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Discovery of a Novel Series of Potent Non-Nucleoside Inhibitors of Hepatitis C Virus NS5B

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Abstract

Hepatitis C virus (HCV) is a major global public health problem. While the current standard of care, a direct-acting antiviral (DAA) protease inhibitor taken in combination with pegylated interferon and ribavirin, represents a major advancement in recent years, an unmet medical need still exists for treatment modalities that improve upon both efficacy and tolerability. Towards those ends, much effort has continued to focus on the discovery of new DAAs, with the ultimate goal to provide interferon-free combinations. The RNA-dependent RNA polymerase enzyme NS5B represents one such DAA therapeutic target for inhibition which has attracted much interest over the past decade. Herein, we report the discovery and optimization of a novel series of inhibitors of HCV NS5B, through the use of structure-based design applied to a fragment-derived starting point. Issues of potency, pharmacokinetics and early safety were addressed in order to provide a clinical candidate in fluoropyridone **19**.

INTRODUCTION

Hepatitis C virus (HCV) represents a major global public health problem. Over 150 million individuals suffer from chronic infection, with over 350,000 deaths each year attributed to hepatitis C-related liver diseases such as cirrhosis and liver cancer.¹ While vaccines exist for some other hepatitis viruses, there are none available for HCV. Up until 2011, standard of care (SOC) treatment consisted of pegylated interferon (Peg-IFN) in combination with the antiviral ribavirin. Numerous shortcomings existed for this treatment regimen, including success rates of only ~ 50% sustained virologic response (SVR) in patients with HCV genotype 1 (GT-1, by far the most common genotype worldwide, accounting for ~ 75% of all cases), and generally poor tolerability.²⁻⁴ Much effort has recently focused on the discovery of direct-acting antivirals (DAAs), which can intervene and interfere at specific points within the viral life cycle.⁵⁻⁹ The first two DAA therapeutics to reach the market,

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ASSOCIATED CONTENT

Supporting Information

Synthetic procedures and spectroscopic data, metabolite ID study of compound **4** (MS/MS/MS), HCV NS5B polymerase protein crystallography, references. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Accession Codes

Atom coordinates and structure factors for HCV NS5B/ligand complexes have been deposited at the Protein Data Bank under the codes 4MK7 (**2**), 4MK8 (**4**), 4MK9 (**12**), 4MKA (**13**), 4MKB (**14**).

NS3/4A protease inhibitors boceprevir and telaprevir, did so in 2011 and are currently being used in combination with the previous SOC, boosting SVR to 70-80% for GT-1.^{10,11} Unmet needs remain, however, to further increase the SVR in both treatment naïve and treatment experienced patients, as well as in patients comorbid with other infections, such as HIV. Also, elimination of side effects associated with IFN is highly desirable in terms of tolerability and patient compliance, and towards that end much research continues into the discovery of new DAAs that may ultimately be used in combination with each other as part of an IFN-free drug cocktail.^{12,13}

HCV is a virus of the family *Flaviviridae*, and contains a positive sense, single-stranded RNA genome. The viral life cycle consists of 8 major stages, including entry (endocytosis), uncoating (fusion), polyprotein synthesis from (+)-RNA, cleavage of the polyprotein into individual proteins (structural and non-structural), RNA replication, viral packaging and maturation, release, and re-infection (Figure 1).¹⁴ Any of those stages represent opportunities for direct-acting antiviral therapeutic intervention. As already mentioned, inhibitors of the NS3/4A protease enzyme have now reached the market and are currently in use in combination with the previous SOC. The RNA-dependent RNA polymerase enzyme NS5B (enlarged section of Figure 1) represents another DAA therapeutic target for inhibition, and has been the focus of intense research in recent years.¹⁵⁻¹⁸ Early efforts focused on identification of nucleoside inhibitors which engage at the catalytic site, ultimately leading to the discovery of some promising therapeutic agents now in late-stage clinical trials.¹⁹ More recently, multiple allosteric sites have been identified on NS5B, including the so-called thumb I, thumb II, palm I and palm II sites.¹⁶ Non-nucleoside inhibitors have been reported for all of these sites, including several drug candidates that are currently under clinical evaluation.²⁰⁻²⁴

Previously, we reported the discovery of a novel series of benzothiazine-substituted quinolinediones as inhibitors of NS5B, acting at the palm I allosteric site.²⁵ One example from that series is compound **1** (Figure 2). Although we identified highly potent inhibitors, oral exposures in preclinical *in vivo* pharmacokinetics studies were not sufficient to reach targeted levels, and ultimately we looked for new chemotypes from which to optimize towards a clinical candidate. More recently, we reported the pioneering use of *de novo* fragment design that enabled us to generate a novel, low molecular weight (MW) lead structure (compound **2**, Figure 2).²⁶ Pyridone **2** exhibits potent inhibition of NS5B enzymatic activity (Table 1), especially considering its small size. Given the low MW and high ligand efficiency (LE = 0.46), we felt **2** was a reasonable starting place from which to develop potent inhibitors of NS5B that would also possess desirable physicochemical and ADMET properties required for a safe, orally delivered drug candidate.

RESULTS AND DISCUSSION

From a structure-based design point of view, we carefully considered the X-ray co-crystal structures of **1** and **2** with NS5B (Figure 3). Both compounds satisfy the large hydrophobic pocket of the palm I allosteric site, with a *p*-fluorobenzyl moiety for **1**, and with *tert*-butylphenyl for **2**. Interestingly for **2**, the core benzene ring engages in a classic edge-to-face π -stack with the side chain of Tyr448, while the *tert*-butyl group completely fills the bottom of the hydrophobic pocket, making close hydrophobic contact with the side chain of Pro197, among other residues. Both ligands engage in *H*-bonding with the adjacent backbone area, via the quinolinedione oxy-anion of **1** and analogously via the pyridone moiety of **2**. As we previously reported, one of the remarkable features of the highly ligand-efficient **2** is the donor-acceptor engagement by the 2(1*H*)-pyridone moiety with two backbone residues (*NH* of Tyr448 and *C=O* of Gln446). In further comparing the co-crystal structures of **1** and **2**, superimposing the two structures on each other (Figure 3C), the major obvious difference is

the filling of the palm I pocket by **1** in the area adjacent to and partially overlapping with the catalytic pocket. In fact, **1** directly interacts with the side-chain of one of the key catalytic aspartates, Asp318, in a hydrogen bond via the sulfonamide *NH*. Our design concept for the evolution of **2** towards a lead series was to “grow” from the small template towards the catalytic site of NS5B, adding some of the key interactions found between **1** and the enzyme that are absent from **2**, while retaining what we felt was a superior, highly efficient starting point regarding the interactions **2** was already making with the palm I site. Towards such an end, linkers were designed to connect the core benzene ring of **2** together with the highly optimized *N*-arylmethanesulfonamide fragment present in **1**. In the X-ray co-crystal structure of **1**, it is apparent that the carbocyclic ring of the benzothiazine moiety makes an edge-to-face π -stack interaction with Phe193 side chain, and all polar atoms of the sulfonamide moiety make productive hydrogen bonds to either polar side chains or a bound water molecule. Molecular modeling using co-crystal structures of **1** and **2** enabled the *in silico* evaluation of a range of possible linker compositions and positions. The goal was to retain the highly optimized interactions between the *N*-arylmethanesulfonamide moiety and residues of the palm I pocket, with particular care taken to ensure low strain energy of the final bound conformation. A simple two-atom connection to the position adjacent to the methoxy substituent of the core benzene ring of **2** satisfied these requirements.

Extension from the core structure of **2** to the catalytic pocket resulted in potent inhibitors of NS5B (Table 1). Comparing **3** and **4**, it can be seen that the optimal connection point of a two carbon linker from the core structure **2** to an *N*-phenylmethanesulfonamide group is at the position *para* to the substituent nitrogen on the pendant benzene ring. Ethylene-linked **4** not only exhibited potent inhibition of NS5B in the enzymatic assay, but was also efficacious in the cell-based replicon assay, with $EC_{50} < 50$ nM for both genotypes 1a and 1b. We further evaluated **4** for its physicochemical and ADME properties (Table 2). Compound **4** demonstrated low solubility (as measured in a high throughput thermal solubility assay) and medium to high permeability (as measured in the Caco2 assay). It was metabolically unstable both *in vitro* (human and rat liver microsomes) and *in vivo* (rat), and gave only modest oral bioavailability in rat. The reason for low %F may be attributed to absorption limited by poor solubility, as well as to high first pass metabolism (*i.v.* clearance > liver blood flow). We also evaluated **4** in some of our cytochrome P450-based early safety *in vitro* assays (Table 3). It exhibited potent reversible ($IC_{50} = 0.34$ μ M) as well as time-dependent (k_{obs} 6-fold greater than ethynylestradiol) inhibition of CYP3A4. Inhibition of four other CYP isoforms evaluated (1A4, 2C9, 2C19, 2D6) was not observed (all $IC_{50} > 5$ μ M, data not shown). Time dependent inhibition (TDI) of CYP3A4 was especially concerning, as it could indicate the formation of reactive metabolites, further compounding the risk for drug-drug interactions (DDIs) from reversible inhibition of CYP3A4, and even potentially leading to autoimmune reactions and severe liver toxicities.²⁷

In vitro metabolite ID studies conducted in microsomal preparations revealed three main regions of oxidative metabolism / hydroxylation: the ethylene linker, the pyridone ring, and the *tert*-butyl group (Figure 4, see also Supporting Information for metabolite ID study of **4**). We felt that any of these areas could be responsible, at least in part, for the observed high first pass clearance as well as for the potential production of reactive metabolites (*e.g.*, quinone methide-type structures from oxidation of the benzylic positions of the ethylene linker, pyridinedione from the pyridone, dimethylacetaldehyde from *tert*-butyl). Our strategy was therefore set to address some of the shortcomings of **4** (poor metabolic stability, DDI / TDI risk) by taking a stepwise approach to modify each of those three key regions with substitutions and changes that we expected to confer resistance to oxidation, either through direct steric blocking or through reduction of relative electron richness, or through a combination. Furthermore, we felt that while compound **4** was quite potent in both

biochemical and cell-based assays, improvement in potency, especially in the cell-based replicon system, would ultimately lead to lower targeted exposures *in vivo* and thus mitigate some of the liabilities a final candidate may have in terms of limited bioavailability or safety margins. Optimization of potency was therefore to be addressed concomitantly with stability and safety.

Beginning our optimization of **4** by further investigation into various linker groups (Table 1), we identified carboxamide **5** as an inhibitor of comparable potency to **4** as measured in the enzymatic assay, and with increased efficacy in the genotype 1b replicon cellular assay.²⁸ Compound **5** is significantly stabilized compared to **4** when incubated with liver microsome preparations (Table 2), and exhibits much reduced DDI and TDI liabilities (Table 3). Presumably oxidative metabolism has both been directly blocked at the ethylene linker of **4**, as well as indirectly decreased by potential reduction of partitioning into the microsomal fractions because of increased overall polarity of **5** (increased PSA, decreased clogP, Table 2). The increased polarity, however, especially by nature of increased hydrogen bonding donors and acceptors, leads to reduced permeability and increased efflux. Reduced permeability, together with poor solubility, led to essentially no observed bioavailability from *p.o.* dosing in rat. From *i.v.* dosing, however, it was noted that *in vivo* clearance did improve relative to **4** (Table 2).

Next, a variety of 2-atom linker analogs of **4** and **5** were designed and synthesized. Compounds **6** – **11** (Table 1) are representative analogs that were predicted via modeling to maintain the critical interactions made by the *N*-phenylmethanesulfonamide moiety while binding in a low strain energy conformation. In addition, some of these linkers were designed to improve solubility by decreasing planarity (**6** – **8**) as well as by introducing an ionizable moiety (**7** and **8**). While solubility was improved (data not shown), significant loss of potency in both biochemical and cell-based assays was seen as an unacceptable trade-off. We anticipated a requirement for exposures at multiples above efficacious concentrations, especially to enable preclinical toxicological studies. Thus we set aggressive targets for potency, especially potency in a serum-shifted cell-based assay (*vide infra*), in addition to looking for ideal ADMET properties to allow for safe, oral delivery. Note that piperidine **8** was also devoid of TDI activity (Table 3), demonstrating that the 1,2-diarylethylene moiety was most likely responsible for the observed effect in **4**. This hypothesis is also supported by the observed low TDI signal for stilbene analog **10**, as well as by the moderate TDI signal observed for the 1,2-*trans*-cyclopropane-linked analog **11**, which has stereoelectronic properties in-between those of the saturated and unsaturated 2 carbon linkers of **4** and **10**, respectively. Compound **10** was also remarkable to us for its robust potency in the replicon assay, for both genotypes 1a and 1b. Its improved *in vitro* (microsomal) stability compared to **4** does not likely owe to a partitioning / polarity difference (similar PSA, clogP, Table 2), and TDI activity is less (Table 3). While we were unable to measure in a Caco-2 assay because of experimental difficulties relating most likely to poor compound solubility, we were hopeful that an expected reasonable permeability of **10** relative to **5** (with fewer *H*-bonding groups and lower PSA for **10**) would make for a better starting point for optimization. Thus considering its advantages in potency and overall ADMET properties, **10** became our lead template upon which we could further refine towards a clinical candidate.

