt both cell-lines. Replacing the ethyl side-chain in 5 with a chloro substituent in 11 retained activity but replacement of the phenyl nucleus with a cyclohexyl group in 13, or a heterocycle as in 15, was detrimental to activity.

We then focussed on the phenethyl substituent as shown in **Table 2**. Relatively conservative changes to the phenyl ring largely retained potency in both replicons, for example the benzofuran 21. However, when heteroatoms were introduced, for example the benzimidazoles 18 and 19, the pyrazine 20, the pyrimidine 26, triazole 28,
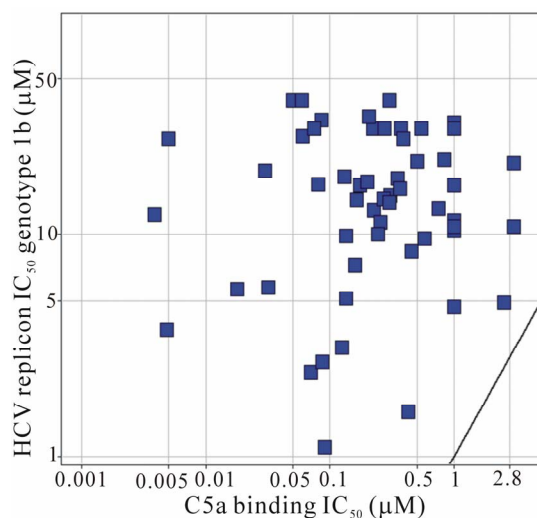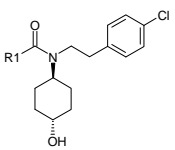

Scheme 1. Preparation of compound 5 from series 1. Reagents and conditions: (a) p-chloro-phenethylamine, Na(AcO)$_3$BH, AcOH, DCM, rt, 18 h, 98%; (b) 2-ethyl-benzoic acid, (COCl)$_2$, DMF, DCM, rt, 1 h, 100%; (c) 2 N HCl, THF, 60°C, 3 h, 84%; (d) NaBH$_4$, EtOH, rt, 3 h, 78% (+ approx. 15% of the corresponding cis diastereoisomer).

Figure 4. Scatterplot of replicon potency and C5a receptor binding potency.

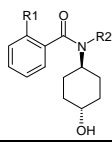**Table 1. Amide group structure-activity relationship data within series 1.**

Compound	R1	gt1b activity EC$_{50}$	gt1a activity EC$_{50}$	WST-1 CC$_{50}$
5		1410	90	>40,000
8		761	17,000	17,300
9		831	44	>40,000
10		994	1820	>40,000
11		1080	227	>40,000
12		1100	135	>40,000
13		1270	4260	18,000
14		1840	188	>40,000
15		7440	822	>40,000
16		3020	>40,000	>40,000
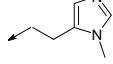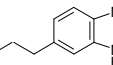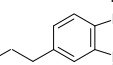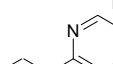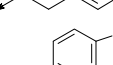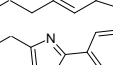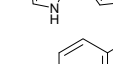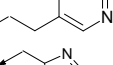
All figures are in nM. Data is a mean of at least two replicates. Assays are as described in [11,13,14].

Table 2. Phenethyl group structure-activity relationship data within series 1.

Compound	R1	R2	gt1b activity EC$_{50}$	gt1a activity EC$_{50}$	WST-1 CC$_{50}$
17	Et		196	5800	>40,000
18	Et		276	2150	>40,000
19	Me		371	1120	>40,000
20	Me		484	40,000	>40,000
21	Me		685	155	>40,000
22	Me		858	40,000	>40,000
23	Me		954	2090	>40,000
24	Me		989	40,000	>40,000
25	Me		2230	40,000	>40,000
26	Me		4000	40,000	>40,000
27	Me		4040	40,000	>40,000
28	Me		5410	40,000	>40,000

imidazole 17 or even the closely analogous pyridine 23, gt1a potency in particular suffered. Other more significant structural changes gave similar results, for example the imidazole linked phenyl group in 22, or its fused equivalent 24 were both weak inhibitors of the gt1a replicon. Linking through the pyrazole ring of 25, or even the cyclohexyl ring of 27 were weak inhibitors of both replicons.

