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Discovery of a novel series of non-nucleoside thumb pocket 2 HCV NS5B polymerase inhibitors

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ABSTRACT

A novel series of non-nucleoside thumb pocket 2 HCV NS5B polymerase inhibitors were derived from a fragment-based approach using information from X-ray crystallographic analysis of NS5B-inhibitor complexes and iterative rounds of parallel synthesis. Structure-based drug design strategies led to the discovery of potent sub-micromolar inhibitors **11a–c** and **12a–c** from a weak-binding fragment-like structure **1** as a starting point.

The hepatitis C virus is a leading cause of liver disease and represents a serious health concern.[1] Treatment of chronic HCV infection is based on the poorly tolerated combination of pegylated interferon-alpha (peg-IFN) and the nucleoside analogue ribavirin (RBV).[2] Historically, this regimen had limited efficacy against the genotype (gt) 1 strains[3] that are predominant in Europe, North America and Japan[4] providing sustained virological response (SVR) rates of 40–50%. In 2011, the first direct acting HCV antivirals, the protease inhibitors telaprevir and boceprevir, were approved in combination with peg-IFN and RBV for the treatment of HCV gt 1 infection. These new agents improved SVR rates to approximately 68–75% while decreasing treatment duration for a significant proportion of patients.[5] The HCV drug development landscape is rapidly evolving towards the use of combinations of direct acting antivirals that will eliminate the need for peg-IFN and provide better efficacy and tolerability for the treatment of HCV infection.[6] We and others have been working on various approaches to find new, complementary oral antivirals that act directly against virally encoded functions.[7]

To complement our efforts in the development of thumb pocket 1 non-nucleoside inhibitors (NNIs) of the HCV NS5B polymerase,[8] we began an initiative to identify new lead structures that bind to one of the other three reported allosteric sites of the enzyme[9] and could be used in combination with our thumb pocket 1 inhibitor BI 207127[8b] and HCV protease inhibitor faldaprevir.[10] In a search for low molecular weight ligands that bind to the NS5B protein and could serve as starting points for a fragment-based approach, we discovered that sulfonamide derivative **1** binds in the previously identified thumb pocket 2 allosteric pocket (Fig. 1).[11,12] It is notable that **1** was also observed in palm site 1. However, it was the interaction of **1** in thumb pocket 2 that was successfully translated into a novel lead series.

A pocket view overlay of fragment **1** with previously reported pyranoindole thumb pocket 2 inhibitor **2**[13] (Fig. 2A) revealed a good overlap of the halogenated benzene rings over the lipophilic surface defined by residues L419 and M423 as well as the occupancy of the deep lipophilic pocket by the methylisoxazole of **1** and the propyl chain of **2**. A key interaction that was observed with the carboxylic acid of pyranoindole **2** but not observed for **1** was a hydrogen bond with S476.

In an attempt to incorporate this interaction into fragment **1**, 41 anthranilic acid sulfonamides **5** were synthesized from anthranilic acid derivatives **4** and 2,4,5-trichlorosulfonyl chloride **3** (Scheme 1). Improved potency was observed as summarized by the representative analogues **5a–c** in Table 1. For example, unsubstituted anthranilic acid **5a** was fourfold more active than **1** (IC_{50} ~100 μM) and halogen substituents at the 4- and 5-positions (compounds **5b** & **5c**) provided inhibitors with IC_{50} values in the 10 μM range.

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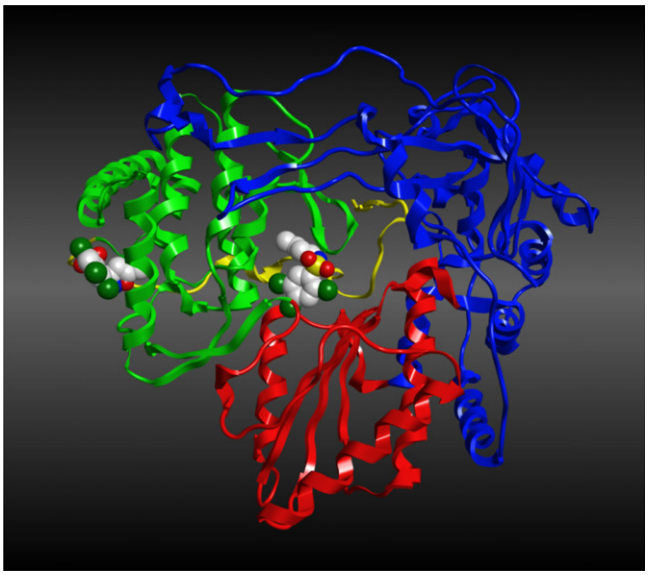
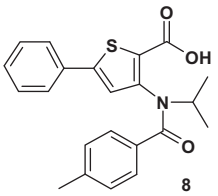