Before we fully committed to the stilbene template of **10**, however, we did design and synthesize a number of analogs of **4** and **5** in which the linker was cyclized onto the aryl ring of the *N*-arylmethanesulfonamide terminus. Design ideas were evaluated and filtered via modeling, where we looked to further restrict the rotational freedom of molecules such as **4** - **11** into what we believed was the most likely active conformation, positioning all of the key interactions as seen in the overlay of crystal structures for **1** and **2** (Figure 3C). Hydrogen

bond accepting moieties were included in the fused ring designs to engage with the water molecule network observed in the palm I pocket. Example successful embodiments of this design concept are oxazole **12** and isocoumarin **13**.²⁹ We obtained X-ray co-crystal structures of **12** and **13** bound with NS5B (Figure 5), and were pleased to observe them in their predicted binding modes. Of interest to note is that the water molecule hydrogen-bonding with the oxazole nitrogen of **12** is effectively replaced by the isocoumarin carbonyl oxygen of **13**. Bicyclic compounds **12** and **13** were both quite potent (Table 1), however both also exhibited deficiencies that ultimately led to their deprioritization. In the case of oxazole **12**, poor oral bioavailability was at issue (Table 2), with near-zero exposure from oral dosing most likely owing to a combination of solubility-limiting absorption and efflux-mediated low permeability. In the case of isocoumarin **13**, low stability when incubated with human and rat liver microsome preparations, as well as observed efflux which could limit oral exposure (PK experiment not performed), led to its deprioritization. Returning to the stilbene template **10**, we next explored modification of the pyridone ring (Table 4). Although change of the saturated to unsaturated linker from **4** to **10** resulted in improved stability to microsomes, as well as reduced TDI potential, we sought to improve stability further so that our aggressive exposure targets could be better met. Recalling that metabolite ID studies had identified the pyridone ring as one of three sites of oxidation (in addition to the linker and *tert*-butyl group), we sought to stabilize the pyridone either by replacement with more electron deficient heterocycles (**14** – **18**), or by introduction of small substituents to potentially block oxidation directly (**19** – **22**). In either case, we viewed retention of the 1,3-donor-acceptor motif of the 2(1*H*)-pyridone as a requirement, so that the unique engagement with the backbone amides of residues Gln446 and Tyr448 would be maintained. Also, at this mature stage of our lead optimization effort we began to focus more on efficacy in the HCV replicon assay performed in the presence of 40% human serum (HuS). We targeted potency in this *in vitro* system that would allow for exposures *in vivo* at 5-fold coverage above the measured EC₉₀. Note that stilbene **10** exhibits a 20-fold shift reduction in efficacy in this assay. However, we still considered it a very potent compound with EC₅₀ and EC₉₀ of 21 and 150 nM, respectively, in the presence of 40% HuS. Note also that enzymatic data is no longer included on Table 4 (and later also not on Table 6), as all of the compounds **14** – **27** exhibited IC₅₀ between 1 and 3 nM (data not shown), even for the least active examples, such as **15**. Under our biochemical assay conditions (enzyme and substrate concentrations at 3 nM each), all IC₅₀ < 3 nM are indistinguishable, as the enzyme is effectively titrated with inhibitor at those potencies.

For heteroaromatic replacements of pyridone, examples **14** – **18** are representative of the effort. We were able to obtain our first X-ray co-crystal structure of a stilbene series compound with **14** (Figure 6). A co-crystal structure of the prototype ethylene-linker **4** is also shown for comparison. It can clearly be seen that the unsaturated C=C linker adopts a more coplanar disposition between the two aryl rings than does the saturated ethylene linker, and this bound conformation is very close to the low-energy ground state conformation typical for stilbenes, which would help account for the intrinsic potency increase over diarylethylenes. Note that in the structure for **4** (Figure 6B), it is curious that the –CH₃ group of the methoxy is rotated in a different direction than that observed in all of our other co-crystal structures, pushing side chain of Cys366 away from the ligand. The reason for this observed difference is unclear.

The significant (4- to 7-fold) potency increase for pyridazinone **14** relative to pyridone **10** in the serum-shifted replicon assay caught our attention. While decreased lipophilicity (clogP, Table 4) may be limiting nonspecific binding to serum proteins for **14**, it is also possible that there is an intrinsic potency increase due to specific interactions with the binding pocket. We observe what could be an n-to- π^* interaction between the ring nitrogen lone pair and the backbone carbonyl carbon of Gly410 (Figure 7), providing an enthalpic improvement to the

binding energy, although the vector angle for this interaction does not appear to be ideal. Pyrimidinone **15** is significantly less potent than the other aza-modified pyridines in Table 4. This observation could be explained by increased acidity, whereby an anion form is dominant, and thus cannot provide an *H*-bond donation to the backbone carbonyl of Tyr448 (we did not measure the pK_a of **15** to test this hypothesis). An alternative explanation would be that the lone pair of the additional nitrogen in **15** is not able to make any productive contact with protein residues, and thus the cost of desolvation is significantly detracting from the binding energy.³⁰ Note that uracil analog **16** recovers potency lost in **15**, which could be due to reduction in relative acidity, the ability to make more productive contacts with residues in the binding pocket (putatively Gly410 backbone and Asn411 side chain, in addition to Gln446 and Tyr448 backbone), or both. Pyrazinone **17** exhibits a similar efficacy profile to the parent pyridone **10**, whereas triazinone **18** is slightly less active.

For single substituents, a sampling of our effort is summarized by pyridones **19** – **22**. Placing the substituents at the 5-position (**19** – **21**) was intended to directly block the putative site of metabolic oxidation, a strategy that was at least partially successful (*vide infra*). In terms of potency, it was interesting to see that no loss was observed with the 5-fluoro substitution in **19**. The slightly larger substituents chloro and methyl in **20** and **21**, respectively, affected modest declines in potency relative to the unsubstituted pyridone **10**, with a trend towards a greater decline in the genotype 1a replicon assay. We were unable to obtain co-crystal structures of our molecules complexed with genotype 1a NS5B, and so structure-based explanations of this trend can only be speculative. Several residues in the palm I region near the pyridone ring binding site are changed in genotype 1a relative to 1b, including Y415F, D444N and Q446E, which could play a role in the observed potency differences. Note that when the substituent is moved from the 5- to the 6-position (methyl group on **22**), potency is restored to the levels observed for **10** and **19**, for both subtypes 1a and 1b.

As a primary motivation behind the design of pyridone analogs **14** – **22** was to improve metabolic stability, we evaluated them under both *in vitro* and *in vivo* conditions (Table 5). In general, pyridone substitutions (**19** – **22**) had a larger impact on reducing clearance than did replacement with heterocyclic analogs containing additional nitrogens (**14** – **18**). Considering stability category as defined in Table 5 (high, medium, low), data from human liver microsomes and hepatocytes were in higher concordance with each other than were the corresponding data from rat. For all of the compounds reported on Table 5, human *in vitro* stability either fell within or very close to the medium range, which represents a significant improvement relative to the *in vitro* stability measured for **10** (Table 2). *In vitro* stability data from rat was not highly predictive of *in vivo*, with no clear advantage to using either microsomal or hepatocytic systems (both predicting the *in vivo* stability category with only ~ 50% accuracy). For the substituted pyridones evaluated, two exhibited high stability *in vivo* (**19** and **22**), with one more in the medium-to-high range (**20**).

While several candidates had been identified from the exploration of pyridone group modifications which exhibited medium to high stability (**18**, **19**, **20** and **22**) *in vitro* and *in vivo*, we decided to extend the effort to block or retard metabolic oxidation to modifications on the *tert*-butyl group. Representative examples of this effort are shown in Table 6,³¹ and will be discussed first in terms of efficacy and cell permeability, before presenting the effects of these changes on clearance. Hydroxy-*tert*-butyl analog **23** is very potent, especially in the serum-shifted assay. Oxetane **24** is less potent than **23**, but similar to **10** and other potent stilbene series compounds. Comparing the polarities (clogP) of **10**, **23** and **24**, it is not clear that increased polarity is driving the increased replicon activities of alcohol **23**, as clogP for **23** and **24** are very close. Unfortunately, it is more clear for both compounds that increasing polarity leads to reduced intrinsic permeability, as well as increased efflux,

with **24** fairs slightly better in this regard than **23**. We generally targeted permeability as measured by the Caco2 assay to be $> 10^{-6}$ cm/s, with efflux ratio < 10 , to advance molecules with the best chance for efficient intestinal absorption, so data for both **23** and **24** fell outside of those targets. Deuterated *tert*-butyl analog **25** was designed to test the impact of a primary kinetic isotope effect on metabolic oxidation of *tert*-butyl CH. Replacement of hydrogens with deuteria does not decrease potency (data only available for genotype 1a, which typically gives lowered measured potencies for this series). We also explored the 1-chloro-1-cyclopropyl group as a replacement for *tert*-butyl that would be a good size mimetic, without presenting any terminal methyl groups for easy oxidation. 1-Chloro-1-cyclopropyl appeared to give an advantage in permeability, with **26** looking much improved relative to *oxo*-substituted *tert*-butyl analogs **23** and **24**. Potency drops only 2-fold (compare **26** to **10**, Table 4), showing that the 1-chloro-1-cyclopropyl moiety is well tolerated in the place of *tert*-butyl. The loss of potency is slightly greater when combined with a pyridone substitution in **27** (3- to 4-fold, compare to data for **19**, Table 4), which was designed to see if blocking metabolism at both sites simultaneously would produce additive or synergistic effects on stability.

We evaluated promising *tert*-butyl replacements for stability (Table 7). Oxetane **24** did show improvement relative to **10** *in vitro*, with all measurements at either medium or high stability. *In vivo* stability observed in rat, however, fell in the low range. 1-Chloro-1-cyclopropyl analog **26** showed a very similar *in vitro* stabilization when incubated with liver microsome preparations from both human and rat. Combination of the *tert*-butyl stabilizing group 1-chloro-1-cyclopropyl with a pyridone stabilizing substitution (5-fluoro), however, did not result in an additive or synergistic effect (compare hepatocyte stability for **27** vs. **19** from Table 5). Interestingly, one of the most effective substitution we found for stabilizing the template *in vitro* through *tert*-butyl modification was deuterium, as in the perdeutero analog **25**, demonstrating that an isotope effect can significantly impact rate of metabolic clearance on this template. Although retarding oxidative metabolism by replacing or substituting at the *tert*-butyl group had some effect on overall rate of clearance (at least *in vitro*), it appeared that the effect achieved by stabilizing through pyridone modification alone was sufficient to reach the desired target of medium to high stability.

We decided to focus on fluoropyridone **19** for further preclinical development based on its balance of high potency with medium to high metabolic stability. For a summary of *in vitro* data for **19** not already presented, see Table 8. Intrinsic permeability was measured at slightly below the desired target of 10^{-6} cm/s, however significant efflux was not observed and the molecule was still advanced. CYP3A4-mediated DDI and TDI liabilities were low. Inhibition of four other CYP isoforms (1A4, 2C9, 2C19, 2D6) was not observed (all $IC_{50} > 50$ μ M, data not shown).