The final area we probed in this initial SAR exercise was in the region of the cyclohexyl alcohol group (**Figure 5**). For example, the difluoro-cyclohexyl group in 29 was much less active than the cyclohexyl alcohol of compound 5. Basic groups as in 30, extended lipophilic groups as in the trifluoromethyl-substituted cyclohexyl 31, and even conservative, close analogues such as the methyl ether 32 and the sulfoxide 33 were no better than micromolar active against both replicons.

For the collection of compounds screened and/or made in this series, we had been unable to identify sub-500nM inhibitors of both replicon genotypes. A further concern within the series was that the most potent compounds
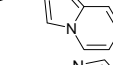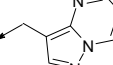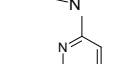

29
gt1b EC$_{50}$ 2460nM
gt1a EC$_{50}$ 1820nM
CC$_{50}$ >40000nM

30
gt1b EC$_{50}$ 1880nM
gt1a EC$_{50}$ 1040nM
CC$_{50}$ 9010nM

31
gt1b EC$_{50}$ 2100nM
gt1a EC$_{50}$ 3170nM
CC$_{50}$ 40000nM

32
gt1b EC$_{50}$ 3120nM
gt1a EC$_{50}$ 6420nM
CC$_{50}$ >40000nM

33
gt1b EC$_{50}$ 3560nM
gt1a EC$_{50}$ 4060nM
CC$_{50}$ 10000nM

Figure 5. Cyclohexyl alcohol derivatives of series 1.

appeared to be among the more lipophilic. Attempts to reduce lipophilicity met with only limited success. The Lipophilic Lipoidal Efficiency (LLE or LipE) [18] of the more potent compounds in the series was approximately 4. Within the series, compounds with cLogP greater than 2 were rapidly turned over in human liver microsomes. Taken together, the inability to find potent compounds against both genotypes, and the rapid *in vitro* metabolism of compounds potent against gt1a, led to the decision to abandon this series.

3.3. Series 2

The purine series represented by 6 was an initially weak starting point, albeit balanced against the gt1a and gt1b replicons. Compound 6 was synthesised acording to **Scheme 2**.

Our early SAR investigations initially focussed on the 6-position of the purine core, and examined simple amine substituents in replacement of the piperidine group (**Table 3**). Growing the size of the substituent to the pyrazine-piperazine 34, the phenyl pyrazole 35 or the analogous pyrazole-phenyl 36 and its isomer 37 resulted in essentially equivalent potency. The saturated substituents 38 and 39 were weaker in the primary assay, and pyrazole substituents in which the substituent was significantly reduced in size down to just a methyl group in 40 was weaker. When the pyrazole substituent was extended out to a benzyl group in 41, a dramatic increase in activity was observed, in both gt1b and gt1a replicons, however this was subsequently confirmed to be due to cytotoxicity.

We devoted some time and a small number of analogues to exploring the cytotoxicity of the benzyl pyrazole analogue 41 through modification of the phenol group (**Table 4**). Aromatic groups such as the methoxy-phenyl 43, the cyanophenyl 42 or the pyridine 45 were all notably more cytotoxic than 41. 5-membered heterocycles 47, or saturated heterocycles 46 maintained essentially no therapeutic index, a similar situation to the contracted
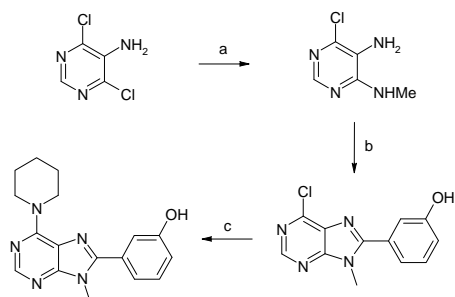
Scheme 2. Preparation of compound 6 from Series 2. Reagents and conditions: (a) Methylamine, diisopropy lethylamine, n-butanol, 120°C, 16 h, 100%; (b) m-hy-droxy-benzaldehyde, acetic acid, ferric chloride, ethanol, rt, 48%; (c) Piperidine, triethylamine, DMSO, 150°C microwave irradiation, 30 min, 78%.
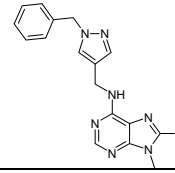
Table 3. C-6 analogues of the Series 2 purine lead.

