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Novel substituted pyrimidines as HCV replication (replicase) inhibitors

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ABSTRACT

Compound **1** was identified as a HCV replication inhibitor from screening/early SAR triage. Potency improvement was achieved via modulation of substituent on the 5-azo linkage. Due to potential toxicological concern, the 5-azo linkage was replaced with 5-alkenyl or 5-alkynyl moiety. Analogs containing the 5-alkynyl linkage were found to be potent inhibitors of HCV replication. Further evaluation identified compounds **53** and **63** with good overall profile, in terms of replicon potency, selectivity and in vivo characteristics. Initial target engagement studies suggest that these novel carbanucleoside-like derivatives may inhibit the HCV replication complex (replicase).

Chronic hepatitis C virus (HCV) infection is a global health burden affecting an estimated 3% of the human population.[1] It is the leading cause of liver cirrhosis, hepatocellular carcinoma and liver failure in humans. HCV is a positive stranded RNA virus belonging to the *Flaviviridae* family. Upon entering hepatocytes, the HCV genome encodes a polyprotein of approximately 3000 amino acid residues. Post-translational cleavage of the polyprotein into functional individual viral proteins is mediated by host and viral proteases, ultimately resulting in viral replication.[2] Until recently, the standard-of-care (SOC) has been pegylated α-interferon and ribavirin combination therapy, which does not target the virus specifically.[3] Moreover, this SOC is effective in less than 50% of genotype 1 patients, a population more prevalent in North America, Europe and Japan, and is accompanied with unwanted side effects. Intense efforts were focused in the past decade to discover novel small molecule direct-acting antivirals (DAA) that inhibit viral replication. These efforts resulted in the recent regulatory approval of NS3 protease inhibitors, boceprevir and telaprevir that improved the cure rates when added to SOC.[4] Several other DAAs (NS3 protease inhibitors, NS5B nucleoside and non-nucleoside inhibitors, and NS5A inhibitors) are in various stages of clinical trials.[5] Despite these advances, the genetic variability of HCV (at least six genotypes, with numerous subtypes) and high mutation rate will likely require a combination of agents to ultimately suppress emergence of viral resistance resulting in clinical cure.

In our continued interest to discover inhibitors that target HCV via novel pathways, screening of a proprietary nucleoside-like library was carried out. From screening and early SAR triage, compound **1** (Fig. 1) was identified as a potential HCV replication inhibitor with encouraging potency in the cell-based genotype 1b subgenomic replicon assay.[6] Compound **1** exhibited replicon EC_{50} of 0.3 μM, with good selectivity window (MTS CC_{50} = 25 μM). A noteworthy feature of the nucleoside-like inhibitor **1** is the presence of carbocyclic ring instead of the ribose moiety required for natural nucleosides. Carbocyclic nucleosides are known to have enhanced stability toward pyrophosphorylases that hydrolytically cleave glycosidic bonds.[7] The difference in conformation of the cyclopentane versus ribose ring could also result in a different

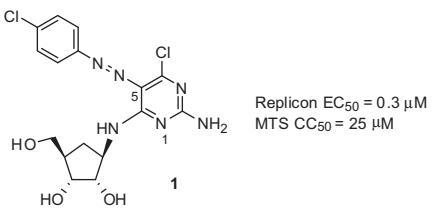

Figure 1. Profile of initial lead **1**.

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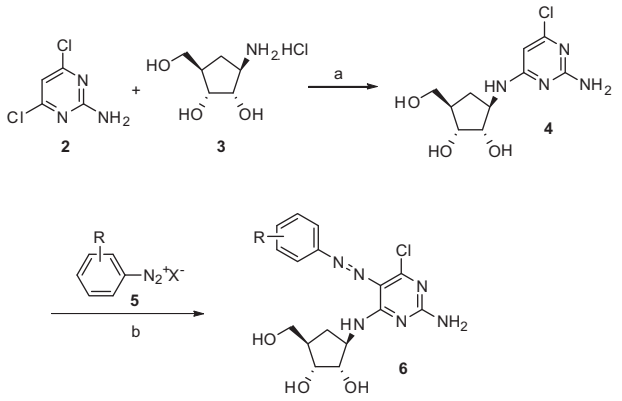
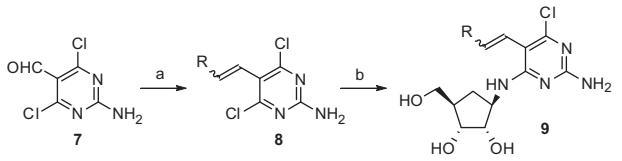
SAR from that of natural nucleosides. Hence we initiated medicinal chemistry efforts around the core structure **1** with a goal to identify suitable candidate for further lead optimization.