Further evaluation of **19** included *in vivo* pharmacokinetic (PK) studies, as well as *in vitro* potency studies against a panel of genotype 1 HCV NS5B clinical isolates. With regards to *in vivo* PK, we felt that in order to enable preclinical toxicology studies, sustained exposures in preclinical species should exceed by at least several multiples the exposures expected to be useful in human efficacy studies (*i.e.*, where plasma trough levels, C_{min} , exceed the 40% human serum-shifted replicon EC_{90}). Thus, a target C_{min} of 303.5 ng/mL, equating to 5-fold above the serum-shifted EC_{90} for GT-1b, was considered a minimum requirement following oral dosing. Furthermore, those levels were targeted to be maintained for > 12 hours following a single dose, so that *b.i.d.* or *q.d.* dosing regimens could be implemented. Studies in rat are summarized in Table 9 and on Figure 8. Upon dosing compound **19** as a suspension in our typical vehicle (hypromellose 2910, polysorbate 80, benzyl alcohol and water), modest oral bioavailability (7.2%) was observed at 2 mg/kg, with a non-dose proportional drop-off to 2.1% observed at the higher 20 mg/kg dose. Importantly, exposures

did not cover the targeted plasma level of 5-fold over serum-shifted EC₉₀, even at C_{max}. Moving into a “nanosuspension”³² and dosing up to 50 mg/kg showed slightly increased bioavailability (now 2.9%), however targeted plasma concentrations were again not reached. Considering low solubility for this series (for **19**, aqueous solubility in pH 6.5 phosphate buffer = 0.1 µg/mL), we viewed it as a strong possibility that oral absorption was being limited by slow dissolution. We therefore investigated solution dosing using a polyethylene glycol / caprylocaproyl macrogol-8 glyceride (Labrasol®) vehicle,³³ in which compound **19** was completely dissolved and not merely in suspension. Indeed, increases in bioavailability and exposures above targeted levels in plasma were observed at the two doses tested. The increased exposures observed during these studies from solution formulation support the hypothesis that absorption was limited because of solubility / dissolution factors in the previous studies using suspensions. At the high solution dose (50 mg/kg), plasma concentrations remained above 303.5 ng/mL for over 8 hours (with extrapolation suggesting up to 12 hours, Figure 8), sufficient coverage to enable toxicology studies in rat. Similar experiments in dog using PEG 400 / Labrasol® solution dosing also demonstrated good kinetics and oral bioavailability (Table 10).

In addition to profiling **19** for its PK properties, we studied its ability to inhibit various HCV strains by measuring its activity against replicons containing NS5B sequences derived from clinical isolates from treatment naïve genotype 1 HCV patients. Preexisting amino acid polymorphisms may confer reduced sensitivity to direct-acting antivirals, and drug pressure-induced selection may increase the prevalence of those strains within a patient's quasispecies, leading to increased potential for resistance.³⁴ Using a transient replicon assay, we measured EC₅₀ against a number patient-derived NS5B isolates from both genotypes 1a and 1b (Table 11 and Figure 9).³⁵ The H77 and Con1 strains were included as references for genotypes 1a and 1b, respectively. Also for comparison, we evaluated another non-nucleoside inhibitor of NS5B, PF-00868554, which binds to a different allosteric site, the “thumb II” site. Pyridone **19** is very potent against all isolates tested from both genotypes, with EC₅₀ ranging from 0.4 – 6.8 nM. Activity for the thumb II inhibitor PF-00868554 is also consistent across all isolates tested, with EC₅₀ measured in the range of 18.7 – 95.8 nM and in agreement with previously published data.³⁶ Interestingly, the largest shift observed for **19** relative to a reference strain was for the GT-1a isolate RO-38 (~ 7-fold), which contains the mutation S556G, a known resistance mutation to palm I non-nucleoside inhibitors.³⁵ Serine 556 is present in the palm I binding site (see Figs. 3, 5 and 6). Even considering this shift, **19** is still an extremely potent inhibitor with an EC₅₀ of 6.8 nM.

CONCLUSION

In summary, a novel, highly potent series of non-nucleoside inhibitors of HCV NS5B has been identified from a fragment-derived starting point, and optimized to a clinical candidate in fluoropyridone **19**. A structure-based approach was applied to the design of various linkers connecting the hydrophobic core to the arylsulfonamide that engages the catalytic residues adjacent to the palm I allosteric site. We identified conformationally restricted linkers such as in carboxamide **5** and *trans*-stilbene **10**, predisposed to their active, target-bound conformations in the ground state, giving them robust intrinsic potency. Evaluation of linkers based on early safety criteria such as time-dependent inhibition of CYP3A4, pharmacokinetic criteria such as *in vitro* and *in vivo* stability towards clearance, and physicochemical criteria such as membrane permeability led to the selection of the *trans*-stilbene connector as the preferred template upon which to further optimize. The results of metabolite ID studies focused our attention on the pyridone and *tert*-butyl moieties as potential regions for tuning properties, especially towards further improving stability. Substituted pyridones provided the largest effect in this direction, and fluoropyridone **19** was ultimately selected as the clinical candidate. While rat *in vivo* pharmacokinetics from *i.v.*

dosing for **19** fell into a desirable range, low solubility limited oral absorption. In order to achieve exposures needed to support preclinical toxicology evaluation at multiples above the human serum-adjusted replicon EC₉₀, we solved the problem of poor oral absorption by moving to a lipid-based solution formulation. Finally, we showed that **19** maintains its robust, low nanomolar potency across 17 different human HCV NS5B clinical isolates from both genotypes 1a and 1b. Outcomes from further development studies of **19** will be reported in due course.

CHEMISTRY

Syntheses of selected representatives from compounds **3** – **27** are depicted in Schemes 1 – 5. Full synthetic details for all compounds **3** – **27** that are not outlined here may be found in the Supporting Information. In Schemes 1 and 2 are depicted the syntheses of a few different linkers from Table 1: 1,2-diarylethylene **4** and *trans*-stilbene **10** (Scheme 1); and carboxamide **5** (Scheme 2). The sequence towards **4** and **10** began with 3-*tert*-butyl-2-hydroxybenzaldehyde **28**, which was brominated and *O*-methylated in high yield to produce 5-bromo-3-*tert*-butyl-2-hydroxy-benzaldehyde **29**. Suzuki cross-coupling with (2-methoxy-3-pyridyl)boronic acid provided biaryl intermediate **30**, which was then reacted with a 4-nitrobenzyl Wittig reagent to give stilbene **31** as the *trans* isomer. Tandem hydrogenation of both the nitro and stilbene groups provided aniline **32** with a saturated ethylene linker. Compound **32** was *N*-mesylated and selectively *O*-dealkylated to afford the final pyridone **4**. Alternatively, intermediate **31** could have its nitro group selectively reduced using elemental iron, resulting in aniline **33**. Mesylation and deprotection as before yielded stilbene **10**. In Scheme 2 is depicted the synthesis of carboxamide **5**. First, 5-bromo-1-*tert*-butyl-2-methoxy-3-nitrobenzene **34** was cross-coupled under Suzuki conditions to give biaryl **35**. Hydrogenation afforded aniline **36**, which was then treated with 4-nitrobenzoyl chloride in the presence of triethylamine to provide carboxamide **37** in high yield. Nitro group reduction was achieved under hydrogenation conditions with catalytic palladium hydroxide to give **38**. Finally, *N*-mesylation followed by *O*-demethylation afforded pyridone **5**.

In Schemes 3 and 4 are shown two different routes used to prepare pyridone replacement analogs pyridazinone **14** and pyrimidinone **15**, respectively. Beginning with Scheme 3, 4-bromo-2-*tert*-butylanisole is converted to an organomagnesium reagent under Grignard conditions, and then reacted in excess (2.5 equiv) with 4,5-dichloro-1*H*-pyridazin-6-one in refluxing THF. Chlorine substitution occurred at the more reactive 4-position, with good selectivity and high yield. The remaining chloro substituent was removed by hydrogenolysis in water / DMF in the presence of palladium(0) on carbon and potassium hydroxide to afford pyridazinone **40**.³⁷ Selective bromination at the benzene ring position *ortho* to the methoxy group was achieved by treatment with *N*-bromosuccinimide in DMF at 50 °C for 18 hours to give **41**. The concise, 4-step synthesis of **14** was completed by a final step cross coupling with *N* [4 [(*E*) 2 (4,4,6-trimethyl-1,3,2-dioxaborinan-2-yl)vinyl]phenyl]methanesulfonamide under Suzuki conditions and microwave heating to 125 °C in a sealed tube. Forcing conditions were required to achieve the modest 18% yield of the final product, as the bromo group of **41** is quite sterically hindered (bulky *tert*-butyl group forces methoxy methyl to rotate away from it, thus hindering access to the bromine). In Scheme 4, we began with 5-bromo-3-*tert*-butyl-2 hydroxy-benzaldehyde **29** (from Scheme 1) and stereoselectively installed the *trans*-stilbene via Horner-Wadsworth-Emmons reaction with the benzaldehyde, followed by reduction of the terminal nitro group with elemental iron to afford aniline **42** in high yield. *N*-mesylation provided **43**, which was then converted to boronate **44**, providing a versatile intermediate which could be used for cross-coupling reactions with heteroaryl bromides to make pyridones and pyridone replacements. In this example, boronate **44** was

reacted with 4-benzyloxy-5-bromopyrimidine to give a corresponding biaryl intermediate in 34% yield. Removal of the *O*-benzyl group via treatment with HBr in acetic acid at room temperature afforded the final product pyrimidinone **15** in 71% yield.

In Scheme 5 is depicted the synthesis of compound **24**, containing one of the *tert*-butyl replacement groups (3-methyloxetan-3-yl) from Table 6. In the first step, 4,6-dibromo-2-iodoanisole **45** is coupled to diethyl malonate in the presence of cesium carbonate and catalytic amounts of copper(I) iodide and 2-phenylphenol following Buchwald's procedure,³⁸ generating α -arylmalonate **46** via selective reaction at aryl iodide position. Methylation of **46** at the α -malonate position, followed by reduction of the esters to alcohols with lithium aluminum hydride at low temperature afforded diol **47**. Ring closure of the 1,3-diol to the oxetane was affected by subjection to Mitsunobu-type conditions (DIAD, triphenylphosphine, toluene) in a sealed tube at 140 °C (microwave), yielding intermediate **48**. Taking advantage of the steric hindrance differential between the two bromines of **48**, a Suzuki cross coupling with (2-oxo-1*H*-pyridin-3-yl)boronic acid occurred with high regioselectivity at the less hindered aryl bromide to give the biaryl product **49**. Also, we found by this point that the pyridone group did not need protection to carry through many of the chemistries that we used to prepare compounds in this series, thus sparing deprotection steps at the ends of syntheses. The final step here was cross-coupling with *N*-[4-[(*E*)-2-(4,4,6-trimethyl-1,3,2-dioxaborinan-2-yl)vinyl]phenyl]methanesulfonamide under Suzuki conditions with microwave heating to 120 °C in a sealed tube, affording *trans*-stilbene **24** in 64% yield.

EXPERIMENTAL

General information

All reactions were performed in round-bottom flasks unless otherwise noted. The flasks were fitted with rubber septa and reactions were conducted under inert atmosphere. Stainless steel syringes were used to transfer air- and moisture-sensitive liquids. Most commercial reagents were purchased from Sigma Aldrich, Alfa Aesar, Strem, Lancaster, AstaTech, or TCI, and used as received. Solvents were purchased from Mallinckrodt and anhydrous dimethylformamide (DMF) and tetrahydrofuran (THF) from Sigma Aldrich in a Sure/Seal system. Flash chromatography was performed using AnaLogix IntelliFlashTM 280 or Teledyne Isco CombiFlash[®] Companion[®] purification systems using silica gel columns RediSep Rf (particle size 40-63 powder). Deuterated solvents were purchased from Sigma-Aldrich. Proton nuclear magnetic resonance (¹H NMR) spectra was recorded on Bruker Avance 300 MHz, 400 MHz, or 500 MHz spectrometers at 25 °C. Chemical shifts for protons are reported in parts per million downfield from tetramethylsilane and are referenced to residual protium in the NMR solvent (CHCl₃ δ 7.27, DMSO δ 2.50). Data is represented as follows: chemical shift, multiplicity (br. = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants in Hertz (Hz), and integration. Liquid chromatography mass spectrometry (LC/MS) analyses were carried out using an Agilent 6140 single quadrupole in ES/APCI dual mode, and Agilent LC with a Zorbax SB-C18 2.1×30mm, 3.5 μ m column using a 0.02% TFA/water-acetonitrile solvent system. Microwave reactions were carried out in a Biotage Initiator microwave synthesizer. Purity of tested compounds was 95% as confirmed by HPLC/MS.

