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Biaryl guanidine inhibitors of in vitro HCV-IRES activity

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Abstract—A structure–activity relationship analysis was carried out on a high-throughput small molecule screening lead for HCV-IRES translation inhibition. The study led to the identification of a guanidine-based structure with low μM inhibitory activity.

Over 170 million people are infected with hepatitis C virus (HCV), representing 1–3% of the world population.[1,2] Persistent HCV infections can lead to liver disorders such as cirrhosis and hepatocellular carcinomas that eventually require organ transplantation.[3] Current therapies, based on combinations of pegylated interferons and ribavirin, provide a sustained response in only a portion of the treated patients and side effects can be quite severe.[4] Since there is still no effective, well-tolerated treatment for HCV infection, alternative novel therapies are desperately needed.[5]

A new approach to combat HCV infection would be a small molecule inhibitor of the HCV internal ribosome entry site (IRES).[6] The IRES, a highly structured non-translated domain of 340–342 bases located at the 5′ terminus of the HCV genome,[7] allows HCV to bypass the normal cap-dependent cellular pathway for protein synthesis. The IRES's unique function and highly conserved sequence make it an interesting target for selective antiviral therapy.[5,8] Furthermore, an HCV-IRES inhibitor is especially attractive as it would intervene early in the life-cycle of the virus. The IRES has been targeted by oligonucleotides[9] and artificial ribozymes,[10] but little progress has been made in the development of small molecule inhibitors.[11–13] New small molecule lead structures need to be identified and optimized for HCV-IRES inhibitory activity to exploit the full potential of this target.

A library of ~180,000 compounds, biased towards structured RNA targets, was evaluated for HCV-IRES transcription/translation inhibitory activity. Inhibition of IRES-mediated translation was detected by use of a dicistronic plasmid, as described by Honda et al.[14] In the dual reporter assay the dicistronic RNA transcript is expressed containing sequences encoding the reporters separated by a functional HCV-IRES sequence. The translation of the upstream Renilla luciferase reading frame is initiated by the cap-dependent mechanism while translation of the downstream firefly luciferase reading frame is controlled by the IRES.

From the high-throughput screening exercise, biaryl guanidines **1** and **2** were identified as inhibitors of HCV-IRES translation, with some selectivity (~2.5-fold) over cap-dependent translation (Fig. 1).[15] Guanidine **2** (protonated guanidinium group at physiological

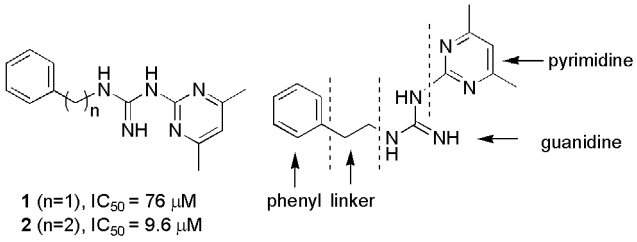

Figure 1. Inhibitors of the HCV-IRES identified from high-throughput screening (left). Components of the SAR study on screening lead **2** (right).

Keywords: HCV-IRES; HCV; Inhibitor; RNA.
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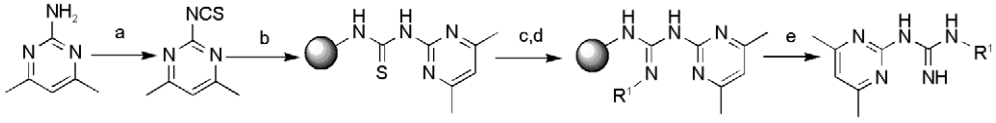
pH) was selected as the starting point for an exploratory structure–activity relationship (SAR) study (Fig. 1).

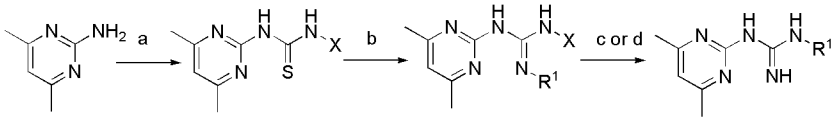
The biaryl guanidines were prepared using either solid or solution-phase methodologies. In the solid phase approach, the pyrimidine isothiocyanate, prepared by treating 2-amino-4,6-dimethylpyrimidine with di-2-pyridyl thionocarbonate,[16] was reacted with ArgoGel®-Rink-NH_2 resin to generate a resin bound thiourea, according to Scheme 1.[17] The thiourea, activated with 1-ethyl-3-(3′-dimethylaminopropyl) carbodiimide hydrochloride (EDC), was then treated with an aryl amine to afford the guanidine derivative, after resin cleavage. The appropriate amines were obtained from commercial sources or prepared using standard protocols, as described in the literature.[18] In the solution-phase approach, the amino pyrimidine was treated with either Fmoc- or benzylcarbonyl-protected isothiocyanate to give the thiourea derivative, according to Scheme 2. The thiourea was then reacted with an amine to afford the guanidine after deprotection. All structures were purified by reversed-phase HPLC to >95% homogeneity (isolated as AcOH salts in most cases). Compounds gave spectral data (LC–MS and ^{1}H NMR) consistent with their assigned structures.

