Bioorganic & Medicinal Chemistry Letters 23 (2013) 3487–3490

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Aryl uracil inhibitors of hepatitis C virus NS5B polymerase: Synthesis and characterization of analogs with a fused 5,6-bicyclic ring motif

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ARTICLE INFO

Article history:
Received 8 February 2013
Revised 12 April 2013
Accepted 22 April 2013
Available online 1 May 2013

Keywords:
Hepatitis C
NS5B polymerase inhibitors
Aryl uracil

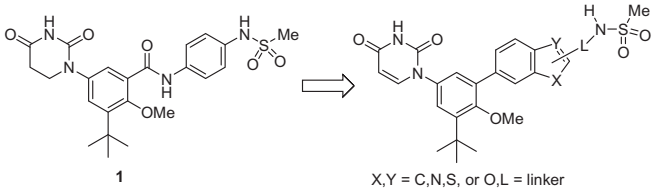
ABSTRACT

The synthesis and structure–activity relationships of a novel aryl uracil series which contains a fused 5,6-bicyclic ring unit for HCV NS5B inhibition is described. Several analogs display replicon cell culture potencies in the low nanomolar range along with excellent rat pharmacokinetic values.

Hepatitis C virus (HCV) is a (+)-strand RNA virus of the *Flaviviridae* family that was first identified in 1989.[1] HCV is a common pathogen that can lead to cirrhosis, hepatocellular carcinoma (HCC) and liver failure. It is estimated that 170 million people were infected worldwide in the year 2000, and that the virus is responsible for at least 10,000 deaths annually in the United States alone.[2] HCV has six major genotype classes, with genotypes 1 and 2 being the most prevalent in the United States, Europe, and Japan.[3] Recently two new protease inhibitor drugs, boceprevir and telaprevir, were approved by the FDA. However these drugs still must be used in combination with the standard of care which imparts several drug-related toxicities. Thus there is still an urgent need for new HCV drugs with diverse modes of action.[4]

Our research team has been pursuing inhibition of the Hepatitis C virus by targeting the HCV polymerase enzyme. Our initial attempts at optimizing a lead series resulted in the generation of a highly potent arylsulfonamide amide (**1**).[5] Unfortunately, the pharmacokinetics of this amide series were poor. For instance when compound **1** was administered as a solution in vehicle in rats at a dose of 5 mg/kg, the bioavailability was only 1.4%. Discovery efforts led to the discovery of a series of substituted aryl uracil analogs that showed improved antiviral potency while also improving upon the poor pharmacokinetic properties (Scheme 1).

This series of substituted aryl uracil analogs were constructed by employing the general sequence as shown in Scheme 2.[6] Key intermediate **3** was generated from a three step sequence employing 2-*tert*-butylphenol as the starting material. Iodide **3** could then be reacted in a Suzuki coupling protocol with boronate **5** to generate inhibitor **6** after subsequent removal of the *para*-methoxybenzyl (PMB) group. The corresponding benzofuran (**10**) and



Scheme 1.

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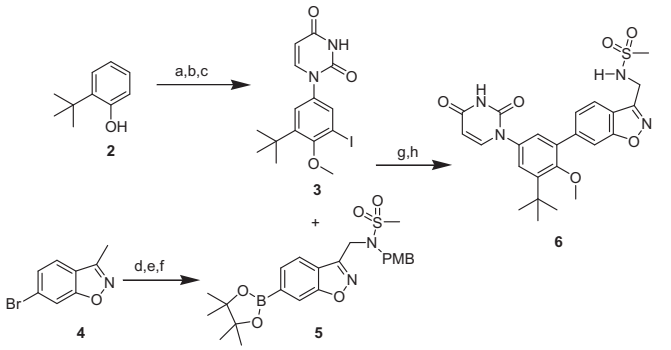
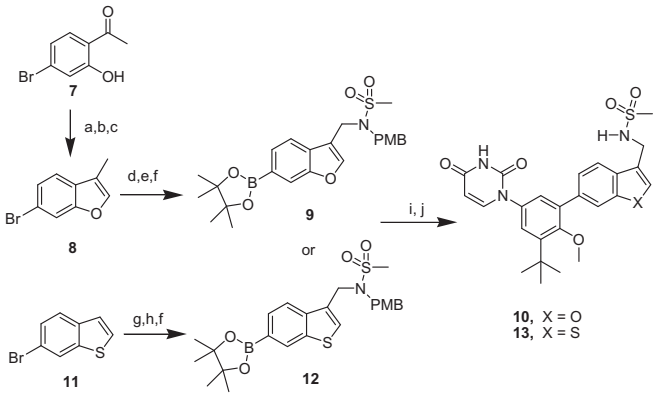
http://dx.doi.org/10.1016/j.bmcl.2013.04.057