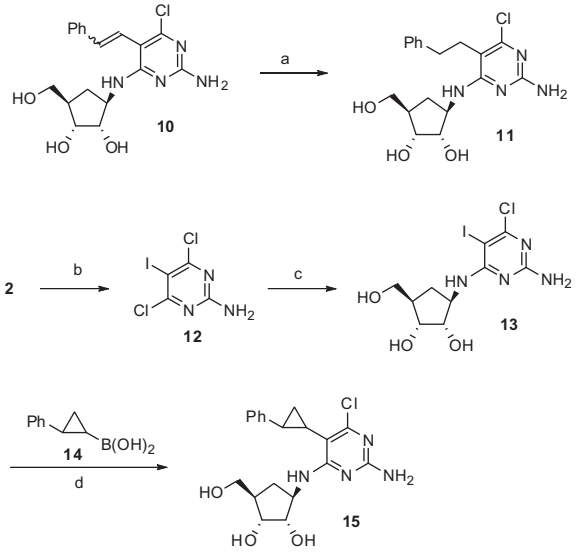
Schemes 1–4 describes the chemistry for preparation of target compounds. SAR studies were initiated with substitution on the phenyl ring connected to the 5-azo linkage of **1**. Synthesis of inhibitors from the 5-azo series is shown in Scheme 1. The key intermediate **4** was prepared by refluxing a mixture of commercially available 2-amino-4,6-dichloropyrimidine (**2**), (1*R*,2*S*,3*R*,4*R*)-2,3-dihydroxy-4-(hydroxymethyl)-1-amino cyclopentane hydrochloride (**3**), and triethylamine in ethanol. The target compounds were subsequently



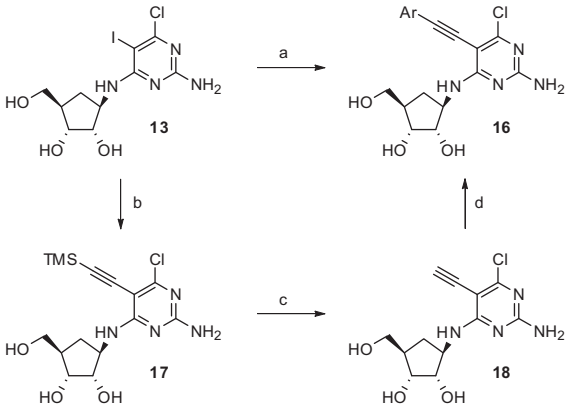
Scheme 1. Reagents and conditions: (a) EtOH, Et_3N, reflux, 16 h (72%); (b) **5** (commercial or generated in situ from commercially available anilines/$NaNO_2$/HCl), NaOAc, gl. AcOH, water (~60%).



Scheme 2. Reagents and conditions: (a) $Ph_3P^+CH_2RX^-$, *n*BuLi, THF, −78 °C (~40%); (b) **3**, EtOH, Et_3N, reflux, 72 h (20–30%).



Scheme 3. Reagents and conditions: (a) 10% Pd/C, cyclohexene, MeOH, THF, 80 °C, 2 h (~75%); (b) ICl, gl. AcOH, 5 h (79%); (c) **3**, EtOH, Et_3N, reflux, 72 h (83%); (d) phenylcyclopropylboronic acid **14**, $Pd(Ph_3P)_4$, K_2CO_3, DMF, 90 °C, 16 h (~20%).



Scheme 4. Reagents and conditions: (a) Arylacetylene, $Pd(Ph_3P)_4$, CuI, Et_3N, DMF, 18 h (~78%); (b) TMSacetylene, $Pd(Ph_3P)_4$, CuI, Et_3N, DMF, 55 °C, 18 h (56%); (c) tetraethylammonium fluoride dihydrate, MeCN, 2 h (94%); (d) aryl halide, $Pd(Ph_3P)_4$, CuI, Et_3N, DMF, microwave, 90 °C, 10 min (50–56%).

obtained by coupling intermediate **4** with various substituted benzenediazonium halides (commercial or prepared by diazotization of appropriately substituted anilines with sodium nitrite and HCl) using established protocol.