Table 1 summarizes the SAR of the initial set of compounds in this study. Extension of the carbon linker to three methylene units (compound **3**), decreased activity ~15-fold, compared to screening lead **2**, with the two carbon linker. The guanidine NH's are crucial for activity as N-methylation removes the inhibitory activity for guanidines **4** and **5**. Replacement of the guanidine core with an urea (**6**)[19] or a thiourea moiety (**7**)[20] also abolishes activity. When the pyrimidine nitrogens are replaced with carbons, activity is also lost (analogs **8** and **9**) and removing a single pyrimidine methyl substituent resulted in a 2.8-fold loss in potency for guanidine **10**. These modifications highlight the importance of the guanidine H-bonding motif for inhibitory activity. Evidently, biaryl guanidine **2** provided the best starting structure for further SAR analysis.

Table 2 summarizes the effect of a variety of aryl substituents on HCV-IRES inhibitory activity. The order of potency of the 4-substituted analogs is H > OMe ~ Me ~ CO_2Me ~ F ~ Cl > NO_2 ~ OH ~ Br ~ NH_2 > Ph ~ OEt ~ OCF_3 ~ CF_3 > $CH(Me)_2$ > CO_2H, $C(Me)_3$. There is no clear SAR trend based on the electronic nature of the substituents. Steric bulk at the 4-position reduced activity significantly as 4-$C(Me)_3$

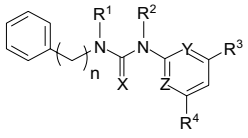


Scheme 1. Reagents and conditions: (a) DPT (di-2-pyridyl thionocarbonate), DCM, reflux, 5 h; (b) ArgoGel®-Rink-NH_2, rt, 12 h; (c) 10 equiv EDC, 20 equiv DIPEA, DCM, rt, 30 min; (d) 10 equiv R^1NH_2, 40 °C, 20 h; (e) 35% TFA, DCM, 2 h, rt, 55–60% over three steps based upon initial resin substitution (HPLC-purified).



Scheme 2. Reagents and conditions: (a) X-NCS, X = Fmoc or COPh, DCM; (b) 2 equiv R^1NH_2, 2 equiv EDC, 4 equiv DIPEA, DCM; (c) X = Fmoc, PS-trisamine resin, DMF; (d) X = COPh, 10% HCl, dioxane, 55–60% over three steps (HPLC-purified).

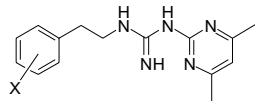
Table 1. Initial SAR for screening lead **2**



Compd	*n*	X	R^1	R^2	Y	Z	R^3	R^4	HCV-IRES IC_{50} (μM)	Selectivity[a]
1	1	NH	H	H	N	N	Me	Me	76	2.6
2	2	NH	H	H	N	N	Me	Me	9.6	2.4
3	3	NH	H	H	N	N	Me	Me	150	1.3
4	2	NH	Me	H	N	N	Me	Me	>200	—
5	2	NH	H	Me	N	N	Me	Me	>200	—
6	2	O	H	H	N	N	Me	Me	>200	—
7	2	S	H	H	N	N	Me	Me	>200	—
8	2	NH	H	H	N	C	Me	Me	>200	—
9	2	NH	H	H	C	C	Me	Me	>200	—
10	2	NH	H	H	N	N	H	Me	46	3.5

[a] Selectivity = (non-IRES IC_{50}/HCV-IRES IC_{50}). Selectivity values are not reported for compounds with non-IRES IC_{50}'s > 200 μM.

Table 2. Aryl SAR



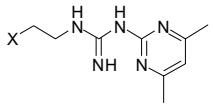
Compd	X	HCV-IRES IC_{50} (μM)	Selectivity[a]
2	H	9.6	2.4
11	4-OMe	25	4.1
12	4-OEt	82	>2.4
13	4-Ph	80	>2.5
14	4-OH	49	3.0
15	4-Me	34	3.8
16	4-OCF_3	92	>2.2
17	4-CO_2H	>200	—
18	4-NO_2	54	1.5
19	4-$C(Me)_3$	>200	—
20	4-$CH(Me)_2$	104	>1.9
21	4-CF_3	82	2.2
22	4-NH_2	61	2.5
23	4-CO_2Me	29	3.4
24	4-F	23	4.1
25	4-Cl	24	2.3
26	4-Br	50	2.5
27	2-Me	15	1.8
28	2-Cl	16	1.8
29	2-OMe	13	1.8
30	3-Cl	12	1.8
31	3-Me	23	2.8
32	2,5-Me	35	5.7
33	2,4-Me	23	3.3
34	2,4,6-OMe	100	>2.0
35	2,4-OMe	68	2.6
36	2,5-OMe	94	>2.1
37	3,5-OMe	26	>7.7