Scheme 2. Reagents and conditions: (a) NaOH, MeOH, NaI, then NaOCl, 93%; (b) MeI, acetone, NaOH, 25 °C, 48 h, 99%; (c) uracil, *N*-(2-cyanophenyl)picolinamide, K_3PO_4, CuI, DMSO, 60 °C, 18 h, 61%; (d) 1-bromopyrrolidine-2,5-dione, dibenzoyl peroxide, CCl_4, reflux, 6 h, 43%; (e) *N*-(4-methoxybenzyl)methanesulfonamide, EtOH, aq NaOH, 80 °C, 1.5 h, 22%; (f) bis(pinacolato)diboron, KOAc, Pd(dppf)$_2$Cl$_2$–CH_2Cl_2, dioxane, 80 °C, 16 h, 79%; (g) Pd(dppf)$_2$Cl$_2$–CH_2Cl_2, EtOH, toluene, 1 M aq Na_2CO_3, 100 °C, microwave, 1 h, 83%; (h) TFA, 40 °C, 6 h, 41%.



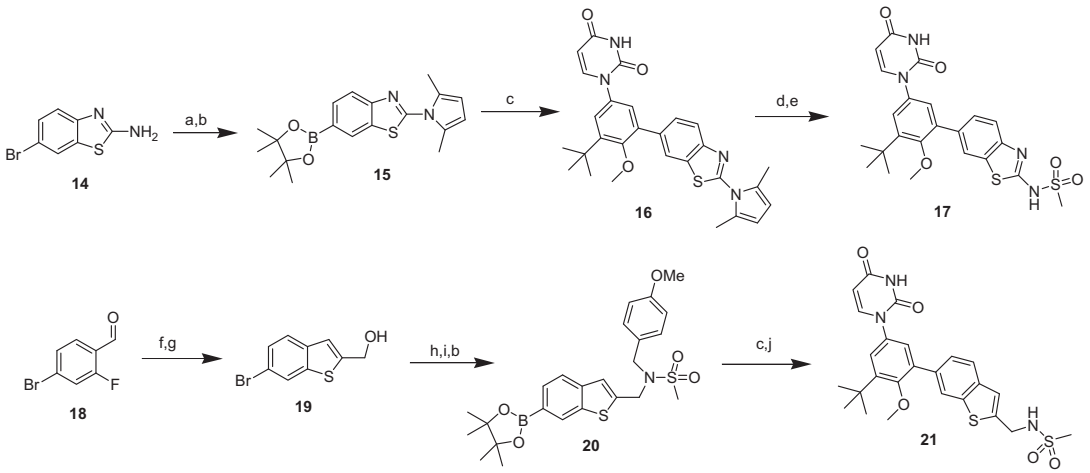
Scheme 3. Reagents and conditions: (a) NaH, DMF, 0.5 h, then methyl bromoacetate, 25 °C, 18 h, 82%; (b) NaOH, H_2O, THF, 25 °C, 3 h, 97%; (c) Ac_2O, NaOAc, reflux, 18 h, 82%; (d) NBS, dibenzoyl peroxide, PhCl, reflux, 2 h, 32%; (e) *N*-(4-methoxybenzyl)methanesulfonamide, K_2CO_3, DMF, 70 °C, 3 h, 35%; (f) bis(pinacolato)diboron, KOAc, Pd(dppf)$_2$Cl$_2$–CH_2Cl_2, dioxane, 80 °C, 16 h, 75–86%; (g) 37% aq formaldehyde, concd HCl, HCl gas, 70 °C, 1 h, 82%; (h) *N*-(4-methoxybenzyl)methanesulfonamide, K_2CO_3, DMA, 25 °C, 3 h, 64%; (i) compound **3**, Pd(dppf)$_2$Cl$_2$–CH_2Cl_2, EtOH, toluene, 1 M aq Na_2CO_3, 100 °C, microwave, 1 h, 43–87%; (j) TFA, DCM, 25 °C, 18 h, 75–84%.

benzothiophene (**13**) inhibitors were also constructed using iodide **3**, utilizing the same Suzuki coupling procedure, as shown in Scheme 3. The 3-bromobenzo[*b*]thiophene (**11**) employed was obtained by following a literature procedure for its preparation.[7]