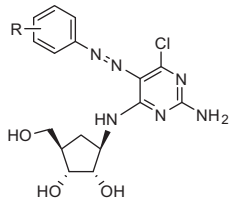
Scheme 2 depicts the synthesis of targets from the 5-alkenyl series, the first group of analogs designed to replace the 5-azo linkage. Wittig reaction of substituted benzyl triphenylphosphonium halide and 2-amino-4,6-dichloro-5-formylpyrimidine **7**, resulted in intermediate **8** as mixture of *cis/trans* isomers (mostly *trans*). Treatment of **8** with carbasugar derivative **3** employing previously described procedures afforded the required compounds as a mixture of *cis/trans* isomers in a modest yield.

Preparation of targets with the 5-alkenyl moiety saturated or cyclopropanated is shown in Scheme 3. Reduction of **10** with catalytic palladium on carbon and cyclohexene at elevated temperatures gave **11**. The cyclopropyl derived target **15** was prepared via Suzuki conditions.[8] Thus, 2-amino-4,6-dichloropyrimidine **2** was iodinated with iodine monochloride to afford **12**, which was subsequently reacted with carbasugar **3** in refluxing ethanol to provide 5-iodo intermediate **13**. Suzuki reaction of 5-iodo intermediate **13** with phenylcyclopropyl boronic acid **14**[9] resulted in target **15**.

Synthesis of the 5-alkynyl series targets is shown in Scheme 4. Sonogashira reaction[8] of previously described 5-iodo intermediate **13** with commercially available arylacetylenes resulted in some of the target compounds of type **16**. Alternatively, to expand our selection of arylacetylenic targets of type **16** the following protocol was employed. Coupling of the 5-iodinated derivative **13** with TMS-acetylene under essentially the above described conditions gave intermediate **17**. Subsequent desilylation of **17** to **18** with tetraethylammonium fluoride dihydrate in acetonitrile, followed by a second Sonogashira reaction with appropriately substituted aryl halides then gave the desired target compounds **16**.

As mentioned above, while screening a nucleoside-like library, compound **1** was identified with interesting HCV replication inhibitory activity. In the subgenomic replicon assay compound **1** exhibited encouraging potency with an EC_{50} = 0.3 μM, and acceptable selectivity (MTS CC_{50} = 25 μM). SAR efforts were initiated with modifications to the phenyl moiety appended to the 5-azo linkage. Compounds listed in Table 1 were prepared as described in Scheme 1. Thus, moving the chloro moiety from *para* (compd **1**) to *meta* position resulted in an equipotent analog, **19**. However, ortho chloro substituted phenyl analog, **20**, and the parent phenyl derivative, **21** resulted in few fold improvement in replicon EC_{50}. Substituting the phenyl moiety with fluoro functionality proved to be advantageous, with analogs **22–24** showing improved potency while retaining the good selectivity window. Introduction

Table 1
Evaluation of 5-azo series



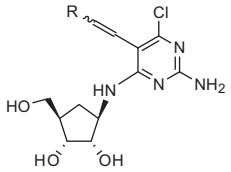
Compd	R	EC_{50} (μM)	CC_{50} (μM)
1	4-Cl	0.3	25
19	3-Cl	0.32	21
20	2-Cl	0.11	13
21	H	0.1	17
22	4-F	0.2	25
23	3-F	0.07	24
24	2-F	0.1	25
25	4-CN	1.2	25
26	3-CN	0.6	25
27	4-NO_2	3.8	25
28	3-NO_2	0.9	20
29	4-Me	0.06	7
30	4-OMe	0.02	25

of strongly electron withdrawing cyano (**25**, **26**) or nitro (**27**, **28**) moiety to the phenyl ring resulted in diminished replicon potency. While the 4-methyl substituted analog, **29** was essentially equipotent to parent phenyl derivative **21**, methoxy substitution at the 4-position of the phenyl ring (compd **30**) resulted in significant improvement in potency (EC_{50} = 20 nM) with an excellent selectivity index (CC_{50}/EC_{50} > 1000). Our attempts to modify the core structure by replacing the carbasugar moiety with numerous cyclic and acyclic alkylamino residues, with and without appended hydroxyl functionality resulted in significant erosion in replicon activity, thus highlighting the importance of the carbasugar motif as a potential recognition element.