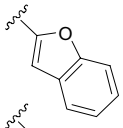
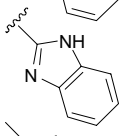
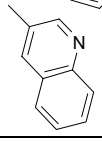
[a] Selectivity = (non-IRES IC_{50}/HCV-IRES IC_{50}). Selectivity values are not reported for compounds with non-IRES IC_{50}'s > 200 μM.

and $-CH(Me)_2$ substitutions were not tolerated. Substitution was slightly better tolerated at the 2 and 3 positions (2-Me > 3-Me > 4-Me and 2-Cl ~ 3-Cl > 4-Cl). The ionizable 4-carboxy substitution abolishes the activity of biaryl guanidine **17**. The aryl ring was substituted for a variety of heterocycles, according to Table 3. The order of potency of the heterocyclic modifications are 2-benzofuran ~ 2-benzimidazole ~ 3-quinoline > 3-pyridine ~ 2-pyridine ~ 2-thiophene > 4-pyridine > 2-furan. The fused heterocyclic substitutions, with better π-stacking potential, were slightly more potent than the others. Since neither the aryl nor the heterocyclic substitutions (Tables 2 and 3) gave better HCV-IRES inhibitory activity over screening lead **2**, the phenyl group was retained for the linker and pyrimidine SAR.

A few substitutions were made on the linker between the phenyl group and the guanidine core, according to Table 4. Methyl substitution decreased inhibitory activity and enantiomer **47** was 1.7-fold more potent than enantiomer **46**. For hydroxy substitution at the same position, inhibitory activity was retained for enantiomer **49** and ~8-fold reduced for enantiomer **48**. The potency difference between enantiomers **48** and **49** may suggest the accessibility of a somewhat structured binding site.

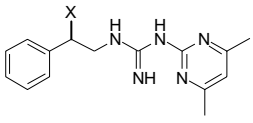
Table 3. Heterocycle SAR



Compd	X	HCV-IRES IC_{50} (μM)	Selectivity[a]
2	Ph	9.5	2.4
38		39	2.4
39		32	4.0
40		55	>3.6
41		97	>2.0
42		38	1.3
43		25	2.8
44		23	1.7
45		23	3.7

[a] Selectivity = (non-IRES IC_{50}/HCV-IRES IC_{50}). Selectivity values are not reported for compounds with non-IRES IC_{50}'s > 200 μM.

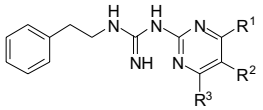
Table 4. Linker SAR



Compd	X	HCV-IRES IC_{50} (μM)	Selectivity[a]
2	H	9.5	2.4
46		35	5.7
47		21	1.9
48		75	>2.7
49		9.7	2.2
50[b]		75	>2.7

[a] Selectivity = (non-IRES IC_{50}/HCV-IRES IC_{50}).
[b] Racemic mixture.

Table 5. Pyrimidine SAR



Compd	R^1	R^2	R^3	HCV-IRES IC_{50} (μM)	Selectivity[a]
2	Me	H	Me	9.6	2.4
51	OMe	H	OMe	35	5.0
52	Et	H	Et	13	2.2
53	Me	Me	Me	4.2	1.3
54	Me	Br	Me	8.1	2.2
55	Me	OH	Me	19	3.6
56	Me	OMe	Me	9.9	3.4
57	Me	$O(CH_2)_3$–$N(Me)_2$	Me	2.1	2.0

[a] Selectivity = (non-IRES IC_{50}/HCV-IRES IC_{50}).

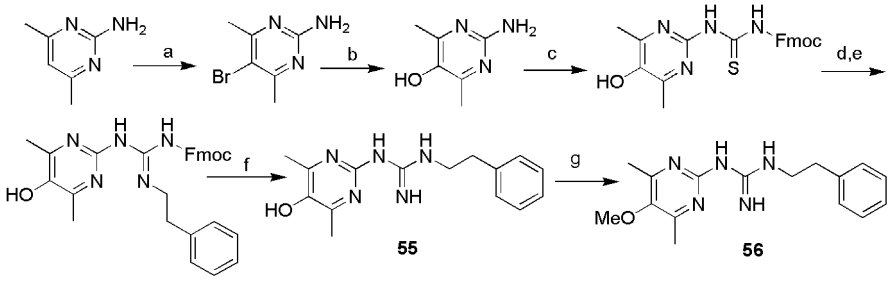
Phenyl substitution on the linker resulted in a ~8-fold reduction in HCV-IRES activity of racemic biaryl guanidine **50**, compared to parent structure **2** (Table 4).