Compound	R	gt1b activity EC_{50}	gt1a activity EC_{50}	WST-1 CC_{50}
6		2720	4880	40,000
34		1290	2520	25,600
35		4270	6480	11,500
36		5310	11,200	31,900
37		5910	8470	31,100
38		5960	5190	40,000
39		6590	12,000	40,000
40		8070	18,600	40,000
41		183	381	668

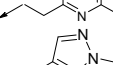All figures are in nM. Data is a mean of at least two replicates. Assays are as described in [11,13,14].

Table 4. Phenol analogues of the purine-based Series 2.
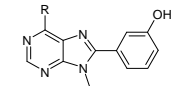
Compound	R	gt1b activity EC_{50}	gt1a activity EC_{50}	WST-1 CC_{50}
41	OH	183	381	668
42	CN	35	79	114
43	OMe	102	258	133
44	Me	153	387	136
45		209		206
46		229	890	433
47		345	547	229
48	H	581	1210	941

All figures are in nM. Data is a mean of at least two replicates. Assays are as described in [11,13,14].

methyl group in 44 or a simple hydrogen atom in 48. These data confirmed that the benzyl-pyrazole purine core carried inherent cytotoxicity through an unknown mechanism.

As a final effort in this series, we were intrigued to see if small changes to the purine core would also maintain cytotoxicity (**Table 5**). Simple nitrogen atom deletion to give the 1-deazapurine group in 49 gave significantly weaker activity, which again appeared to be due to cytotoxicity. The analogous 3-deazapurine in 50 showed extremely high potency in the WST-1 assay, highlighting how subtle the cytotoxicity effects in this chemotype were. Substructure searching around the purine core uncovered several analogues such as the imidazopyrazine 51 which again showed potent cellular toxicity. More significant structural changes to a quinoline 52 or quinazoline 53 core were required to see drop-offs in cytotoxicity, accompanied by much weaker activity in the primary replicon assays.

At this stage, we were satisfied that this series offered no viable opportunities for a chemistry programme and all futher work was stopped in this chemotype.

Table 5. Purine analogues based on the Series 2 lead structure.

Compound	Structure	gt1b activity EC$_{50}$	gt1a activity EC$_{50}$	WST-1 CC$_{50}$
49		5730	3120	4000
50		22	42	62
51		191	52	15
52		3130	>4000	5690
53		6500	>12700	40,000

All figures are in nM. Data is a mean of at least two replicates. Assays are as described in [11,13,14].

3.4. Series 3

The pyrazolopyrimidine 7 emerged from the high throughput screen as a promising hit, with reasonably balanced, albeit weak cell-based activity. This hit originated from a commercially available compound set [19,20] and contained potentially reactive thiophene rings, which could be readily substituted out, but in normal handling was observed to have poor solubility. It is notable that this structure also bears significant resemblance to similar compounds proposed to be inhibitors of HCV NS4B from Viropharma Inc. based on data from a cell proliferation assay [21]. A substructure search of the Pfizer compound collection uncovered a range of similar compounds featuring diverse core structures 54 - 57, depicted in **Figure 6** below. Of these, attention was immediately focussed on the pyrrolopyridine core of 56, which possessed better gt1b potency, while retaining spectrum against the gt1a replicon, compared to the starting pyrazolopyrimidine.

Synthetic efforts quickly moved towards expanding the SAR around this core, through modifications to the aryl group pendant to the pyridine ring, and to the amide grouping using the chemistry described in **Scheme 3**.