Figure 1. Ribbon structure of the HCV NS5B polymerase. Colored in red is the palm domain that includes the active site. The finger domain is colored in blue and the thumb domain is green. Sulfonamide **1** is depicted as a CPK model bound to thumb pocket 2 (green domain) and palm site 1 (green/red domain interface).

Analysis of the inhibitor:enzyme complex of 4-chloroanthranilic acid derivative **5c** indicated that this analogue was partially successful in achieving the desired hydrogen bonding interaction. Figure 2B shows the overlay with fragment **1** where the carboxylic acid of **5c** forms a water-mediated H-bond with the backbone amide N–H of S476.

The SAR of the arylsulfonamide substituent was explored in combination with the 5-bromo- and 4-chloroanthranilic acid substitution of **5b**–**c**. Halogenated anthranilic acids **4b**–**c** were coupled with arylsulfonyl chlorides under the same reaction conditions described in Scheme 1 to produce compound series **6** ($n = 74$) and **7** ($n = 54$). Unfortunately, no significant improvements in potency were achieved. Representative inhibitors **6a**–**d** and **7c**–**d** are shown in Table 2. The unsubstituted phenyl sulfonamide **6a** was approximately fourfold less potent than 2,4,5-trichlorosulfonylamide **5b**. Halogenated aryl- and heteroarylsulfonamides such as **6b**–**d** and **7b**–**d** resulted in a relatively flat SAR. Despite providing only a marginal improvement over **5c**, the most important inhibitor to emerge from this exercise was 5-bromothien-2-ylsulfonamide **7d**. Analysis of the binding mode of this compound provided a key observation that led us towards inhibitors with improved potency.

Analysis of the polymerase: **7d** complex highlighted an unexpected change in binding mode as shown in Figure 2C. In the overlay of **7d** and **5c**, the anthranilic acid and sulfonamide moieties were transposed to enable bidentate hydrogen bonding between the inhibitor carboxylic acid and the backbone N–H's of S476 and Y477. These hydrogen bonding patterns in thumb pocket 2 are similar to those reported for the thiophene carboxylic acid inhibitor **8**.[11f]



The new binding mode prompted an exploration of the 4- and 5-positions of the anthranilic acid ring in order to improve potency by exploiting hydrophobic contacts with the protein surface in a similar fashion to the halogenated phenyl rings of **2** and **5c** in Figure 2A and B. As shown in Table 3, introduction of a phenyl group to the anthranilic acid scaffold, analogous to thiophene carboxylic acid **8**, at either the 5- (R_5 = Ph, **9**) or 4- (R_4 = Ph, **10**) position did not improve potency. After examining a variety of substituents at these positions, incorporation of an oxygen linker between the two phenyl rings was found to provide a 10-fold improvement in potency. This improvement was observed for both the 4-phenoxy **11a** and 5-phenoxy **12a** derivatives.

Scheme 2 describes the introduction of phenyl substitution at the 4- and 5-positions of the anthranilic acid scaffold. Phenylboronic acid was coupled to 5-bromoanthranilic acid methyl ester **13** or 4-bromo-2-nitrobenzoic acid **14** using a Suzuki–Miyaura coupling to produce the corresponding intermediates **15** and **16**. The 5-phenylanthranilic acid methyl ester **15** was reacted with 5-bromo-2-thiophenesulfonyl chloride then saponified to afford inhibitor **9**. Methyl-2-nitro-4-phenylbenzoate **16** was reduced with $Pd(OH)_2/C$ prior to sulfonylation and saponification to prepare inhibitor **10**. The synthesis of the 4- and 5-phenoxy substituted anthranilic acids **11a**–**c** and **12a**–**c** commenced with the S_NAr