For the exploratory pyrimidine SAR study (Table 5), a limited number of amino pyrimidine precursors were commercially available. Compounds **51**–**53** were prepared according to Scheme 1, with the appropriate amino pyrimidines. The amino pyrimidine precursor required for the synthesis of **51** was commercially available, while precursors for guanidines **52**, **53** and **54** (See Scheme 3) were prepared using standard literature protocols.[21] Guanidines **55** and **56** were prepared using straightforward procedures, according to Scheme 3.

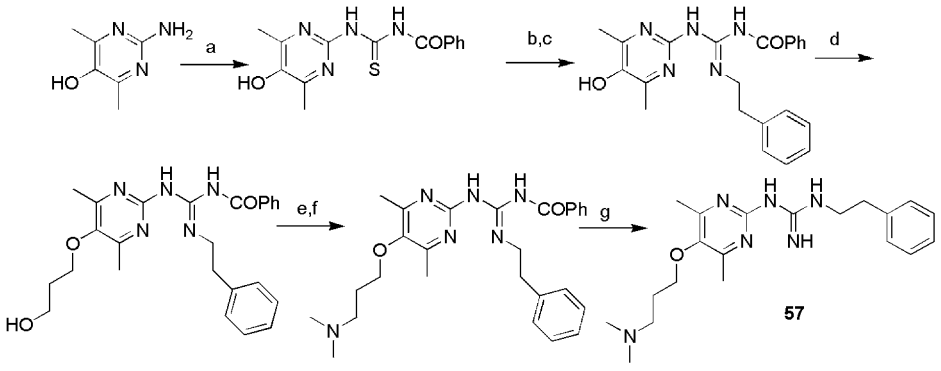
In the pyrimidine SAR set, replacing methyl with methoxy substituents in guanidine **51** resulted in a 3.6-fold loss in activity. Activity was only slightly reduced for **52** with the ethyl substitution. Substitution was tolerated at the 4-position (R^2) of the pyrimidine and the order of potency is Me > H ~ Br ~ OMe > OH.

Since substitution was tolerated at the 4-position of the pyrimidine ring, we chose to introduce a dimethylaminopropoxy side-chain, a potential RNA-binding motif, at this position.[22,23] Guanidine **57** was prepared according to Scheme 4 in a straightforward manner[24] and was found to be 4.5-fold more potent than screening lead **2**. Unfortunately, an improvement in selectivity did not accompany the enhanced inhibitory activity for **57**.

In summary, the SAR study led to the identification of guanidine **57** as a 2 μM inhibitor of HCV-IRES translation. Overall, the screening lead structure **2** was rather resistant to chemical optimization as attempts to identify additional binding contacts with numerous H-bonding and π-stacking functionalities did not improve activity. A modest 4.5-fold improvement in HCV-IRES inhibitory activity was observed for biaryl guanidine **57** with the cationic dimethylaminopropoxy side-chain substitution. Future SAR studies can be focused on improv-



Scheme 3. Reagents and conditions: (a) Br_2, AcOH, 0 °C, 50%; (b) $Ba(OH)_2$, Cu–bronze powder, H_2O, 180 °C, 20 h, 22%; (c) Fmoc-NCS, acetone, 12 h; (d) EDC, DIPEA, 0 °C, DCM, 15 min; (e) Ph–$(CH_2)_2$–NH_2, rt, 12 h; (f) PS-trisamine resin, DMF, 12 h, rt; (g) MeOH, PPh_3, diisopropyl diazodicarboxylate (DIAD), THF, 12 h, rt, 25% over five steps.



Scheme 4. (a) Reagents and conditions: (a) PhCONCS, acetone–DCM, 1:1, 12 h; (b) EDC, DIPEA, DCM, 0 °C, 15 min; (c) Ph$(CH_2)_2$–NH_2, 12 h, rt; (d) Br–$(CH_2)_3$–OH, acetone, K_2CO_3, 12 h, reflux; (e) MsCl, TEA, DMAP, DCM, 0 °C then 12 h, rt; (f) 2 M $(CH_3)_2$–NH in THF, 40 °C, 12 h; (g) 10% HCl/dioxane, 12 h, reflux.

ing the selectivity and potency of new lead structure **57**. In general, the pharmacophoric information presented in this paper is useful for the design and optimization of HCV-IRES small molecule inhibitors.

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24. Structural data for **57**: ^{1}H NMR (CD_3OD, 300 MHz): δ 7.21 (m, 5H), 3.77 (t, 2H, $J = 6.2$), 3.58 (t, 2H, $J = 6.8$), 2.90 (t, 2H, $J = 6.2$), 2.56 (m, 2H), 1.91 (m, 2H), 1.80 (s, 6H); MS (ES^+) 371.1 (MH^+).