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Identification of [(naphthalene-1-carbonyl)-amino]-acetic acid derivatives as nonnucleoside inhibitors of HCV NS5B RNA dependent RNA polymerase

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Abstract—A novel series of HCV NS5B RNA dependent RNA polymerase inhibitors containing a naphthalene carboxamide scaffold were identified by high throughput screening. Optimization of substituents by parallel synthesis and the iterative design towards understanding structure–activity relationship to improve potency are described. Tetra substituted naphthalene **31** displayed potent activity with IC_{50} of 120 nM against HCV NS5B enzyme and was selective over a panel of polymerases.

Hepatitis C infection is a major form of post-transfusion hepatitis.[1] Eighty percent of the infected patients develop chronic hepatitis, of these 20% progress to develop cirrhosis, bridging fibrosis and 1–5% to develop hepatoculluar carcinoma. To date, there is no vaccine for prevention of the infection. The currently approved treatments, interferon monotherapy or interferon and ribavirin combination therapy, are effective in up to 56% of naive patients. Considerable side effects are associated with these regimens causing up to 20% of the patients to discontinue the therapy.[2] As a result, there is an unmet need for developing a safe and effective antiviral agent.

Hepatitis C virus genomic RNA encodes three structural and seven nonstructural proteins. The nonstructural protein 5B (NS5B) is an RNA dependent RNA polymerase, which is a key component in the viral genome replication.[3] The enzymatic activities of this enzyme have been extensively characterized in vitro.[4] Recently, a cell-culture model system containing a subgenomic replicon capable of supporting HCV replication has been developed.[5] The availability of these in vitro systems makes it possible to screen for inhibitors that might have clinical utility for treatment of diseases caused by HCV. Several classes of HCV NS5B polymerase inhibitors, both nucleoside[6] and nonnucleosides[7] (Fig. 1) have been reported. The diketo acid **1** is reported as an inhibitor capable of interacting directly with the metal ions present in the enzyme active site. The benzo-1,2,4-thiadiazine derivative **2**, identified from screening is reported to interact directly with the viral polymerase and inhibit RNA synthesis. Benzimidazole class of polymerase inhibitors **3** is reported to have been undergoing clinical trials.[7c] Crystal structures of another class of

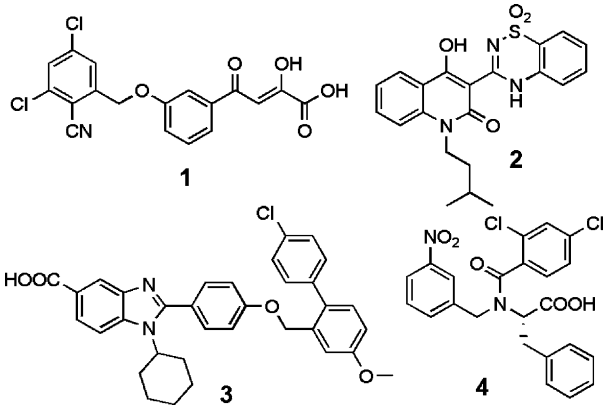

Figure 1. Small molecule inhibitors of HCV NS5B polymerase.

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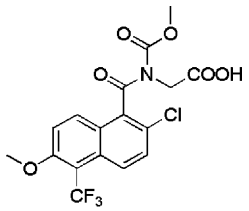
inhibitors characterized by a N,N-disubstituted phenylalanine moiety **4** and NS5B HCV polymerase has identified an allosteric binding site located about 35 Å from active site.

Our effort towards identifying a HCV polymerase inhibitor started with the high throughput screening of various compound libraries. The effort culminated in the identification of naphthalene carboxamide **5** (Fig. 2) that had an IC_{50} of 5.0 μM. It was found to be inactive against human polymerase β (IC_{50} = >100 μM), calf thymus polymerase α (IC_{50} = >100 μM), helicase (IC_{50} = >75 μM) as well as HIV reverse transcriptase (IC_{50} = >100 μM). Employing fluorescence spectroscopy techniques, the apparent K_D of 1.6 μM was determined from the changes in the endogenous tryptophan fluorescence of the enzyme upon inhibitor binding, at the emission and excitation wavelength of 340 and 295 nm, respectively. From these experiments the stoichiometry of binding was found to be in a 1:1 ratio indicative of specific binding. The SAR studies to improve the potency of this novel lead are described below.