While the initial SAR activities were carried out with the screening lead containing an azo linkage, it was highly desirable to find a suitable alternative for the azo functionality due to potential toxicological concern. We decided first to replace the 5-azo linkage with alkenyl moiety. Inhibitors with the 5-alkenyl functionality were prepared as described in Scheme 2, and the results are shown in Table 2. Thus, the parent phenyl substituted 5-alkenyl derivative **10** was almost a log less potent than the corresponding 5-azo compound, **21**. Introduction of a 4-methyl (**31**) or 4-methoxy (**32**) group on the phenyl ring, substituents that showed the best potency in the 5-azo series, were less potent as well. Position of the methoxy group on the phenyl ring had minimal impact on replicon potency, with 3-methoxy derivative **33** being essentially equipotent and 2-methoxy analog **34**, less potent than the parent, **10**. However, introduction of an additional methoxy functionality, the dimethoxy derivative **35**, resulted in restoring most of the replicon potency (EC_{50} = 0.12 μM) with a very good selectivity window. Next, halo groups were installed on the phenyl ring. Among the halo (chloro and fluoro) groups studied, 2-chloro (**37**) and 3-fluoro (**38**) substituted inhibitors displayed good EC_{50}, 0.3 and 0.2 μM, respectively. As observed for the 5-azo series, strongly electron withdrawing substitutions on the phenyl moiety (**40**–**42**) resulted in loss in potency. Finally, our attempts to replace the phenyl ring with a pyridyl moiety (**43**) resulted in no improvement in replicon potency. The *trans* and *cis*-3-pyridyl isomers, **44** and **45**, respectively, were essentially equipotent, thus displaying no potency bias for aromatic group disposition on the alkenyl linkage.

Modification of the 5-alkenyl linkage, via saturation or cyclopropanation of the double bond was studied next. Preparation of the two derivatives is shown in Scheme 3. Unfortunately, saturation of the 5-alkenyl group (compd **11**) or cyclopropanation (compd **15**)

Table 2
5-Alkenyl series SAR data

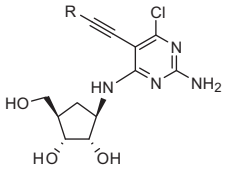


Compd	R	EC_{50} (μM)	CC_{50} (μM)
10	Ph	0.9	>25
31	4-MePh	1.9	25
32	4-MeOPh	1.9	25
33	3-MeOPh	0.6	25
34	2-MeOPh	2.5	25
35	3,4-DiOMePh	0.12	>25
36	4-ClPh	3.3	~30
37	2-ClPh	0.3	>25
38	3-FPh	0.2	>25
39	2-FPh	0.5	25
40	4-CF_3Ph	4.2	25
41	4-CNPh	5.5	>25
42	3-CNPh	0.6	>25
43	4-Pyridyl	3.4	25
44	*trans*-3-Pyridyl	1	25
45	*cis*-3-Pyridyl	4	25
46	2-Pyridyl	2.3	25

were not tolerated. Both compounds displayed significantly diminished potency when subjected to replicon assay (EC_{50} > 25 μM). The above described results indicated that sp2-type (or pi-character) at the 5-position was necessary for potency.