5-Bromo-3-*tert*-butyl-2-methoxy-benzaldehyde (29)—To a solution of 3-*tert*-butyl-2-hydroxybenzaldehyde (**28**) (5.0 g, 28.0 mmol) in CH₂Cl₂ (20 mL) at 0 °C was added dropwise a solution of Br₂ (1.45 mL, 28.0 mmol) in CH₂Cl₂ (15 mL) over a period of 30 min. After the addition was complete, the reaction was stirred for 1 h before the organic volatiles were removed under reduced pressure to afford 5-bromo-3-*tert*-butyl-2-hydroxy-

benzaldehyde (7.23 g, 100%) as a light yellowish solid. ^1H NMR (300 MHz, CDCl_3) δ 11.76 (s, 1 H), 9.84 (s, 1 H), 7.67–7.48 (m, 2 H), 1.44 (s, 9 H) ppm.

A mixture of 5-bromo-3-*tert*-butyl-2-hydroxy-benzaldehyde (3.83 g, 15.0 mmol), MeI (2.32 mL, 37.0 mmol) and K_2CO_3 (6.18 g, 45.0 mmol) in DMF (50 mL) was heated at 50 °C for 1 h then cooled to rt and diluted with Et_2O and water. The organic layer was thrice washed with water then brine, dried (MgSO_4) and concentrated to afford **29** (3.99 g, 99%) as a yellow solid. ^1H NMR (300 MHz, CDCl_3) δ 10.30 (s, 1 H), 7.86 (d, J = 2.3 Hz, 1 H), 7.67 (d, J = 2.6 Hz, 1 H), 3.97 (s, 3 H), 1.43 (s, 9 H) ppm.

3-*tert*-Butyl-2-methoxy-5-(2-methoxy-3-pyridyl)benzaldehyde (30)—A sealed tube containing **29** (2.0 g, 7.38 mmol), (2-methoxy-3-pyridyl)boronic acid (1.68 g, 10.98 mmol), $\text{Pd}(\text{PPh}_3)_4$ (0.66 g, 0.57 mmol), Na_2CO_3 (1.96 g, 18.49 mmol) in a mixture of MeOH (11 mL) and CH_2Cl_2 (3 mL) was heated with a microwave reactor at 115 °C for 30 min. The reaction mixture was concentrated, diluted with EtOAc, washed with brine, and dried over Na_2SO_4 . Chromatography on SiO_2 (EtOAc/hexane, 0 to 10%) gave **30** (2.123 g, 96%) as a pale yellow oil. ^1H NMR (400 MHz, CDCl_3) δ 10.60–10.15 (m, 1 H), 8.25–8.14 (m, 1 H), 7.97–7.88 (m, 1 H), 7.87–7.75 (m, 1 H), 7.70–7.54 (m, 1 H), 7.12–6.91 (m, 1 H), 4.08–3.98 (m, 6 H), 1.48 (s, 9 H) ppm.

3-[3-*tert*-Butyl-4-methoxy-5-[(*E*)-2-(4-nitrophenyl)vinyl]phenyl]-2-methoxy-pyridine (31)—To a slurry of NaH (0.17 g, 4.159 mmol, 60% in mineral oil) in THF (5 mL) containing 0.1 equivalent of 15-crown-5 cooled to 0 °C was added a solution of diethyl (4-nitro-phenyl)-phosphonate (1.14 g, 4.16 mmol) in THF (5 mL). When gas evolution ceased, a slurry of **30** (1.13 g, 3.78 mmol) was added and stirred at rt for 18 h. An additional equivalent of NaH was added to the partially reacted mixture and stirring continued for 1 h. The reaction mixture was partitioned between water and Et_2O and the organic extract was sequentially washed with water and brine, dried (Na_2SO_4), filtered, and evaporated. The crude product was purified over SiO_2 by chromatography (EtOAc/hexane, 50%) to afford **31** as a sole geometric isomer. ^1H NMR (400 MHz, CDCl_3) δ 8.23 (d, J = 8.6 Hz, 2 H), 8.17 (d, J = 4.5 Hz, 1 H), 7.73–7.61 (m, 4 H), 7.57 (d, J = 16.2 Hz, 1 H), 7.53–7.47 (m, 1 H), 7.13 (d, J = 16.7 Hz, 1 H), 7.03–6.95 (m, 1 H), 4.01 (s, 3 H), 3.84 (s, 3 H), 1.45 (s, 9 H) ppm.

4-[2-[3-*tert*-Butyl-2-methoxy-5-(2-methoxy-3-pyridyl)phenyl]ethyl]aniline (32)—To a solution of **31** (351 mg, 0.840 mmol) in EtOAc (15 mL) and MeOH (15 mL), was added $\text{Pd}(\text{OH})_2$ (20% on carbon, 166 mg, 0.237 mmol). H_2 gas was bubbled into the solution for 15 min. The reaction was stirred under an atmosphere of H_2 for 18 h. The reaction was filtered, concentrated, and purified over SiO_2 by chromatography (EtOAc/hexane, 5% to 20%) to give **32** (106 mg, 32%) as a colorless oil. LC/MS (ES/APCI): 391 ($\text{M} + \text{H}$) $^+$.

***N*-[4-[2-[3-*tert*-Butyl-2-methoxy-5-(2-oxo-1*H*-pyridin-3yl)phenyl]ethyl]phenyl]methanesulfonamide (4)**—To a solution of **32** (106 mg, 0.272 mmol) in pyridine (5 mL) at 0 °C was added methanesulfonyl chloride (0.035 mL, 0.450 mmol). The reaction was gradually warmed to rt and stirred for 1.5 h. The solution was diluted with EtOAc, washed with saturated CuSO_4 solution, 1N HCl solution, and dried over Na_2SO_4 . Purification over SiO_2 by chromatography (EtOAc/hexane, 10% to 25%) gave *N*-[4-[2-[3-*tert*-butyl-2-methoxy-5-(2-methoxy-3-pyridyl)phenyl]ethyl]phenyl]methanesulfonamide (104 mg, 82%) as a colorless oil. ^1H NMR (300 MHz, CDCl_3) δ 8.13 (dd, J = 4.9, 1.5 Hz, 1 H), 7.57 (dd, J = 7.3, 1.7 Hz, 1 H), 7.43–7.27 (m, 2 H), 7.25–7.10 (m, 4 H), 6.96 (dd, J = 7.2, 5.3 Hz, 1 H), 6.39 (s, 1 H), 3.98 (s, 3 H), 3.83 (s, 3 H), 2.99 (s, 4 H), 2.97 (s, 3 H), 1.43 (s, 9 H) ppm.

A solution of *N*-[4-[2-[3-*tert*-butyl-2-methoxy-5-(2-methoxy-3-pyridyl)phenyl]ethyl]phenyl]methanesulfonamide (104 mg, 0.222 mmol), 48% HBr (0.1 mL, 0.87 mmol) in AcOH (3 mL) was heated at 65 °C for 18 h in a sealed tube. The reaction mixture was carefully poured into saturated aqueous NaHCO₃ ice solution, extracted with EtOAc, and dried over Na₂SO₄. Purification by SiO₂ preparative TLC (EtOAc/hexane, 1:1 followed by EtOAc/hexane, 2:1) gave **4** (51 mg, 51%) as a white solid. ¹H NMR (300 MHz, DMSO-*d*₆) δ 11.68 (br s, 1 H), 9.59 (s, 1 H), 7.54 (dd, *J* = 6.9, 2.1 Hz, 1 H), 7.51 (d, *J* = 2.2 Hz, 1 H), 7.46 (d, *J* = 2.2 Hz, 1 H), 7.34 (dd, *J* = 6.4, 2.0 Hz, 1 H), 7.25 (d, *J* = 8.5 Hz, 2 H), 7.13 (d, *J* = 8.5 Hz, 2 H), 6.26 (t, *J* = 6.8 Hz, 1 H), 3.76 (s, 3 H), 2.93 (s, 3 H), 2.88 (s, 4 H), 1.36 (s, 9 H) ppm. LC/MS (ES/APCI): 455 (M + H)⁺.

4-[(*E*)-2-[3-*tert*-Butyl-2-methoxy-5-(2-methoxy-3-pyridyl)phenyl]vinyl]aniline (33**)**—To a suspension of **31** (0.29 g, 0.693 mmol) in MeOH (5 mL) and water (5 mL) was added NH₄Cl (0.37 g, 6.93 mmol) followed by Fe powder (0.19 g, 3.464 mmol). The solution was heated at reflux for 1 h, cooled and filtered. The filtrate was concentrated *in vacuo*. The filtrate was taken up in EtOAc, washed with brine, dried (Na₂SO₄), filtered and evaporated. The crude product was purified over SiO₂ by chromatography (EtOAc/hexane, 30%) to afford **33** (0.26 g, 100%) as a yellow foam. ¹H NMR (300 MHz, CDCl₃) δ 8.15 (dd, *J* = 4.9, 1.9 Hz, 1 H), 7.63 (d, *J* = 2.3 Hz, 2 H), 7.44 7.33 (m, 3 H), 7.17 (d, *J* = 15.8 Hz, 1 H), 7.02 6.93 (m, 2 H), 6.69 (d, *J* = 8.3 Hz, 2 H), 4.00 (s, 3 H), 3.83 (s, 3 H), 3.81 3.69 (m, 2 H), 1.44 (s, 9 H) ppm.

***N*-[4-[(*E*)-2-[3-*tert*-Butyl-2-methoxy-5-(2-oxo-1*H*-pyridin-3-yl)phenyl]vinyl]phenyl] methanesulfonamide (**10**)**—To a solution of **33** (0.153 g, 0.394 mmol) in pyridine (4 mL) at 0 °C was added methanesulfonyl chloride (0.037 mL, 0.055 g, 0.473 mmol). Stirring was continued at 0 °C for 2 h, then at rt for an additional 2 h. The reaction mixture was diluted with EtOAc, washed sequentially with aqueous CuSO₄ and 2N HCl, dried (Na₂SO₄), filtered, and concentrated. The crude residue was purified over SiO₂ by chromatography (EtOAc/hexane, 40%) to afford *N*-[4-[(*E*)-2-[3-*tert*-butyl-2-methoxy-5-(2-methoxy-3-pyridyl)phenyl]vinyl]phenyl] methanesulfonamide (0.178 g, 96%) as a yellow foam. ¹H NMR (400 MHz, CDCl₃) δ 8.16 (dd, *J* = 4.5, 1.5 Hz, 1 H), 7.64 (d, *J* = 2.5 Hz, 2 H), 7.54 (d, *J* = 8.6 Hz, 2 H), 7.46 (d, *J* = 2.0 Hz, 1 H), 7.36 (d, *J* = 16.7 Hz, 1 H), 7.23 (d, *J* = 8.1 Hz, 2 H), 7.05 (s, 1 H), 7.02-6.95 (m, 1 H), 6.42 (s, 1 H), 4.00 (s, 3 H), 3.83 (s, 3 H), 3.04 (s, 3 H), 1.45 (s, 9 H) ppm.

A solution of *N*-[4-[(*E*)-2-[3-*tert*-butyl-2-methoxy-5-(2-methoxy-3-pyridyl)phenyl]vinyl]phenyl]methanesulfonamide (0.14 g, 0.3 mmol), AcOH (3 mL) and 48% HBr (0.105 mL) was heated at 60 °C for 18 h. The reaction mixture was cooled to rt, diluted with EtOAc and water to give a precipitate, which was filtered and washed with water and ether to give **10** (115 mg, 67%) as an off-white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.84 (d, *J* = 1.5 Hz, 1 H), 7.67 (dd, *J* = 6.9, 2.0 Hz, 1 H), 7.58-7.51 (m, 3 H), 7.37 (dd, *J* = 6.3, 1.8 Hz, 1 H), 7.17 (d, *J* = 16.4 Hz, 1 H), 7.17-7.07 (m, 3 H), 6.29 (t, *J* = 6.6 Hz, 1 H), 3.75 (s, 3 H), 2.91 (s, 1 H), 1.39 (s, 9 H) ppm. LC/MS (ES/APCI): 453 (M + H)⁺.