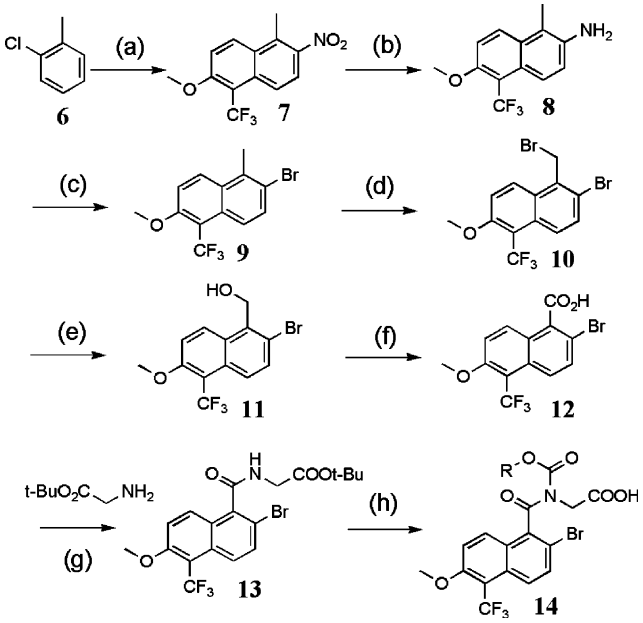


5

Figure 2. HTS lead for HCV NS5B polymerase.

The compounds for this series were synthesized as shown in Scheme 1 starting from the in-house intermediate **7** (prepared in several steps starting from **6**[8]). Reduction to the amino compound **8** followed by diazotization and bromination afforded the bromo compound **9**. The required acid **12** was obtained by benzylic bromination followed by hydrolysis and oxidation of the alcohol **11**. Coupling of the acid with amino acid esters using standard coupling conditions followed by acylation of the nitrogen and ester hydrolysis gave the desired targets.[9]

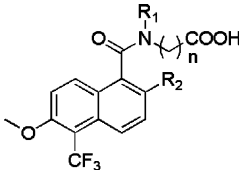
Our initial efforts towards understanding SAR of the lead molecule were focused on replacing the highly substituted naphthalene core by other mono and bis substituted naphthalene scaffolds. Towards that end, 5-bromo, 4-methyl, 4-fluoro, 4-N,N-dimethylamino, 6-methoxy, 5-trifluoromethyl-6-methoxy, and 2-naphthyl analogs of the lead were synthesized and were found to be inactive against HCV NS5B polymerase (IC_{50} = >50 μM). This preliminary information on the substituents requirement on the naphthalene scaffold prompted us to retain the 6-methoxy-5-trifluormethyl substituents in place and explore variations in the C-2 substituent and the carbamate moiety. The results of this study are summarized in Table 1.



Scheme 1. (a) Ref. 8; (b) H_2, Pd/C, MeOH, 12 h, 88%; (c) $NaNO_2$, CuBr; HBr, 3 h, 55%; (d) NBS, benzoyl peroxide, CCl_4, reflux, 18 h, 62%; (e) NaOH, EtOH, 48 h, 43%; (f) Jones reagent, acetone, 2 h, 67%; (g) EDCI, HOBt, DIEA, DMF, 18 h, 88%; (h) (1) NaH, THF, 60 °C; 30 min. (2) ROCOCl; 18 h, rt. (3) Formic acid.

As seen from example **15** replacing chloro on C-2 position with bromo slightly improved the potency. However a more electron withdrawing fluoro group (example **16**) or electron releasing methoxy group (example **17**) decreased the potency significantly. Removing the halogen from C-2 carbon to give the unsubstituted derivative **18** or replacing it with a bulky 4-chlorophenyl group as in example **19** lead to complete loss of activity. From these examples it is clear that the

Table 1. HCV NS5B Inhibitory activity of naphthalene derivatives



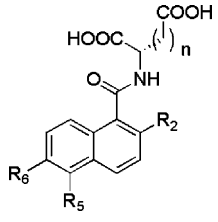
Example	R_1	R_2	*n*	IC_{50} (μM)[10]
5	$-COOCH_3$	Cl	1	5.0
15	$-COOCH_3$	Br	1	1.4
16	$-COOCH_3$	F	1	15.2
17	$-COOCH_3$	OMe	1	11.3
18	$-COOCH_3$	H	1	>26
19	$-COOCH_3$	4-ClPh	1	>20
20	$-COOCH_2CH_3$	Br	1	3.1
21	$-COO(CH_2)_2CH_3$	Br	1	4.9
22	$-COOCH_2C{\equiv}CH$	Br	1	2.9
23	$-COOCH_2CH{=}CH_2$	Br	1	3.3
24	$-COOCH_2CH(CH_3)_2$	Br	1	7.7
25	$-COOCH_2C(CH_3)_3$	Br	1	6.4
26	$-COOCH_3$	Br	2	>10
27	$-CH_3$	Cl	1	5.1
28	$-CH_3$	Br	1	2.0
29	$-CH_2COOH$	H	1	0.9