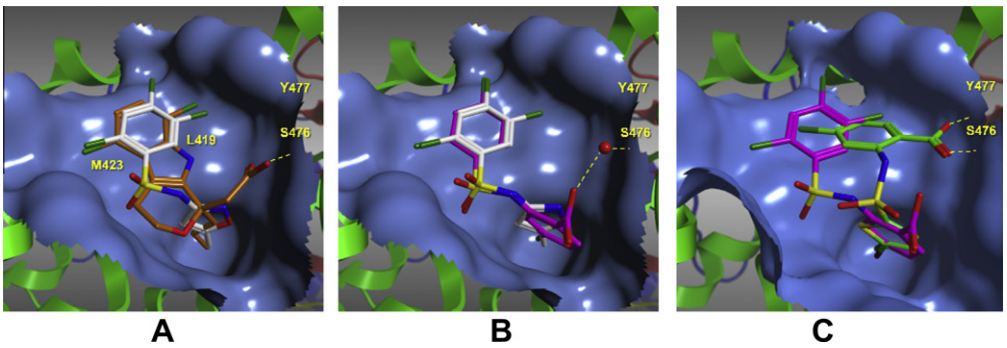
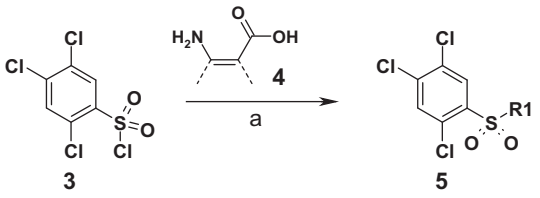


Figure 2. X-ray co-structure overlays in thumb pocket 2. (A) Fragment hit **1** (white) and the pyranoindole derivative **2** (orange). (B) Fragment hit **1** (white) and the anthranilic acid derivative **5c** (magenta). (C) Anthranilic acid derivatives: trichlorophenylsulfonamide derivative **5c** (magenta) and the 5-bromothiophenylsulfonamide **7d** (green).



Scheme 1. Sulfonylation of anthranilic acid derivatives (a) **4**, aniline, satd. aq. Na_2CO_3, 2 day, rt.

Table 1
Carboxylic acid containing inhibitors

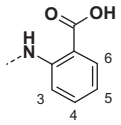
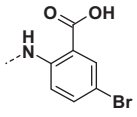
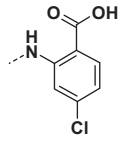
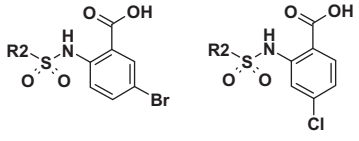
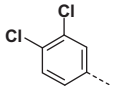
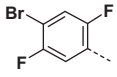
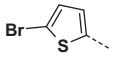
R		IC_{50} (μm)
5a		26
5b		9.0
5c		11

Table 2
5-Bromo- and 4-chloroanthranilic acid sulfonamides



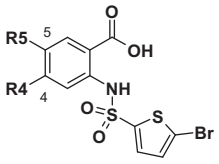
6 **7**

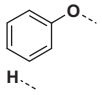
R2		IC_{50} (μm)		IC_{50} (μm)
	**6a**	43		
	**6b**	8.4		
	**6c**	4.5	**7c**	6.8
	**6d**	6.1	**7d**	4.6

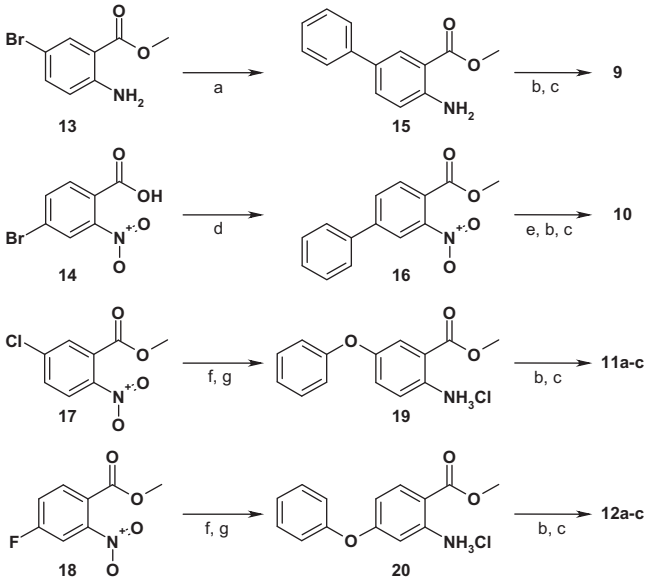
addition of phenol on methyl-5-chloro-2-nitrobenzoate **17** or methyl-4-fluoro-2-nitrobenzoic ester **18.** The nitro groups were reduced by hydrogenolysis followed by treatment with HCl to produce aniline salts **19** and **20**. Sulfonylation and saponification afforded 4- and 5-phenoxyanthranilic acid inhibitors **11a–c** and **11a–c**.