3-(3-*tert*-Butyl-4-methoxy-5-nitro-phenyl)-2-methoxy-pyridine (35**)**—A mixture of 5-bromo-1-*tert*-butyl-2-methoxy-3-nitrobenzene (**34**) (0.48 g, 1.7 mmol), (2-methoxy-3-pyridyl)boronic acid (0.28 g, 1.8 mmol), Pd(PPh₃)₄ (0.19 g, 0.17 mmol), and Na₂CO₃ (0.53 g, 5.0 mmol) in CH₂Cl₂/MeOH (3:1, 8 mL) was subjected to microwave heating to 115 °C for 35 min. The reaction mixture was filtered and the filtrate concentrated. The crude residue was purified over SiO₂ by chromatography (EtOAc/hexane, 0 to 20%) to afford **35** (0.44 g, 82%) as a yellow solid. ¹H NMR (300 MHz, CDCl₃) δ 8.19 (dd, *J* = 4.9, 1.5 Hz, 1 H), 7.87

(d, $J = 2.3$ Hz, 1 H), 7.72 (d, $J = 1.9$ Hz, 1 H), 7.61 (dd, $J = 7.5, 1.9$ Hz, 1 H), 7.00 (dd, $J = 7.3, 5.1$ Hz, 1 H), 3.99 (s, 3 H), 3.85 (s, 3 H), 1.44 (s, 9 H) ppm.

3-*tert*-Butyl-2-methoxy-5-(2-methoxy-3-pyridyl)aniline (36)—To a solution of **35** (0.98 g, 3.1 mmol) in EtOAc/MeOH (2:1, 30 mL) was added 10% Pd/C (0.10 g). A stream of H₂ was bubbled through the solution for 20 min then the solution was stirred at rt for 16 h. The reaction mixture was filtered and concentrated to afford **36**. ¹H NMR (300 MHz, CDCl₃) δ 8.12 (dd, $J = 4.9, 1.9$ Hz, 1 H), 7.59 (dd, $J = 7.36, 1.7$ Hz, 1 H), 7.02–6.84 (m, 3 H), 3.97 (s, 3 H), 3.84 (s, 3 H), 3.70 (br s, 2 H), 1.41 (s, 9 H) ppm.

***N*-[3-*tert*-Butyl-2-methoxy-5-(2-methoxy-3-pyridyl)phenyl]-4-nitro-benzamide (37)**—To a solution of **36** (0.24 g, 0.85 mmol) and Et₃N (0.18 mL, 1.3 mmol) in CH₂Cl₂ (10 mL) at 0 °C was added 4-nitrobenzoyl chloride (0.19 g, 1.0 mmol). The resulting mixture was stirred at 0 °C for 3 h, and then warmed to rt. The mixture was then diluted with additional CH₂Cl₂, washed sequentially with water and brine, dried (Na₂SO₄), filtered, and concentrated. The crude residue was purified over SiO₂ by chromatography to afford **37** (0.36 g, 97%) as a light yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 8.42–8.35 (m, 3 H), 8.27 (br s, 1 H), 8.16 (dd, $J = 5.1, 1.5$ Hz, 1 H), 8.10 (d, $J = 8.56$ Hz, 2 H), 7.66 (dd, $J = 7.6, 1.5$ Hz, 1 H), 7.40 (d, $J = 2.0$ Hz, 1 H), 6.98 (dd, $J = 7.1, 5.0$ Hz, 1 H), 4.00 (s, 3 H), 3.84 (s, 3 H), 1.45 (s, 9 H) ppm.

4-Amino-*N*-[3-*tert*-butyl-2-methoxy-5-(2-methoxy-3-pyridyl)phenyl]benzamide (38)—To a solution of **37** (0.36 g, 0.82 mmol) in EtOAc/MeOH (2:1, 15 mL) was added 10% Pd/C (0.035 g). A stream of H₂ gas was bubbled through the solution for 20 min, and the white solid precipitate that formed was recovered by filtration to afford **38** (quantitative) which was carried onto the subsequent step without further purification. ¹H NMR (300 MHz, CDCl₃) δ 8.38 (d, $J = 2.3$ Hz, 1 H), 8.13 (d, $J = 5.0, 1.9$ Hz, 1 H), 8.08 (br s, 1 H), 7.77 (d, $J = 8.7$ Hz, 2 H), 7.66 (dd, $J = 7.3, 1.7$ Hz, 1 H), 7.34 (d, $J = 1.9$ Hz, 1 H), 6.95 (dd, $J = 7.2, 4.9$ Hz, 1 H), 6.73 (d, $J = 8.3$ Hz, 2 H), 4.04 (br s, 2 H), 3.99 (s, 3 H), 3.83 (s, 3 H), 1.44 (s, 9 H) ppm.

***N*-[3-*tert*-Butyl-2-methoxy-5-(2-oxo-1*H*-pyridin-3-yl)phenyl]-4-(methanesulfonamido) benzamide (5)**—To a solution of **38** (0.10 g, 0.25 mmol) in pyridine (2 mL) at 0 °C was added methanesulfonyl chloride (0.034 g, 0.30 mmol). Stirring was continued at 0 °C for 2 h, then at rt for an additional 2 h. The reaction mixture was diluted with EtOAc, washed sequentially with aqueous CuSO₄ and 2N HCl, dried (Na₂SO₄), filtered, and concentrated. The crude residue was purified over SiO₂ by chromatography (EtOAc/hexane, 50% to 100%) to afford *N*-[3-*tert*-butyl-2-methoxy-5-(2-methoxy-3-pyridyl)phenyl]-4-(methanesulfonamido)benzamide (0.12 g, 99%) as a colorless oil. ¹H NMR (300 MHz, CDCl₃) δ 8.38 (d, $J = 1.9$ Hz, 1 H), 8.23–8.10 (m, 2 H), 7.94 (d, $J = 8.7$ Hz, 2 H), 7.65 (dd, $J = 7.2, 1.9$ Hz, 1 H), 7.40–7.30 (m, 3 H), 6.95 (dd, $J = 7.3, 5.0$ Hz, 1 H), 6.90 (br s, 1 H), 3.99 (s, 3 H), 3.84 (s, 3 H), 3.10 (s, 3 H), 1.45 (s, 9 H) ppm.

A solution of *N*-[3-*tert*-butyl-2-methoxy-5-(2-methoxy-3-pyridyl)phenyl]-4-(methanesulfonamido)benzamide (0.12 g, 0.25 mmol), 48% HBr (40 μ L) and AcOH (3 mL) was heated in a sealed tube at 60 °C for 15 h. The reaction mixture was cooled to rt, diluted with EtOAc, and washed with water. Upon addition of saturated aqueous NaHCO₃, a solid precipitated, which was collected and triturated with Et₂O to afford **5** (70 mg, 60%) as a white solid. mp 274.0–276.0 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.74 (br s, 1 H), 10.20 (s, 1 H), 9.87 (s, 1 H), 8.04 (d, $J = 8.6$ Hz, 2 H), 7.71 (d, $J = 2.0$ Hz, 1 H), 7.61 (dd, $J = 6.8, 1.8$ Hz, 1 H), 7.53 (d, $J = 2.0$ Hz, 1 H), 7.36 (dd, $J = 6.1, 1.5$ Hz, 1 H), 7.30 (d, $J = 8.6$ Hz, 2

H), 6.29 (t, $J = 6.6$ Hz, 1 H), 3.72 (s, 3 H), 3.10 (s, 3 H), 1.39 (s, 9 H) ppm. LC/MS (ES/APCI): 470 ($M + H$)⁺.

5-(3-*tert*-Butyl-4-methoxy-phenyl)-1*H*-pyridazin-6-one (40)—A dry round-bottom flask was charged with 4-bromo-2-*tert*-butylanisole (**39**) (2.933 g, 12 mmol), THF (15 mL) and magnesium turnings (0.29 g, 12 mmol) were added. The reaction mixture was heated to reflux and stirred for 45 min then cooled to rt. The resulting solution was added dropwise at rt to a stirred solution of 4,5-dichloro-1*H*-pyridazin-6-one (0.796 g, 4.8 mmol), THF (10 mL) and Et₂O (20 mL). The reaction mixture was then heated at reflux for 18 h. The reaction was cooled to 0 °C and quenched with saturated aqueous NH₄Cl and extracted with EtOAc (150 mL). The aqueous phase was withdrawn and re-extracted with EtOAc (150 mL). Each extract was washed sequentially with water and brine. The combined organic extracts were dried (Na₂SO₄), filtered, and concentrated *in vacuo*. The residue was triturated with EtOAc/hexane (1:1) to afford 5-(3-*tert*-butyl-4-methoxy-phenyl)-4-chloro-1*H*-pyridazin-6-one (1.087 g, 77%) as a white solid. ¹H NMR (300 MHz, CDCl₃) δ 11.25 (br s, 1 H), 7.85 (s, 1 H) 7.53-7.35 (m, 2 H), 6.96 (d, $J = 8.3$ Hz, 1 H), 3.89 (s, 3 H), 1.39 (s, 9 H) ppm.

A Parr Shaker bottle was charged with 5-(3-*tert*-butyl-4-methoxy-phenyl)-4-chloro-1*H*-pyridazin-6-one (1.08 g), a solution of KOH (0.52 g in 11 mL of H₂O), and DMF (1.3 mL). To this mixture was added 10% Pd/C and the bottle was connected to a Parr shaker and flushed three times with H₂ then shaken for 18 h at rt under an atmosphere of ca. 50 psi of H₂. To the resulting solution was added 5M KOH to dissolve the precipitate then the solution was filtered through a glass microfiber filter and rinsed with 5M KOH and H₂O. The filtrate was acidified with concentrated HCl and the resulting mixture extracted with CH₂Cl₂ (100 mL). The aqueous layer was withdrawn and re-extracted with EtOAc. The combined extracts were dried (Na₂SO₄), filtered, and evaporated to afford **40** (0.791 g, 83%) as a white powder. ¹H NMR (400 MHz, CDCl₃) δ 10.78 (br s, 1 H), 7.83 (d, $J = 4.0$ Hz, 2 H), 7.72 (d, $J = 2.0$ Hz, 1 H), 7.30 (d, $J = 4.0$ Hz, 1 H), 6.95 (d, $J = 8.6$ Hz, 1 H), 3.89 (s, 3 H), 1.40 (s, 9 H) ppm.

5-(3-Bromo-5-*tert*-butyl-4-methoxy-phenyl)-1*H*-pyridazin-6-one (41)—To a solution of **40** (0.1 g, 0.39 mmol) and DMF (2 mL) was added NBS (0.069 g, 0.39 mmol) and the resulting solution stirred at 50 °C for 18 h. The reaction was concentrated *in vacuo* and the residue partitioned between Et₂O and water. The aqueous layer was withdrawn and re-extracted with Et₂O. The organic layers were twice washed with water (5 mL) and once with brine (5 mL). The organic layers were combined, dried (Na₂SO₄), filtered, and evaporated. The crude product was purified over SiO₂ by chromatography (EtOAc/hexane, 0 to 30%) to afford **41** (0.068 g, 52%) as an off-white solid. ¹H NMR (300 MHz, CDCl₃) δ 7.97 (d, $J = 2.3$ Hz, 1 H), 7.91-7.86 (m, 1 H), 7.77-7.73 (m, 1 H), 7.32 (d, $J = 4.1$ Hz, 1 H), 3.97 (s, 3 H), 1.42 (s, 9 H) ppm.

***N*-[4-[(*E*)-2-[3-*tert*-Butyl-2-methoxy-5-(6-oxo-1*H*-pyridazin-5-yl)phenyl]vinyl]phenyl]methanesulfonamide (14)**—A microwave tube was charged with **41** (0.068 g), *N*-[4-[(*E*)-2-(4,4,6-trimethyl-1,3,2-dioxaborinan-2-yl)vinyl]phenyl]methanesulfonamide (0.078 g), Na₂CO₃ (0.064 g), Pd(PPh₃)₄ (0.023 g), MeOH (1.8 mL), and CH₂Cl₂ (0.6 mL). The tube was flushed with Ar, sealed and irradiated in a microwave synthesizer at 125 °C for 40 min. The reaction mixture was cooled and concentrated *in vacuo*. The residue was partitioned between CH₂Cl₂ (25 mL) and water (5 mL). The organic layer was washed with brine (5 mL). The aqueous phase was twice extracted with CH₂Cl₂ (25 mL). The organic layers were combined, dried (Na₂SO₄), filtered, and evaporated. The crude product was purified over SiO₂ by chromatography

(EtOAc/hexane, 0 to 60%) to give **14** (0.175 g, 18%) as a yellow powder. mp: 240.0-242.0 °C. ¹H NMR (400 MHz, CDCl₃) δ 11.19 (s, 1 H), 8.50 (s, 1 H), 8.25 (d, *J* = 2.2 Hz, 1 H), 7.93 (d, *J* = 4.2 Hz, 1 H), 7.56-7.50 (m, 3 H), 7.41 (d, *J* = 4.2 Hz, 1 H), 7.38-7.30 (m, 3 H), 7.07 (d, *J* = 16.4 Hz, 1 H), 3.84 (s, 3 H), 3.03 (s, 3 H), 1.45 (s, 9 H) ppm. LC/MS (ES/APCI): 454 (M + H)⁺.