Initial data suggested that a lipophilic group at the 6-position, adjacent to the pyridine ring of the pyrrolopyridine core, would be essential for high potency. Groups such as *tert*-Bu, phenyl, substituted phenyl or a methyl-cyclopropyl group did indeed support high potency (**Table 6**) while polar functionality, for example the alcohol 70, ablated all activity. On the opposite side of the molecule, amide substituents of quite diverse character such as the cyclohexyl alcohol in 58, or the cyclopropyl and cyclopentyl funtionalised alcohols in 60 and 63 respectively were well-tolerated, and while the gt1a activity of the series was inferior to their gt1b potency, these were good starting points in an encouraging region of physicochemical space for further optimisation. When these alcohol-containing groups were altered to incorporate further expressions, for example the substituted amino-ethanol unit of 68, although primary potency against the gt1b replicon declined, this compound was a more balanced starting point against the gt1a replicon.
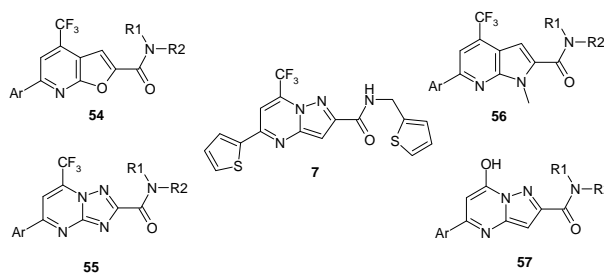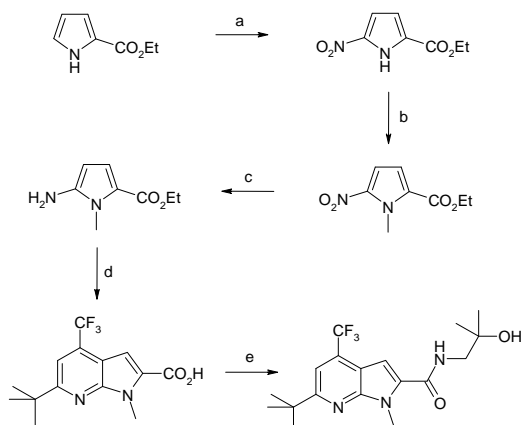

Figure 6. Core variations explored around the pyrazolopyrimidine lead 7.

Scheme 3. Preparation of compound 77 from series 3. Reagents and conditions: (a) Acetic anhydride, HNO₃, −78°C→rt, 16 h, 41%. (b) Cs₂CO₃, DMF, MeI, rt, 16 h, 41%; (c) EtOAc, 10% Pd; (c) 50 bar H₂ pressure, 60°C, 16 h, 93%; (d) Acetic acid, microwave irradiation, 120°C, 15 min followed by MeOH, LiOH, 50°C, 3 h, 88% over 2 steps; (e) 1-amino-2-methyl-propan-2-ol, 1-propane-phosphonic acid cyclic anhydride, Et3N, DMF, rt, 16 h, 98%.

Table 6. Amide analogues based on the Series 3 pyrrolopyridine structure.

Compound	R1	R2	gt1b activity EC_{50}	gt1a activity EC_{50}	WST-1 CC_{50}
58			3460	3300	>10,000
59			70	1900	>10,000
60			1370	2470	>10,000
61			125	>10,000	>10,000
62			2460	4060	>10,000
63			435	1880	>10,000
64		Me	826	8400	>10,000
65			837	5320	>10,000
66			4300	16700	32,800
67			1060	3410	>10,000
68			3550	4740	>10,000
69			444	5510	>10,000
70			>10,000	>10,000	>10,000

All figures are in nM. Data is a mean of at least two replicates. Assays are as described in [11,13,14]..

Heterocycles exemplified by the tetrahydrofuran in 59, the piperidine amide in 61, the sulfones in 65 and 66 and the oxazole in 69 all retained sub-1μM activity in the gt1b replicon assay. Contracting the amide group down to just a methyl group in 64 or a propyl group in 67 were all approximately micromolar potency. We were encouraged that a basic group in the piperidine unit 62 retained significant activity against the gt1b replicon.

It was encouraging that none of the initial set of analogues appeared to have significant cytotoxicity concerns, but we were concerned that the majority of examples appeared to have a genotype disconnect in favour of gt1b. For this reason, we selected the balanced analogue 68 as the starting point for further structural modification of the core template, described in **Table 7**. In the 6-position, small changes to the starting trifluoromethyl group, for example through modification to chloro-difluoro in compounds 72 and 73, or difluoromethyl as in 71 offered modest gains in activity. Bulking

Table 7. Core substitution variations around the pyrrolopyridine template.

Compound	Structure	gt1b activity EC_{50}	gt1a activity EC_{50}	WST-1 CC_{50}
71		553	3040	>10,000
72		1330	4620	>10,000
73		1370	7280	>10,000
74		1750	3810	>10000
75		2240	ND	ND
76		3220	2200	>10,000
77		4240	10,400	>10,000
78		>10,000	>10,000	>10,000
79		13,900	21,900	>40,000

All figures are in nM. Data is a mean of at least two replicates. Assays are as described in [11,13,14].

up the 2-position group to, for example, a cyclopentyl unit as in 74 again offered a very modest potency gain. An *N,N*-dimethyl group in 75 or a homologated core template as in the ethyl-analogue 76 were essentially equipotent.