Based on the above observations, replacement of the 5-alkenyl moiety with an alkynyl linkage was deemed appropriate. A series of 5-alkynyl containing inhibitors were synthesized as described in Scheme 4, and evaluated in the replicon assay. Results are shown in Table 3. The parent phenyl alkynyl substituted derivative, **47** displayed good replicon potency, with EC_{50} = 0.2 μM. Introduction of a 4-methyl (**48**) or 4-methoxy (**49**) functionality on the phenyl ring provided equipotent analogs. A fluoro group at the 4 or 2-position, **50** and **51**, respectively, had minimal impact on the replicon potency compared to parent **47**. Incorporation of a series of ester (**52**–**54**), amide (**55**–**57**), sulfone (**58**, **59**) and sulfonamide (**60**) groups were studied next. While substituents at the 2-position of the phenyl ring were essentially equipotent, significant loss in replicon potency was observed for 4-substitutions, **56** and **59**. Additionally, analogs **55**, **57**, **58** and **60** displayed very good selectivity (CC_{50} > 25 μM). Replacement of the phenyl group with small heterocyclic rings (**61**–**63**) was well tolerated, except the imidazolyl analog **64** that resulted in some loss in potency. Installation of 2- or 4-pyridyl group (**65** and **67**, respectively) on the alkyne moiety resulted in loss in potency, while the 3-pyridyl replacement (**66**) restored most of the potency. Hence further modifications were carried out on the 3-pyridyl analog **66**. Introduction of an ester functionality on the pyridyl motif, compounds **68** and **69**, provided significant improvement in replicon potency (EC_{50} of 0.04 and 0.02 μM, respectively), albeit with some loss in selectivity. Small alkyl introduction on the pyridyl ring was studied next, with 2-methyl derivative (**70**) and 2,4-dimethyl analog (**72**) showing improved profile. Substitution of alkoxy group onto the pyridyl ring proved advantageous as well. Thus, 4-methoxy substituent, **73**, and 4-ethoxyl substituent, **74**, were highly potent with EC_{50} values of 0.03 and 0.02 μM, respectively. Finally, a disubstituted pyridyl moiety containing both alkyl and alkoxy groups, resulted in inhibitors with very good potency profile; analog **75** displayed replicon EC_{50} = 0.01 μM and excellent selectivity index (CC_{50}/EC_{50} = 1000).

Table 3
SAR of 5-alkynyl series



Compd	R	EC50 (μM)	CC50 (μM)
47	Ph	0.2	15
48	4-MePh	0.14	15
49	4-MeOPh	0.18	30
50	4-FPh	0.34	23
51	2-FPh	0.23	25
52	(2-CO_2Me)Ph	0.13	13
53	(2-CO_2Et)Ph	0.1	20
54	(2-CO_2*i*Pr)Ph	0.3	13
55	(2-$CONH_2$)Ph	0.2	>25
56	(4-$CONH_2$)Ph	5	>25
57	(2-CONHEt)Ph	0.2	>25
58	(2-SO_2Me)Ph	0.1	>25
59	(4-SO_2Me)Ph	1.9	>25
60	(2-SO_2NH_2)Ph	0.25	>25
61	2-Thiazolyl	0.2	23
62	2-Thiophenyl	0.15	25
63	3-Thiophenyl	0.1	25
64	2-Imidazolyl	0.5	>25
65	2-Pyridyl	0.8	25
66	3-Pyridyl	0.4	25
67	4-Pyridyl	0.9	25
68	(2-CO_2Me)-3-pyridyl	0.04	3
69	(2-CO_2Et)-3-pyridyl	0.02	3
70	(2-Me)-3-pyridyl	0.07	20
71	(4-Me)-3-pyridyl	0.15	4
72	(2,4-diMe)-3-pyridyl	0.05	15
73	(4-MeO)-3-pyridyl	0.03	20
74	(4-EtO)-3-pyridyl	0.02	8
75	(4-MeO-2-Me)-3-pyridyl	0.01	10
76	(4-EtO-2-Me)-3-pyridyl	0.03	11

Selected compounds with good replicon potency from the 5-alkynyl series were then subjected to rat in vivo studies, and the results are depicted in Table 4. Animals were dosed at 10 mpk (PO) or 2 mpk (IV) in a suitable vehicle. Plasma samples were collected at various time points post-dose, and AUC is reported as a measure of exposure. The animals were then sacrificed at 6 h post-dose for measuring concentration of the dosed inhibitor in liver, target organ for the viral disease. The parent compound **47** showed acceptable oral AUC levels (1 μM/h), and minimal target organ exposure. 4-Methyl and 4-methoxy substituted analogs, **48** and **49**, respectively, showed similar in vivo profile. The fluoro substituted compounds **50** and **51** displayed poor oral exposure. Interestingly, inhibitor **53** demonstrated good exposure with an AUC = 4.8 μM/h, and acceptable target organ distribution (liver $conc_{6h}$ = 850 ng/g). The isopropyl ester substituted compound, **54** displayed excellent oral exposure (AUC = 20 μM/h) and target organ concentration (liver $conc_{6\ h}$ = 4725 ng/g). Inhibitor **58** containing the sulfone moiety that exhibited good potency and excellent selectivity did not show any appreciable plasma levels. 2-Thiazolyl and 3-thiophenyl substituted alkynyl derivatives, **61** and **63**, respectively, displayed similar profile when subjected to rat PK studies with acceptable oral bioavailability of 18–19%. While the substituted pyridyl group attached to the alkynyl moiety demonstrated some of the best replicon potency in this series, most of those analogs displayed poor oral exposure. Thus, compounds **68**, **69**, **72**, and **75** showed no detectable levels in plasma or liver, while inhibitors **73** and **76** exhibited minor amounts of plasma exposure and liver distribution on oral dosing. Based on the good potency, selectivity and rat oral bioavailability, inhibitor **63** was dosed in dogs at 2 mpk. Analog **63** demonstrated very good oral exposure in dogs, with an $AUC_{0-24\ h}$ = 2.2 μM/h.