4-[(E)-2-(5-Bromo-3-*tert*-butyl-2-methoxy-phenyl)vinyl]aniline (42)—To a suspension of NaH (0.35 g, 8.73 mmol, 60% mineral oil dispersion) and dry THF (9 mL) was added 15-crown-5 (0.27 mL, 1.36 mmol) and the resulting mixture cooled to 0 °C. A solution of diethyl (4-nitro-benzyl)-phosphonate (2.33 g, 8.53 mmol) in dry THF (10.5 mL) was added slowly while maintaining the temperature at 0 °C. The reaction was stirred for 15 min then a solution of 5-bromo-3-*tert*-butyl-2-methoxy-benzaldehyde (**29**) (1.93 g, 7.12 mmol) and dry THF (21 mL) was added. The reaction mixture was stirred at 0 °C for 2 h then allowed to warm to rt and stirred for 18 h. The reaction mixture was quenched with water (50 mL) and the resulting solution thrice extracted with Et₂O (3 × 60 mL). The combined extracts were washed with brine, dried (Na₂SO₄), filtered, and concentrated *in vacuo*. The crude product was purified over SiO₂ by chromatography (EtOAc/hexane, 5%) to afford 5-bromo-1-*tert*-butyl-2-methoxy-3-[(E)-2-(4-nitrophenyl)vinyl]benzene (2.70 g, 98%). ¹H NMR (300 MHz, CDCl₃) δ 8.24 (d, *J* = 8.7 Hz, 2 H), 7.66 (d, *J* = 8.7 Hz, 2 H), 7.60 (d, *J* = 2.3 Hz, 1 H), 7.51-7.34 (m, 2 H), 7.09 (d, *J* = 16.6 Hz, 1 H), 3.78 (s, 3 H), 1.40 (s, 9 H) ppm.

A mixture of 5-bromo-1-*tert*-butyl-2-methoxy-3-[(E)-2-(4-nitrophenyl)vinyl]benzene (0.788 g, 2.02 mmol), iron (0.471 g, 8.44 mmol), NH₄Cl (0.867 g, 16.2 mmol), MeOH (25 mL), and water (25 mL) was heated at reflux for 4 h. The reaction mixture was cooled to rt and filtered. The filtrate was thrice extracted with EtOAc and the combined extracts washed with brine, dried (Na₂SO₄), filtered, and concentrated *in vacuo* to afford **42** (0.71 g, 95%) as a yellow solid which was used without further purification.

N-[4-[(E)-2-(5-Bromo-3-*tert*-butyl-2-methoxy-phenyl)vinyl]phenyl]methanesulfonamide (43)—To a solution of **42** (0.161 g, 0.45 mmol) and pyridine (5 mL) cooled to 0 °C was added methanesulfonyl chloride (38 μL, 0.493 mmol). After stirring for 45 min at 0 °C, the reaction mixture was washed with 1N HCl and extracted three times with EtOAc. The organic extracts were washed with saturated CuSO₄ solution three times and once with brine. The extracts were dried over Na₂SO₄, filtered, and concentrated to give **43** (193 mg, 99%) as a brown oil. ¹H NMR (300 MHz, CDCl₃) δ 7.57 (d, *J* = 2.3 Hz, 1 H), 7.53 (d, *J* = 8.3 Hz, 2 H), 7.34 (d, *J* = 2.3 Hz, 1 H), 7.29-7.17 (m, 3 H), 6.99 (d, *J* = 16.4 Hz, 1 H), 6.50 (s, 1 H), 3.77 (s, 3 H), 3.05 (s, 3 H), 1.39 (s, 9 H) ppm.

N-[4-[(E)-2-[3-*tert*-Butyl-2-methoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]vinyl]phenyl]methanesulfonamide (44)—A mixture of **43** (0.476 g, 1.086 mmol), 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (0.303 g, 1.19 mmol), PdCl₂(PPh₃)₂ (0.177 g, 0.217 mmol), and KOAc (0.32 g, 3.26 mmol) under an Ar atmosphere was dissolved in DMSO (25 mL). The reaction mixture was then heated to 90 °C for 18 h. The reaction was cooled to rt and partitioned between EtOAc and water. The aqueous phase was extracted with EtOAc and the combined extracts were washed five times with water and once with brine. The organic layers were dried, filtered, and evaporated. The crude product was purified over SiO₂ by chromatography (EtOAc/hexane, 0 to 30%) to give **44** (0.311 g, 53%) as a yellow oil. ¹H NMR (300 MHz, CDCl₃) δ 7.92 (s, 1 H) 7.78-7.67 (m, 1 H) 7.54 (d, *J* = 8.3 Hz, 2 H),

7.38-7.06 (m, 4 H), 6.37 (s, 1 H), 3.80 (s, 3 H), 3.04 (s, 3 H), 1.47-1.41 (m, 9 H), 1.38-1.33 (m, 12 H) ppm.

***N*-[4-[(*E*)-2-[3-*tert*-Butyl-2-methoxy-5-(6-oxo-1*H*-pyrimidin-5-yl)phenyl]vinyl]phenyl]methanesulfonamide (15)**—A tube was charged with **44** (0.130 g, 0.144 mmol), 4-benzyloxy-5-bromopyrimidine (0.070 g, 0.264 mmol), Pd(PPh₃)₄ (0.028 g, 0.024 mmol), Na₂CO₃ (0.077 g, 0.726 mmol), MeOH (3 mL), and CH₂Cl₂ (1 mL). The tube and solution were sparged with Ar, sealed and heated at 120 °C for 40 min. The solution was cooled, filtered through CELITE®, and the filtrate concentrated *in vacuo*. The crude product was purified over SiO₂ by chromatography (EtOAc/hexane, 0 to 50%) to afford *N*-[4-[(*E*)-2-[5-(4-benzyloxy-5-pyrimidin-5-yl)-3-*tert*-butyl-2-methoxy-phenyl]vinyl]phenyl]methanesulfonamide (45 mg, 34%) as an off-white solid. ¹H NMR (400 MHz, CDCl₃) δ 8.78 (s, 1 H), 8.58 (s, 1 H), 7.67 (d, *J* = 2.0 Hz, 1 H), 7.56-7.43 (m, 5 H), 7.34 (d, *J* = 7.6 Hz, 4 H), 7.22 (d, *J* = 8.6 Hz, 2 H), 6.94 (d, *J* = 16.7 Hz, 1 H), 6.34 (s, 1 H), 5.53 (s, 2 H), 3.83 (s, 3 H), 3.05 (s, 3 H), 1.39 (s, 9 H) ppm.

To a solution of *N*-[4-[(*E*)-2-[5-(4-benzyloxy-5-pyrimidin-5-yl)-3-*tert*-butyl-2-methoxy-phenyl]vinyl]phenyl]methanesulfonamide (0.044 g, 0.081 mmol) and AcOH (1.5 mL) at rt was added 48% HBr (30 μL). The reaction was stirred at rt for 18 h, diluted with EtOAc and poured into saturated NaHCO₃. The aqueous layer was extracted with EtOAc and the combined extracts dried, filtered, and evaporated. The crude product was triturated with EtOAc/Et₂O to give **15** (26 mg, 71%) as an off-white solid. mp: 253.0-255.0 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.73 (br s, 1 H), 9.83 (s, 1 H), 8.19 (d, *J* = 11.6 Hz, 2 H), 7.84 (s, 1 H), 7.63 (d, *J* = 8.1 Hz, 2 H), 7.57 (s, 1 H), 7.34-7.15 (m, 4 H), 3.77 (s, 3 H), 3.01 (s, 3 H), 1.39 (s, 9 H) ppm. LC/MS (ES/APCI): 454 (M + H)⁺.

***N*-[4-[(*E*)-2-[3-*tert*-Butyl-5-(5-fluoro-2-oxo-1*H*-pyridin-3-yl)-2-methoxy-phenyl]vinyl]phenyl]methanesulfonamide (19)**—A microwave vial was charged with **43** (0.193 g, 0.442 mmol), (5-fluoro-2-methoxy-3-pyridyl)boronic acid (0.0771 g, 0.451 mmol), Na₂CO₃ (0.123 g, 1.16 mmol), Pd(PPh₃)₄ (0.027 g, 0.023 mmol), MeOH (1.2 mL), and CH₂Cl₂ (0.3 mL), sealed and irradiated in a microwave synthesizer at 115 °C for 35 min. The vial was cooled to rt, filtered, and the solid washed with EtOAc. The filtrate was washed sequentially with water and brine, dried (Na₂SO₄), filtered, and concentrated *in vacuo*. The crude product was purified over SiO₂ by chromatography (EtOAc/hexane, 20 to 50%) to afford *N*-[4-[(*E*)-2-[3-*tert*-butyl-5-(5-fluoro-2-methoxy-3-pyridyl)-2-methoxy-phenyl]vinyl]phenyl]methanesulfonamide (0.127 g, 59%) as a yellow oil. ¹H NMR (300 MHz, CDCl₃) δ 7.99 (d, *J* = 3.0 Hz, 1 H), 7.74-7.28 (m, 9 H), 7.03 (d, *J* = 16.2 Hz, 1 H), 3.98 (s, 3 H), 3.83 (s, 3 H), 3.04 (s, 3 H), 1.45 (s, 9 H) ppm.

A vial was charged with *N*-[4-[(*E*)-2-[3-*tert*-butyl-5-(5-fluoro-2-methoxy-3-pyridyl)-2-methoxy-phenyl]vinyl]phenyl]methanesulfonamide (0.087 g, 0.18 mmol), AcOH (2 mL) and 48% HBr (38 μL), sealed and heated at 60 °C for 18 h. The reaction was cooled to rt and added to a mixture of ice and saturated aqueous NaHCO₃. The resulting mixture was thrice extracted with EtOAc and the combined extracts washed sequentially with saturated aqueous NaHCO₃ and brine, dried (Na₂SO₄), filtered, and concentrated *in vacuo* to afford **19** (0.071 g, 84%) as a solid. ¹H NMR (400 MHz, DMSO *d*₆) δ 9.82 (s, 1 H), 7.88 (d, *J* = 2.0 Hz, 1 H), 7.83 (dd, *J* = 8.8, 3.3 Hz, 1 H), 7.68-7.52 (m, 4 H), 7.35-7.13 (m, 4 H), 3.77 (s, 3 H), 3.01 (s, 3 H), 1.39 (s, 9 H) ppm. LC/MS (ES/APCI): 471 (M + H)⁺.

Diethyl 2-(3,5-dibromo-2-methoxy-phenyl)propanedioate (46)—A mixture of 1,5-dibromo-3-iodo-2-methoxy-benzene (**45**) (5.4 g, 14 mmol), diethyl malonate (4.0 g, 25 mmol), CuI (0.13 g, 0.69 mmol), 2-phenylphenol (0.24 g, 1.4 mmol) and Cs₂CO₃ (6.8 g, 21 mmol) in THF (14 mL) was stirred at 70 °C for 15 h. The resulting mixture was cooled to rt

and partitioned between EtOAc and saturated aqueous NH_4Cl . The organic extract was then washed sequentially with water and brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The crude product was purified over SiO_2 by chromatography (EtOAc/hexane, 0 to 10%) to afford **46** (4.1 g, 55%) as a pale yellow oil. ^1H NMR (400 MHz, CDCl_3) δ 7.65 (d, J = 2.0 Hz, 1 H), 7.28 (d, J = 2.0 Hz, 1 H), 4.24 (dd, J = 7.1, 4.5 Hz, 4 H), 4.12 (q, J = 7.1 Hz, 1 H), 3.85 (s, 3 H), 1.33–1.17 (m, 6 H) ppm.