The SAR of sulfonamide substituents on the phenoxyanthranilic acid inhibitors was similar to that reported for the thiophene carboxylic acid chemotype (e.g., **8**).[14] Parallel SAR was observed for the 4- and 5-phenoxyanthranilic acid isomers. The 4-methylphenylsulfonamides-**11b**/**12b** and the 4-bromo-2-fluorophenylsulfonamides **11c**/**12c** (Table 4) were the most potent inhibitors

Table 3
Anthranilic acid substitution at the 5- and 4-positions



R4		R5		IC_{50} (μm)
9				3.7
10				6.7
11a				0.55
12a				0.61

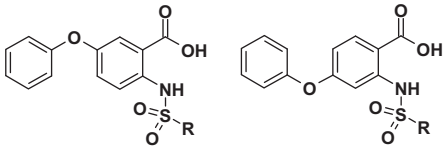


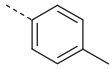
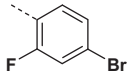
Scheme 2. Synthesis of phenyl- and phenoxyanthranilic acid analogues **9**–**12**. Reagents and conditions: (a) Phenylboronic acid (1.2 equiv), $(Ph_3P)_4Pd$ (0.2 equiv), 2 M aq Na_2CO_3 (2 equiv), DME, 80 °C, 20 h, 57%; (b) sulfonyl chloride (3 equiv), pyridine, 50 °C, 8 h, 80%; (c) NaOH (5 equiv), DMSO, rt, 0.5 h, 60%; (d) same as (a) followed treatment of the crude product with CH_2N_2 in ether prior to purification, 88%; (e) $Pd(OH)_2/C$ (0.2 equiv), MeOH, 1 atm H_2, 16 h, 96%; (f) phenol (1.5 equiv), K_2CO_3 (1.5 equiv), DMSO, 90 °C, 6 h, 57%; (g) same as (e) follow by filtration and treatment with HCl in ether, 79% 2 steps.

identified from a combined total of 47 inhibitors prepared with the 4- and 5-phenoxyanthranilic acids.

We studied the binding mode of the 4-phenoxy- (**11b**) and 5-phenoxy- (**12b**) anthranilic acid inhibitors in thumb pocket 2. The binding orientation of the anthranilic acid scaffold remains consistent with **7d** as can be seen by comparing Figure 2C with the overlay of **11b/12b** in Figure 3. However, the phenoxy substituent of both isomers induced an important change in the pocket conformation. A coordinated movement of residues M423 and L497 created a shallow pocket for the phenoxy group while concomitantly expanding the deep hydrophobic pocket to accommodate larger substituents such as the 2-fluoro-4-bromophenyl group of **11c** and **12c**.[11h] The observation that the phenoxy group occupied the same shallow lipophilic pocket whether they were

Table 4
5- and 4-phenoxy anthranilic acid sulfonamide inhibitors



R			IC_{50} (μm)		IC_{50} (μm)
	**11b**	**11b**	0.22	**12b**	0.16
	**11c**	**11c**	0.34	**12c**	0.31