Table 4
Rat in vivo data of selected compounds

Compd	$AUC_{0-6\ h}$ (μM/h) [a]	F (%)	Liver $conc_{6\ h}$ (ng/g)
47	1	—	70
48	0.73	—	20
49	0.5	—	<10
50	0.06	—	<100
51	ND[b]	—	<100
53	4.8	—	850
54	20	—	4725
58	ND	—	—
61	0.6[c]	18	—
63	1.6[c]	19	—
68	ND	—	—
69	ND	—	—
72	ND	—	—
73	0.11	—	40
75	ND	—	—
76	0.03	—	20

[a] Only PO (10 mpk), vehicle—0.4% mc.
[b] ND—not detected after PO dosing.
[c] IV (2 mpk)/PO (10 mpk), $AUC_{0-24\ h}$.

Target engagement of the aforementioned HCV replication inhibitors are not fully understood as yet. Based on some structural resemblance to nucleosides, it would seem reasonable to hypothesize that these compounds are behaving as traditional nucleoside polymerase inhibitors. However, phosphorylated metabolites were not detected for compounds from similar structural class when dosed in replicon cells. This result suggests that the above described compounds do not impart their replicon activity via classical nucleoside-type mechanisms. Furthermore, representative compounds from the above series displayed only weak inhibitory activity (IC_{50} = 200–1000 μM, for **63** and **73**) in the polymerase enzymatic assay, and no activity in the protease or helicase assay (up to 1 mM, for **63**) (manuscript in preparation). Thus, despite the lack of in vitro enzymatic activities, potent replicon inhibition can be achieved with these compounds, suggesting a mechanism of action which inhibits the HCV replication complex (replicase). Additional studies will be required to further elucidate inhibitor mechanism.

Inhibitor **1** was identified as a screening lead based on the interesting potency in the subgenomic replicon assay, with good selectivity index. While modifications to the core structure, carbasugar replacement, were not tolerated, selected substitutions on the phenyl ring appended to the 5-azo linkage improved replicon potency. Potential toxicological issues associated with the azo linkage prompted the investigation of 5-alkenyl moiety as a suitable alternative, albeit with no improvement in potency. Saturation or cyclopropanation of the alkenyl group proved detrimental in terms of potency. Based on the requirement of sp^2-like substitution at 5-position of the pyrimidine ring, alkynyl functionality was explored. The parent phenyl substituted analog **47** restored replicon potency, and more importantly demonstrated acceptable oral exposure when subjected to rat PK study. Furthermore, compounds from the 5-alkynyl series, **53** and **54**, displayed good potency, selectivity and very good rat plasma and target organ exposure on oral dosing. Disubstituted pyridyl functionality appended to the 5-alkynyl linkage resulted in best in vitro profile, with inhibitor **75** exhibiting replicon EC_{50} = 0.01 μM and excellent selectivity index (CC_{50}/EC_{50} = 1000). Unfortunately, the potent disubstituted pyridyl analogs evaluated were plagued with poor rat PK profile. Finally, inhibitors **53** and **63** exhibited good overall profile, in terms of potency, selectivity index, rodent plasma (for **63**) and liver exposure (for **53**), and dog plasma exposure (for **63**). Thus we have identified carbanucleoside-like core structure with 5-arylacetylenic linkage

on the pyrimidine ring as potential lead structure. Further lead optimization efforts to discover HCV replicase inhibitors from the novel carbanucleoside-like pharmacophore, with improved in vitro and in vivo profiles will be the subject of future communication.

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