2-(3,5-Dibromo-2-methoxy-phenyl)-2-methyl-propane-1,3-diol (47)—To a suspension of NaH (0.86 g, 22 mmol, 60% dispersion in mineral oil) in THF (50 mL) cooled to 0 °C was added dropwise a solution of **46** (4.1 g, 7.7 mmol) in THF (10 mL). The resulting mixture was stirred at 0 °C for 20 min then iodomethane (3.6 g, 26 mmol) was added dropwise and the reaction warmed to rt and stirred for 48 h. The reaction mixture was partitioned between Et_2O and saturated aqueous NH_4Cl . The organic extract was washed sequentially with water and brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The crude product was purified over SiO_2 by chromatography (EtOAc/hexane, 0 to 10%) to afford diethyl 2-(3,5-dibromo-2-methoxy-phenyl)-2-methyl-propanedioate (2.9 g, 84%) as a pale yellow oil. ^1H NMR (400 MHz, CDCl_3) δ 7.64 (s, 1 H), 7.28 (s, 1 H), 4.30–4.15 (m, 4 H), 3.85 (s, 1 H), 1.81 (s, 3 H), 1.27 (t, J = 7.1 Hz, 6 H) ppm.

To a solution of diethyl 2-(3,5-dibromo-2-methoxy-phenyl)-2-methyl-propanedioate (0.919 g, 2.1 mmol) in THF (4 mL) cooled to 0 °C was added dropwise LiAlH_4 (2.40 mL, 2.4 mmol, 1.0 M solution in THF). The reaction mixture was stirred at 0 °C for 1 h, and then saturated aqueous potassium sodium tartrate was added and the resulting mixture was stirred at rt for 2 h. The mixture was extracted with EtOAc, and the organic extract washed sequentially with water and brine, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The crude product was purified over SiO_2 by chromatography (EtOAc/hexane, 0 to 50%) to afford **47** (0.369 g, 52%) as a pale yellow oil. ^1H NMR (400 MHz, CDCl_3) δ 7.64 (d, J = 2.5 Hz, 1 H), 7.56 (d, J = 2.0 Hz, 1 H), 4.15–4.06 (m, 2 H), 3.94 (s, 3 H), 3.90 (dd, J = 11.1, 5.6 Hz, 2 H), 2.46 (t, J = 5.6 Hz, 2 H), 2.05 (s, 3 H).

3-(3,5-Dibromo-2-methoxy-phenyl)-3-methyl-oxetane (48)—A tube was charged with **47** (0.34 g, 0.97 mmol) and PPh_3 (0.51 g, 1.93 mmol), flushed with Ar and sealed. A solution of diisopropyl azodicarboxylate (0.39 g, 1.93 mmol) and toluene (6 mL) was added via syringe and the resulting mixture was irradiated in a microwave reactor at 140 °C for 30 min. The reaction mixture was concentrated *in vacuo* and the crude product purified over SiO_2 by chromatography (EtOAc/hexane, 0 to 10%) to afford **48** (0.138 g, 43%) as a colorless oil. ^1H NMR (400 MHz, CDCl_3) δ 7.57 (d, J = 2.5 Hz, 1 H), 6.91 (d, J = 2.5 Hz, 1 H), 4.98 (d, J = 6.1 Hz, 2 H), 4.50 (d, J = 5.6 Hz, 2 H), 3.87 (s, 3 H), 1.76 (s, 3 H) ppm.

3-[3-Bromo-4-methoxy-5-(3-methyloxetan-3-yl)phenyl]-1H-pyridin-2-one (49)—A microwave vial was charged with **48** (0.138 g, 0.411 mmol), (2-oxo-1H-pyridin-3-yl)boronic acid (0.063 g, 0.453 mmol), Na_2CO_3 (0.131 g, 1.24 mmol), $\text{Pd}(\text{PPh}_3)_4$ (0.047 g, 0.041 mmol), MeOH (3 mL), and CH_2Cl_2 (1 mL), sealed and irradiated in a microwave synthesizer at 115 °C for 30 min. The vial was cooled to rt, filtered, and concentrated *in vacuo*. The crude residue was partitioned between CH_2Cl_2 and water. The organic extract was washed with brine. The aqueous layers were extracted with CH_2Cl_2 . The organic layers were combined, dried over Na_2SO_4 , filtered, and concentrated. The crude product was purified over SiO_2 by chromatography (EtOAc/hexanes, 0 to 100%) to afford **49** (0.095 g, 66%) as an off-white powder. ^1H NMR (400 MHz, CDCl_3) δ 11.79 (br s, 1 H), 7.80 (d, J = 2.0 Hz, 1 H), 7.54 (dd, J = 7.1, 2.0 Hz, 1 H), 7.35 (dd, J = 6.6, 2.0 Hz, 1 H), 7.15 (d, J = 2.0 Hz, 1 H), 6.37 (t, J = 6.8 Hz, 1 H), 5.07 (d, J = 5.6 Hz, 2 H), 4.55 (d, J = 5.6 Hz, 2 H), 3.92 (s, 3 H), 1.80 (s, 3 H) ppm.

***N*-[4-[(*E*)-2-[2-Methoxy-3-(3-methyloxetan-3-yl)-5-(2-oxo-1*H*-pyridin-3-yl)phenyl]vinyl]phenyl]methanesulfonamide (**24**)**—A microwave vial was charged with **49** (0.095 g, 0.271 mmol), *N*-[4-[(*E*)-2-(4,4,6-trimethyl-1,3,2-dioxaborinan-2-yl)vinyl]phenyl]methanesulfonamide (0.132 g, 0.408 mmol), Na₂CO₃ (0.086 g, 0.811 mmol), Pd(PPh₃)₄ (0.031 g, 0.027 mmol), MeOH (3 mL), and CH₂Cl₂ (1.5 mL), sealed and irradiated in a microwave synthesizer at 120 °C for 1 h. The vial was cooled to rt, filtered, and concentrated *in vacuo*. The crude residue was partitioned between CH₂Cl₂ and water. The organic extract was washed with brine. The aqueous layers were extracted with CH₂Cl₂. The organic layers were combined, dried over Na₂SO₄, filtered, and concentrated. The crude product was purified over SiO₂ by chromatography (MeOH/CH₂Cl₂, 0 to 10%) to afford **24** (0.081 g, 64%) as a white powder. mp: 279.0–281.0 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ 11.76 (br s, 1 H), 9.85 (s, 1 H), 7.89 (d, *J* = 2.1 Hz, 1 H), 7.70 (dd, *J* = 7.0, 2.1 Hz, 1 H), 7.62 (d, *J* = 8.7 Hz, 2 H), 7.39 (d, *J* = 5.3 Hz, 1 H), 7.30–7.12 (m, 5 H), 6.30 (t, *J* = 6.8 Hz, 1 H), 4.91 (d, *J* = 5.7 Hz, 2 H), 4.45 (d, *J* = 5.7 Hz, 2 H), 3.74 (s, 3 H), 3.01 (s, 3 H), 1.72 (s, 3 H) ppm. LC/MS (ES/APCI): 467 (M + H)⁺.

Computational chemistry—Molecular modeling was performed using the molecular operating environment (MOE).³⁹ Figures were generated using Pymol 1.5.0.4.⁴⁰

HCV NS5B polymerase biochemical assay protocol—The enzymatic activity of HCV polymerase (NS5B570n-Con1) was measured as described previously²⁶ with only two changes as follows: concentration of RNA template was 3 nM and concentration of NS5B570n-Con1 enzyme was 3 nM.

Stable HCV replicon assay—The stable GT 1b Con1 and GT 1a H77 subgenomic replicon cell lines expressing the renilla luciferase as a reporter gene (cell lines 2209-23 and pRLucH771b75S/I, respectively) were used as described by Le Pogam *et al.*⁴¹

Transient HCV replicon assay using a panel of GT 1 NS5B clinical isolates—The GT 1b Con1 and GT 1a H77 transient replicons in which the wild type NS5B gene has been replaced by NS5B isolates obtained from untreated patients were used as described by Le Pogam *et al.*³⁵

Time Dependent CYP3A4 Inhibition—Stock solutions of test compound, midazolam, and 17 α-ethynylestradiol were prepared in 5% DMSO in acetonitrile. Midazolam, 17 α-ethynylestradiol, and α-nicotinamide adenine dinucleotide phosphate, reduced form (0-NADPH), were obtained from Sigma Chemical Co. Human liver microsomes (HLM, lot no. 0510222) were purchased from XenoTech, LLC (Lenexa, KS), prepared from a mixed-gender pool of 50 human livers. The assay was performed with a two stage robotic procedure in a 96-well format using Biomek 2000 (Beckman) for liquid handling. Inactivation mixtures (180 μL/well) containing 1 mg/mL HLM (Xenotech), 3 mM MgCl₂, 1 mM EDTA, and concentration 10 μM of test compound in 100 mM potassium phosphate buffer (pH 7.4) were incubated at 37 °C in a 96-well plate. After 3 minutes of pre incubation, the reactions were initiated by adding 20 μL of 10 mM NADPH and allowed to proceed up to 20 minutes. Verapamil and Ethynylestradiol at 10 μM were included as positive controls for time dependent CYP3A4 inactivation.^{42–43} At 0, 2, 6, 12, and 20 minutes after the start of the pre incubation, 10 μL of the preincubation mixtures were transferred to activity assay mixtures (90 μL/well, pre-warmed at 37 °C) containing 3 mM MgCl₂, 1 mM EDTA, 1 mM NADPH, and 10 μM of midazolam in 100 mM potassium phosphate buffer (pH 7.4). The final mixtures were incubated for 5 minutes and quenched with 100 μL of 0.1% formic acid in acetonitrile containing 2 Pg/mL 7-hydroxycoumarin as

the internal standard. The quenched mixtures were transferred and further diluted in a deep well plate. After centrifugation, the supernatant was analyzed by HPLC-MS/MS.

Metabolite ID—Human liver microsomal proteins were purchased from BD GENTEST, Woburn, MA. The incubation mixture contained 50 mM potassium phosphate pH 7.4, 5 mM magnesium chloride, 2mM NADPH, test compound (20 μ M) and microsomal protein (0.5 mg/mL). The total volume of each incubation mixture was 0.5 mL. All reagents except NADPH were combined in a 13 mL round bottom glass test tube on ice. The samples were pre incubated for approximately 2 minutes at 37 °C in a thermostatically controlled shaking water bath. The reaction was started by the addition of NADPH. The reaction was stopped at 30 minutes by quenching with 0.5 mL of methanol. For each microsomal incubation, one control sample (30 minutes) without NADPH was incubated together with the samples. The quenched samples were kept on ice (10-15 minutes) then at room temperature, vortexed briefly, and centrifuged. The supernatant was transferred to test tubes, evaporated to partial dryness (~ 0.2 mL), then transferred to an autosampler vial. Samples analyzed by RP-HPLC/UV/MS. Low resolution mass spectrometric analysis was carried out on a ThermoFinnigan iontrap mass spectrometer (model LTQ) equipped with an electrospray ionization source. The heated capillary temperature in the source was held at 250 °C. In the standard screening mode, the trap collected positive m/z 150 to m/z 800 scans. Collision-induced dissociation tandem MS was conducted with 40% relative collision energy and an isolation width of 3 amu. Product ion analyses were performed following collision induced dissociation of selected precursor ions to obtain substructural fragment ion information. MS³ experiments were also performed.

Pharmacokinetics—Male Wistar/Han (CRL:WI) rats (Charles River Laboratories, Hollister, CA or Raleigh, NC) used in these studies weighed between 180 and 300 g and were surgically implanted with cannulas in the jugular and/or femoral veins. All studies were conducted under IACUC approved protocols, and animals were allowed free access to food and water. Single intravenous bolus doses were administered into the tail vein or into the jugular cannula of dual cannulated animals using dosing formulations consisting of DMSO (< 10%) / PEG 400 / water; the remaining cannulas were used for blood collection. Single oral doses were administered by gavage using a dosing formulation consisting of hypromellose 2910 (0.5%), polysorbate 80 (0.4%) and benzyl alcohol (0.9%) in water, unless otherwise specified. Blood was collected into tubes containing EDTA at typically 0.08 (iv only), 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h after dosing. Plasma concentrations were determined by a liquid chromatography/tandem mass spectrometry method (LC/MS/MS) following protein precipitation with acetonitrile.