bromine meets the steric and electronic requirements for this position, hence further optimization of the carbamate region was carried out by retaining the bromo substituent for the C-2 position. Changing the methyl carbamate **15** to other alkyl carbamates like ethyl, *n*-propyl, propargyl, allyl, *i*-butyl or neopentyl carbamates (examples **20**–**25**) did not decrease the potency significantly. Homologation of the acid side chain by 1 carbon (example **26**) rendered the molecule inactive. Attempts to remove the carbamate moiety leading the unsubstituted glycinamide resulted in inactive compound. However, a small alkyl group like methyl can replace the carbamate moiety and the resulting N-methylated glycinamides were equipotent (examples **27** and **28**). Of the other groups experimented for the R_1 region, an acetic acid moiety was found to be more favourable than a carbamate or methyl group as shown by example **29**.

The above observation prompted us to explore substituted naphthyl amides using bis carboxylic acids as part of the amino acid component as shown in Table 2.[11] When comparing the four pairs of compounds (examples **30**–**37**), it was very clear that using L-glutamic acid had significant improvement in potency compared to L-aspartic acid. Also, it was interesting to see that the bis carboxylic acid moiety seems to have a better impact on the inhibitory effect than the aromatic substituents itself. Although the trend we observed in the carbamate series was noticed in these analogs as well, simpler disubstituted analogs like **35** and **37** also displayed sub micromolar potency. However, the bromo group seems to be the ideal group for the C-2 position in this series as well and an electron releasing methoxy group was undesirable.

The most potent inhibitor **31** from this SAR study was selected for further characterization. Compound **31** displayed potency against various isolates of NS5B enzyme derived from HCV 1b genotype with IC_{50}s ranging from 0.12–1.8 μM. It showed no inhibitory activity against a panel of human polymerases including mitochondrial DNA polymerase gamma, and other unrelated viral polymerases up to 80 μM, demonstrating its specificity for the HCV polymerase. However, the low permeability characteristic of bis carboxylic acids rendered these compounds without significant effect in the replicon system, but provides a novel chemotype specific for HCV polymerase for further exploration.

Table 2. HCV NS5B inhibitory activity of naphthyl amide bis carboxylic acid derivatives



Example	R_2	R_5	R_6	*n*	IC_{50} (μM)[10]
30	Br	CF_3	OMe	1	0.91
31	Br	CF_3	OMe	2	0.12
32	F	CF_3	OMe	1	2.1
33	F	CF_3	OMe	2	1.4
34	H	Br	OMe	1	1.2
35	H	Br	OMe	2	0.59
36	H	CF_3	OMe	1	1.3
37	H	CF_3	OMe	2	0.49
38	OMe	H	H	1	>10

In conclusion, we have identified [(naphthalene-1-carbonyl)-amino]-acetic acid derivatives as a novel class of HCV NS5B RNA dependent RNA polymerase inhibitors. We have explored the structure activity requirement for this class of inhibitors and identified simpler naphthyl amide bis carboxylic acid analogs as sub micro-molar inhibitors.

Acknowledgements

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9. Compounds were purified by HPLC and the purity was >90%. LC conditions: HP 1100, 23 °C, 10 μL injected; Column: YMC-ODS-A 4.6×5.05μ; Gradient A: 0.05% TFA/water, B: 0.05% TFA/acetonitrile; time 0–1 min: 98% A and 2% B; 7 min: 10% A and 90% B; 8 min: 10% A and 90% B; 8.9 min: 98% A and 2% B; Post time 1 min; Flow rate 2.5 mL/min; Detection: 215 and 254 nm, DAD. Semi-Prep HPLC: Gilson with Unipoint software; Column: Phenomenex C18 Luna 21.6 mm×60 mm, 5 μM; Solvent A: water (0.02% TFA buffer); Solvent B: acetonitrile (0.02% TFA buffer); Solvent gradient: Time 0 min: 5% B; 2.5 min: 5% B; 12 min: 95% B; Hold 95% B 3 min; Flow rate: 22.5 mL/min; Detection: 215 and 254 nm.
10. The recombinant C-terminally truncated NS5B enzyme used in the assay was derived from genotype 1b, BK strain. Inhibitors were pre-incubated with the enzyme for 15 min followed by an addition of an RNA template, NTPs and [α-^{32}P]GTP. The reaction was carried out at room temperature for 2 h. Product RNA containing incorporated radioactive nucleotides was collected by filtration and the amount of radioactivity was quantified using a scintillation counter.
11. From the analogous SAR work carried out with 1,8-naphthalimide scaffold all the other natural amino acids were found to be inactive indicating the importance of the bis carboxylic acids and the D-Glu and D-Asp amino acids were 20-fold less potent.