It also became clear that the combination of the C2 and C6 groups were critical to the observed activity and genotype coverage. A simple change from the methyl cyclopropyl group of 68 to a *tert*-butyl in 77 lost gt1a potency. Changing to the synthetically simpler dimethyl analogue 78 was inactive against both genotypes, while the bis-trifluoromethyl 79 was somewhat weaker, but retained the balanced genotype activity. At this point, we were faced with what appeared to be quite flat SAR, and little prospect of being able to rapidly analogue into significantly more potent compounds. The physiccochemistry space occupied by compounds from this series was however attractive, with molecular weights in the 400 region and measured lipophilicity values in the range 1.5 - 4. Additional *in vitro* data was obtained on a selection of compounds from the pyrrolopyridine series, including potency in a fully infectious HCV cell culture (HCVcc) assay [22] which incorporated not just the non-structural proteins of HCV but also the structural viral proteins that support viral infectivity (**Table 8**).

The basic group in 62 introduced a significant hERG liability, and the most lipophilic example 60 carried the most human liver microsome turnover, with the intermediate lipophilicity examples 77 and 79 showing moderate microsomal stability and the polar sulfone 66 showing good stability.

The most interesting piece of data we obtained on this subset of compounds was the HCVcc activity in comparison to the replicon assay data. The pyrrolopyridine compounds were consistently much more potent in the HCVcc assay than the replicon assay, and when we subsequently profiled compounds from the other series investigated in the project, a finding which contrasted with all other series (**Figure 7**).

The infectious assay is a different genotype (gt2a)

compared to the replicon assays we routinely used (gt1b and gt1a), and we could not rule out the possibility that the difference between replicon and infectious assay potency was not due to genotype difference as we did not have access to a gt2a replicon. The most difficult to treat HCV genotypes are of gt1, and this was the primary target we set out to inhibit. Alternatively, it is also possible that the azaindole series interacted with a target in the HCVcc system that was not represented in the replicon cell assay, and that the mismatch between replicon and HCVcc potencies would translate across genotypes. An important question became whether translation to a human infection would scale from the replicon or from the infectious *in vitro* assay potency values.

Taking compound 77 as an exemplar, rat pharmacokinetics were obtained as shown in **Table 9**. Predictions of human pharmacokinetics were made by scaling from the rat and dose predictions then made using the infectious assay data.

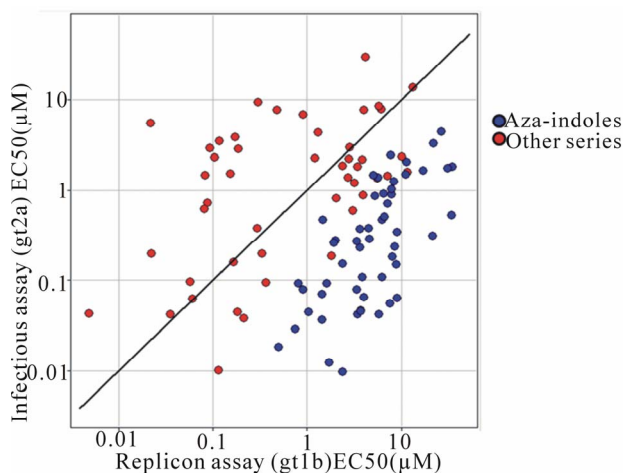Taking the efficacious target concentration for 77 as the infectious assay IC_{50} of 46 nM, it was predicted that

Figure 7. Infectious assay potency (gt2a) vs replicon potency (gt1b) for compounds of the azaindole series compared to those from other series.

Table 8. *In vitro* potency and ADMET data for selected pyrrolopyridines.

Compound (MWt.)	gt1b replicon IC50, nM[a]	gt1a replicon IC50, nM[a]	gt2a HCVcc IC50, nM[b]	MDCK[c] AB/BA, 10^{-6} cm/s	cLogP (LogD[d])	HLM, l/min/mg[e]	Dof. Binding IC50, nM[f]
60 (383)	1370	2470	NT	2/4	3.2 (3.8)	52	>20,000
62 (410)	2460	4060	10	0.1/1	4.4 (1.8)	20	1230
66 (429)	4300	16,700	29	5/8	1.6 (ND)	9	>40,000
77 (371)	4240	10,400	46	7/11	3.8 (3.1)	41	25,600
79 (383)	13,900	21,900	916	24/24	2.9 (3.6)	10	>20,000

All figures are in nM. Data is a mean of at least two replicates. [a]Replicon assays are as described in [11] and [14]. [b]The HCVcc assay is described in [22]. [c]Methodological details for the MDCK permeability assay have been published previously [23]. [d]LogD measured at pH7.4 in octanol/neutral buffer. [e]Details of the HLM assay used have been published previously [24]. [f]Details of the hERG assay used, in which compounds are assessed for their ability to displace labelled dofetilide has been reported previously [25]. NT = not tested.