Supplementary Material

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Acknowledgments

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ABBREVIATIONS

HCV	hepatitis C virus
NS5B	non-structural 5B

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Figure 1.
HCV life cycle.¹⁴

1 **2**

Figure 2.
Previously reported Roche inhibitors of NS5B.

Figure 3.

(A) X-ray co-crystal structure of compound **1** complexed with NS5B at 2.2 Å, showing the palm I allosteric site;²⁵ (B) compound **2** with NS5B at 2.8 Å; (C) overlay **1** and **2** in binding pocket from (A).

Figure 4. Regions of microsomal oxidation of compound **4**, as indicated by the observation of hydroxylated species during metabolite ID studies.

Figure 5.

(A) X-ray co-crystal structure of compound **12** bound in the palm I allosteric site of NS5B at 2.05 Å; (B) compound **13** with NS5B at 2.05 Å.

Figure 6.

A) X-ray co-crystal structure of compound **14** bound in the palm I allosteric site of NS5B at 1.90 Å; B) compound **4** with NS5B at 2.09 Å.

Figure 7.
Enlargement from co-crystal structure of **14** with NS5B (Figure 6), showing potential n-to- π^* interaction between pyridazinone ring nitrogen and Gly410 backbone carbonyl

Figure 8.

Exposure curves for compound **19** following oral dosing in rat, with 5X EC₉₀ line marking 303.5 ng/mL.

Figure 9. Antiviral activity of **19** and PF-00868554 against replicons from genotype 1 NS5B clinical isolates; reference strains shown as solid shapes, geometric means shown as dotted lines.

Scheme 1.

Scheme 2.

Scheme 3.

Scheme 4.

Scheme 5.

Table 1

Core-to-Sulfonamide Linkages.

Cmpd	R	NS5B Enzyme IC ₅₀ (nM) ^a	GT1a-Replicon EC ₅₀ (nM) ^b	GT1b-Replicon EC ₅₀ (nM) ^b
2	H	435	8260	3860
3		45	--	912
4		1	44	30
5		2	48	9
6		23	1560	278
7		40	1170	218
8		40	861	196
9		6	61	38
10		4	6	1
11		15	84	53

Cmpd	R	NS5B Enzyme IC ₅₀ (nM) ^a	GT1a-Replicon EC ₅₀ (nM) ^b	GT1b-Replicon EC ₅₀ (nM) ^b
12		9	28	13
13		1	2	1

^aIC₅₀ measured using GT 1b, Con1 strain (*n* = 2)

^bEC₅₀ measured using GT 1a or GT 1b stable HCV subgenomic replicon (*n* = 2).

Table 2

Physicochemical and ADME comparison for linkers.

Cmpd	PSA ^a	clogP ^b	Caco2 AB ^c	Caco2 ER ^d	Sol. ^e	HLM ^f	RLM ^f	<i>i.v.</i> Cl ^g	%F ^h
4	75.3	4.60	1.5	3.4	< 1	185	1240	82	11
5	98.1	2.47	0.3	48	< 1	50	26	41	0
10	75.8	4.85	--	--	< 1	78	103	--	--
12	93.0	3.70	0.9	19	< 1	40	302	29	0.4
13	96.8	3.50	0.3	15	--	638	388	--	--

^a polar surface area, calculated in Å²^b calculated log *P*^c permeability coefficient, 10⁻⁶ cm/s^d ratio of BA/AB permeability coefficients^e thermal aqueous solubility, pH 6.4 (high throughput assay), µg/mL^f human or rat liver microsome intrinsic clearance, µL/min/mg protein^g clearance in rat after 0.5 mg/kg *i.v.* injection, mL/min/kg^h bioavailability in rat after 2 mg/kg *p.o.* administration.

Table 3

Inhibition of CYP3A4.

Cmpd	IC ₅₀ ^a	% TDI ^b	k _{obs} ^c	k _{obs} ratio ^d
4	0.34	66	0.0921	6.01
5	> 50	4	0.0023	0.11
8	> 50	3	0.0003	0.01
10	1.1	< 1	0.0028	0.15
11	0.04	36	0.0386	1.78

^a inhibition of midazolam turnover, μM ^b normalized percent activity remaining (12 min preincubation with cmpd at 10 μM)^c inactivation rate, min^{-1} ^d ratio of inactivation rates between compound and ethynylestradiol (< 1 considered low TDI potential).

Table 4

Pyridone Replacements and Substitutions.

Cmpd	R	clogP ^a	GT1a EC ₅₀ (nM) ^b	GT1b EC ₅₀ (nM) ^b	40% HuS EC ₅₀ (nM) ^c	40% HuS EC ₉₀ (nM) ^c
10		4.85	6	1	21	150
14		4.16	1	1	5	20
15		4.60	30	25	276	1830
16		4.38	4	2	8	58
17		4.17	9	4	29	134

Cmpd	R	clogP ^a	GT1a EC ₅₀ (nM) ^b	GT1b EC ₅₀ (nM) ^b	40% HuS EC ₅₀ (nM) ^c	40% HuS EC ₉₀ (nM) ^c
18		2.84	8	7	57	311
19		5.17	8	3	22	129
20		5.74	39	6	43	211
21		5.34	63	6	43	193
22		5.34	9	2	25	145

^aCalculated log *P*

^bEC₅₀ measured using GT 1a or GT 1b stable HCV subgenomic replicon (*n* = 2)

^cEC₅₀ and EC₉₀ measured using GT 1b stable HCV subgenomic replicon in the presence of 40% human serum (*n* = 2).

Table 5Pyridone replacement *in vitro* and *in vivo* stability.^a

Cmpd	HLM ^b	HH ^c	RLM ^d	RH ^e	<i>i.v.</i> Cl ^f
14	24 (M)	21 (L)	202 (L)	29 (M)	53 (L)
16	30 (M)	4 (M)	36 (M)	20 (M)	56 (L)
17	--	20 (L)	--	65 (L)	75 (L)
18	20 (M)	7 (M)	78 (M)	42 (L)	31 (M)
19	17 (M)	10 (M)	48 (M)	16 (M)	17 (H)
20	5 (H)	9 (M)	10 (H)	9 (M)	23 (M)
21	29 (M)	10 (M)	92 (L)	18 (M)	44 (L)
22	15 (M)	6 (M)	25 (H)	5 (H)	10 (H)

^aStability category H/M/L = high/medium/low^bhuman liver microsome intrinsic clearance, $\mu\text{L}/\text{min}/\text{mg}$ protein (high stability < 6.5, low stability > 35)^chuman hepatocyte intrinsic clearance, $\mu\text{L}/\text{min}/10^6$ cells (high stability < 2.8, low stability > 15)^drat liver microsome intrinsic clearance, $\mu\text{L}/\text{min}/\text{mg}$ protein (high stability < 15, low stability > 90)^erat hepatocyte intrinsic clearance, $\mu\text{L}/\text{min}/10^6$ cells (high stability < 5.4, low stability > 29)^fclearance in rat after 0.5 mg/kg *i.v.* injection, mL/min/kg (high stability < 20, low stability > 40).

Table 6

tert-Butyl Modifications and Replacements.

Cmpd	R1	R2	clogP ^a	GT1a EC ₅₀ ^b	GT1b EC ₅₀ ^b	40% HuS EC ₅₀ ^c	40% HuS EC ₉₀ ^c	Caco2 AB ^a	Caco2 ER ^f
23	H		3.01	5	3	7	39	0.19	77
24	H		2.79	16	8	30	195	0.36	52
25	H		4.85	2	--	7 ^d	104 ^d	--	--
26	H		4.15	10	4	41	286	1.56	3.6
27	F		4.47	30	8	85	566	--	--

^aCalculated log *P*^bEC₅₀ measured using GT 1a or GT 1b stable HCV subgenomic replicon, nM (*n* = 2)^cEC₅₀ and EC₉₀ measured using GT 1b (accept where noted) stable HCV subgenomic replicon in the presence of 40% human serum (HuS), nM (*n* = 2)^dGT 1a used

e permeability coefficient, 10^{-6} cm/s

f ratio of BA/AB permeability coefficients.

Table 7*tert*-Butyl substitution effect on *in vitro* and *in vivo* stability.^a

Cmpd	HLM	HH	RLM	RH	<i>i.v.</i> CI
24	22 (M)	2 (H)	74 (M)	21 (M)	50 (L)
25	19 (M)	2 (H)	28 (M)	13 (M)	--
26	17 (M)	--	77 (M)	--	--
27	--	9 (M)	--	27 (M)	--

^a refer to Table 5 for explanation of abbreviations.

Table 8Additional *in vitro* data for **19**.

Caco2			CYP3A4		
AB ^a	ER ^b	IC ₅₀ ^c	% TDI ^d	k _{obs} ^e	k _{obs} ratio ^f
0.87	1.8	>50	12	0.0109	0.60

^a permeability coefficient, 10⁻⁶ cm/s^b ratio of BA/AB permeability coefficients^c inhibition of midazolam turnover, μM^d normalized percent activity remaining (12 min pre-incubation with cmpd at 10 μM)^e inactivation rate, min⁻¹^f ratio of inactivation rates between compound and ethynylestradiol (< 1 considered low TDI potential).

Table 9*In vivo* pharmacokinetics for **19** in rat.

Dose / Route	Cl ^b	Vd _{ss} ^c	t _{1/2} ^d	C _{max} ^e	AUC ^f	%F
0.5 mg/kg <i>i.v.</i>	17.4	5.12	5.3	281	470	--
2 mg/kg <i>p.o.</i> , suspension	--	--	--	12.7	138	7.2
20 mg/kg <i>p.o.</i> , suspension	--	--	--	32.4	407	2.2
50 mg/kg <i>p.o.</i> , nanosuspension	--	--	--	104	1370	2.9
14 mg/kg <i>p.o.</i> , solution ^a	--	--	--	398	4460	33
50 mg/kg <i>p.o.</i> , solution ^a	--	--	--	817	10100	21

^a 1:1 PEG 400 / Labrasol^b clearance, mL/min/kg^c volume of distribution at steady state, L/kg^d half-life, hr^e peak concentration, ng/mL^f area under curve, ng·h/mL.

Table 10*In vivo* pharmacokinetics for compound **19** in dog.

Dose / Route	Cl ^b	Vd _{ss} ^c	t _{1/2} ^d	C _{max} ^e	AUC ^f	%F
0.5 mg/kg <i>i.v.</i>	3.28	1.11	4.9	666	2410	--
2 mg/kg <i>p.o.</i> , solution ^a	--	--	--	301	4260	45

^a 1:1 PEG 400 / Labrasol^b clearance, mL/min/kg^c volume of distribution at steady state, L/kg^d half-life, hr^e peak concentration, ng/mL^f area under curve, ng·h/mL.

Table 11Antiviral activity of **19** and PF-00868554 against GT 1a and GT 1b NS5B clinical isolates.

GT	Isolate	Cmpd 19 EC ₅₀ (nM) ^a	PF-00868554 EC ₅₀ (nM) ^a
1a	H77	1.0±0.3	95.8±9.7
	RO-18	1.3±0.1	47.0±6.2
	RO-22	4.1±1.0	42.8±7.6
	RO-24	3.1±0.1	37.0±1.2
	RO-28	1.8±0.1	38.8±2.3
	RO-32	2.3±0.1	26.0±1.2
	RO-33	1.1±0.1	34.4±3.4
	RO-34	2.2±0.1	25.0±0.8
	RO-35	0.4±0.1	22.0±4.9
	RO-38	6.8±0.5	56.0±6.0
	RO-41	1.5±0.2	48.0±6.8
	(mean) ^b	1.8	39.5
1b	Con1	1.4±0.3	51.1±11.2
	RO-1	0.5±0.1	39.3±3.0
	RO-2	0.7±0.1	21.3±4.9
	RO-3	1.7±0.4	28.5±7.2
	RO-4	0.4±0.1	25.5±7.4
	RO-7	1.8±0.7	20.8±5.4
	RO-8	2.0±0.2	18.7±4.8
	RO-9	3.0±0.1	23.5±6.9
	RO-10	2.8±0.1	39.7±7.2
	(mean) ^b	1.3	28.2

^aEC₅₀ measured using transient HCV subgenomic replicon assay ($n = 2$), nM±SEM (Standard Error)^bcalculated geometric mean.