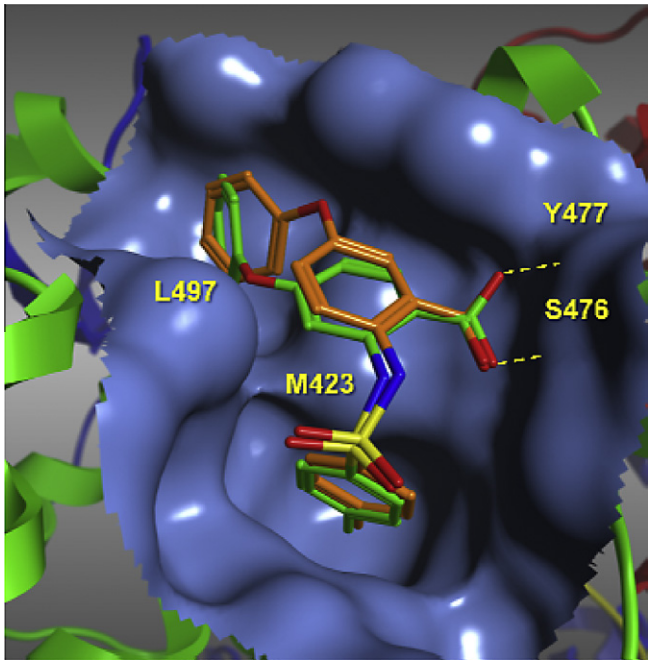


Figure 3. X-ray co-structure overlay of 5-phenoxy (**11b**, orange) and 4-phenoxy (**12b**, green) anthranilic acid inhibitors bound in the induced conformation of thumb pocket 2.

projected from the 5- (**11b**) or 4- (**12b**) position of the anthranilic acid also provides a rationale for the comparable potency.

Three compounds (**11b**, **12b** and **11c**) were profiled against a panel of thumb pocket 1,[15,16] thumb pocket 2[17,18] and palm site 1[19] single amino acid site-specific mutants of the HCV NS5B polymerase and the results are shown in Table 5. Consistent with binding in thumb pocket 2, the potency of each compound shifted approximately 15-fold against the previously disclosed L419 M mutant.[17] Minimal changes in sensitivity were observed with the M423T thumb pocket 2 mutant. This is notable as mutations at M423 have been predominately selected in HCV infected patients dosed with thumb pocket 2 inhibitors.[20,21] As expected, no shift was observed between the wild type genotype 1b and thumb pocket 1 (P495S) or palm site 1 (M414T) mutants. High selectivity was observed versus the RNA-dependent RNA-polymerase from poliovirus and the mammalian RNA polymerase II isolated from calf thymus.[22] Unfortunately, inhibitors **11b**, **12b** and **11c** were not active in the HCV cell-based replicon assay.

Table 6
Summary of in vitro ADME properties of selected anthranilic acid sulfonamide inhibitors

Compds	Solubility[a] (μg/mL)	Caco-2[b] ($\times 10^{-6}$ cm/s)	HLM[c] ($t_{1/2}$, min)	CYP450 IC_{50} 1A2, 2C9, 2C19, 2D6, 3A4
11b	>900	2.2	11	1.8, 3.0, >30, >30, >30
12b	>700	2.4	17	>30, 3.1, 7.9, >30, >30
11c	>900	<0.1	27	>30, 1.1, 1.1, >30, 26

[a] Determined on amorphous solids, 24 h solubility in pH 7.2 buffer using the shaking flask method.
[b] Apical to basal permeability at pH 7.4.
[c] Half-life measured in the presence of human liver microsomes at an initial concentration of 10 μM.

The available in vitro ADME profiles of representative compounds **11b**, **12b** and **11c** are summarized in Table 6. Moderate to poor Caco-2 permeability may be a reflection of poor membrane permeability and would be consistent with the lack of cell culture activity of these compounds. Poor stability in the presence of human liver microsome and low micromolar CYP450 inhibition against select isozymes will need to be addressed during the optimization of the chemical series. By virtue of the presence of a carboxylic acid, excellent solubility was observed at neutral pH.

We have used multiple iterations of parallel synthesis and structure based drug design to discover a novel lead series of thumb pocket 2 anthranilic acid inhibitors from a low affinity fragment. A 650-fold improvement in biochemical potency was achieved for compound **12b** from fragment **1**. Additional profiling confirmed that these inhibitors are selective NS5B thumb pocket 2 inhibitors. Efforts directed towards establishing cell culture activity and improving in vitro ADME properties for this class of allosteric HCV polymerase inhibitors will be reported in due course.

Acknowledgments

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Table 5
Biochemical profiling of selected phenoxyanthranilic acid sulfonamide inhibitors

Compds	Wild type NS5B IC_{50} (μM)	Thumb 2 M423T IC_{50}(μM)	Thumb 2 L419 M IC_{50} (μM)	Thumb 1 P495S IC_{50} (μM)	Palm 1 M414T IC_{50} (μM)	Polio virus polymerase IC_{50} (μM)	Calf thymus polymerase IC_{50} (μM)
11b	0.086	0.17	1.2	—	—	>100	>100
12b	0.072	0.10	1.2	0.077	0.052	>100	>100
11c	0.11	0.20	1.8	0.093	0.052	>100	>100

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