Table 9. Rat pharmacokinetics and predicted human pharmacokinetics for compound 77.

	Dose	Cl (mL/min/kg)	Vdss (L/kg)	f_u	F (%)
Rat pharmacokinetics	2 mg/kg	39	5	0.007	ND
Human predictions to achieve C_{av} above IC_{50} concentration at trough	100 mg *bid*	0.8	5	0.01	~90

ND = not determined.

a 100 mg *bid* dose of this compound would provide adequate coverage at steady state. While this constitutes a reasonable dose prediction, any predictions based on the replicon IC_{50} value would clearly give an unacceptably high dose. Further *in vivo* profiling to allow deconvolution of these very different scenarios is yet to be carried out.

4. Conclusion

We have described a full file high throughput screen of the Pfizer corporate compound collection using a sub-genomic HCV replicon cell-based screen to identify several attractive starting points for medicinal chemistry follow-up. Through hit expansion and SAR activities, a series of azaindoles were prioritised as being most likely to deliver a useful clinical profile, particularly based on their high potency in a fully infectious HCV cell screen.

5. Acknowledgements

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[11] Genotype 1b (Con1, Licensed from Reblikon GmbH) HCV Replicon Cells Were Resuspended to a Concentration of 1.4×10^5 Cells/ml by Addition of Pre-Warmed Medium (DMEM + 10% FCS). 45 µl of This Suspension Was Added to Each Well a 384-Well Assay Plate (Lumitrac, Greiner) Already Containing 0.5 l of Test Compound. All Plates Were Covered with gas Permeable Seals and Incubated at 37ºC, 5% CO_2 for 48 Hours. After 48 Hours, the Plate Was Removed from the Incubator and Left to Cool to Room Temperature for 15 - 30 mins. An Equal Volume of Reconstituted Lyophilised Britelite plus Substrate (PerkinElmer) to Medium Was Added to Each Well. Luminescence Was Read Immediately on an EnVision (Perkin Elmer) Plate Reader.

[12] 1[2-(4-Iodophenyl)-3-(4-Nitrophenyl)-5-(2,4-Disulfophenyl)-2H-Tetrazolium], CAS Registry Number, 150849-52-8.

[13] Genotype 1b (Con1) HCV Replicon Cells Were Resuspended to a Concentration of 1.4×10^5 cells/ml by Addition of Pre-Warmed Medium (DMEM +10% FCS). 45 µl of This Suspension Was Added to Each Well of a 384-Well Assay Plate (Greiner) Already Containing 0.5 l of

Test Compound. All Plates Were Covered with Gas Permeable Seals and Incubated at 37ºC, 5% CO_2 for 48 Hours. After 48 Hours, 5 µl of WST-1 Cell Proliferation Reagent (Roche) Was Added to Each Well and the Plate Returned to the Incubator for 1 Hour. After This Incubation Period Absorbance Was Read at 450 nm on an EnVision (Perkin Elmer) Plate Reader.

[14] Genotype 1a (H77, Licensed from Apath LLC) HCV Replicon Cells Were Resuspended to a Concentration of 1.4×10^5 Cells/ml by Addition of Pre-Warmed Assay Medium (DMEM + 10% FCS). 45 µl of This Suspension Was Added to Each Well of a 384-Well Assay Plate (Lumitrac, Greiner), Already Containing 0.5 l of Test Compound. All Plates Were Covered with Gas Permeable Seals and Incubated at 37ºC, 5% CO_2 for 48 Hours. After 48 Hours, the Assay Plate Was Removed from the Incubator and Left to Cool to Room Temperature for 15 - 30 mins. Medium Was Removed from the Wells and 5 µl Lysis Buffer (Renilla Luciferase Assay Kit, Promega) Was Added to Each Well. The Plate Was Incubated at Room Temperature on a Rocker for 15 mins then 15 µl Assay Substrate was Added to Each Well. Luminescence Was Read Immediately Using an EnVision Plate Reader.

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