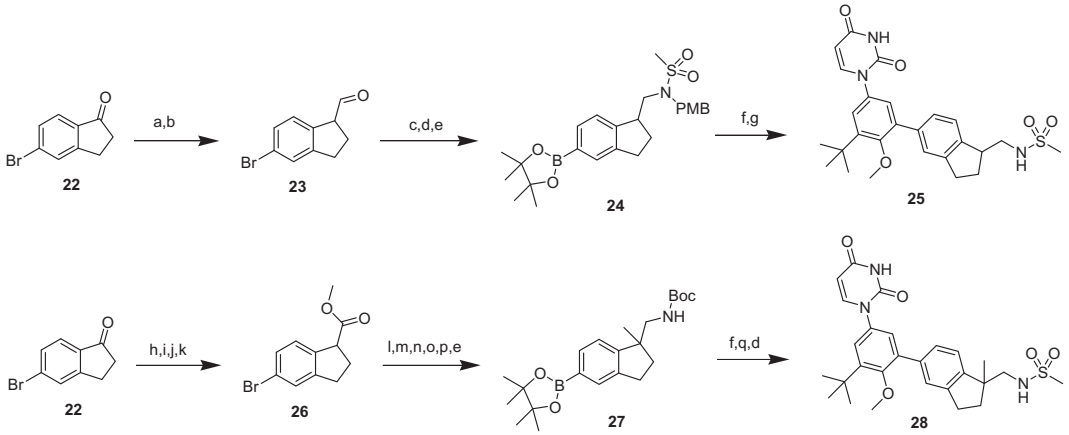
Two heterocyclic inhibitors, benzothiazole **17** and benzothiophene **21**, both of which have the sulfonamide functionality in a different orientation than the previous analogs, were constructed according to the synthetic sequence shown in Scheme 4. Again, a Suzuki reaction was utilized as one of the key reactions in the overall synthetic sequence. Finally, several all carbon bicyclic sulfonamide inhibitors were also generated as shown in Schemes 5 and 6.

Commercially available 5-bromo-2,3-dihydro-1*H*-indene-1-one (**22**) was utilized as the starting material for six analogs. The first, racemic sulfonamide indane **25**, was synthesized by homologating the ketone group in **22** to an aldehyde, then performing a reductive amination followed by the key Suzuki coupling once again as shown in Scheme 5. An additional methyl group could be incorporated onto the indane unit of inhibitor **25** by employing a longer synthetic route. A different set of reagents transformed the starting material **22** to the homologated ester **26**, which was then alkylated with methyl iodide and the subsequent racemic ester was hydrolyzed, made into an amide, then the amide was reduced with borane to give the primary amine. Protection of the amine, then formation of the boronate provided **27**, which was carried on to analog **28**, as shown in Scheme 5. Starting material indanone **22** could also be transformed into indeneamine **29**, which after conversion to the sulfonamide boronate **30**, is subjected to another Suzuki reaction. In this instance, the Suzuki coupling between boronate **30** and aryl iodide **3** was observed to be very sensitive to the amount of unwanted oxygen present in the reaction mixture and thus the yield of inhibitor **31** was much improved upon the utilization of an argon gas inert atmosphere for the desired transformation. Boronate **30** was also methylated on the nitrogen of the sulfonamide and then converted to the corresponding inhibitor **32**. Additionally the dimethylated indene sulfonamide linker analog (**33**) was generated by yet a different set of reagents also shown in Scheme 6. Finally, the methanesulfonylhydrazone analog **34**, and the corresponding hydrazine analog **35** were constructed employing the short synthetic sequence as shown in Scheme 7.

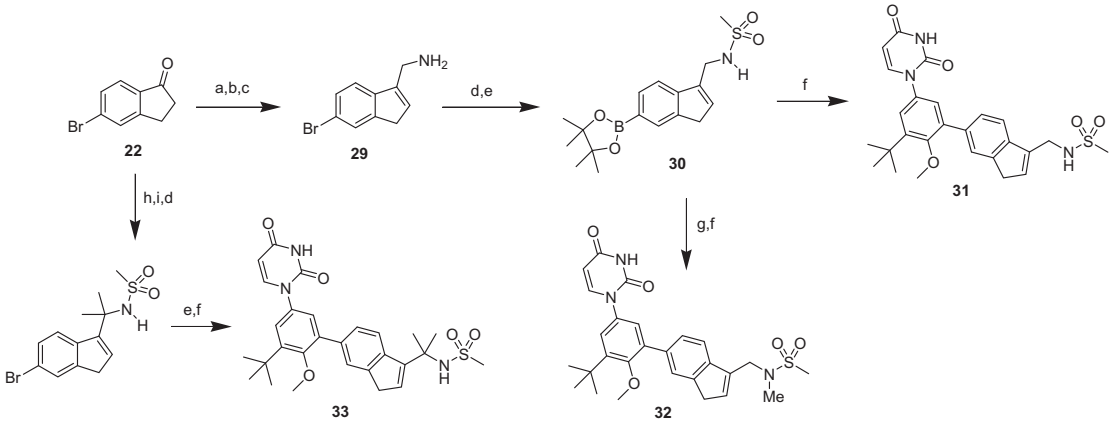
Benzoxazole inhibitor **6** was initially synthesized. This analog (**6**) displays roughly a 20–50 fold loss in in vitro potency versus the starting amide (**1**) comparator as shown in Table 1. Even more disappointing was the poor replicon potency results with benzoxazole **6** against both genotypes 1a and 1b. Undeterred by these



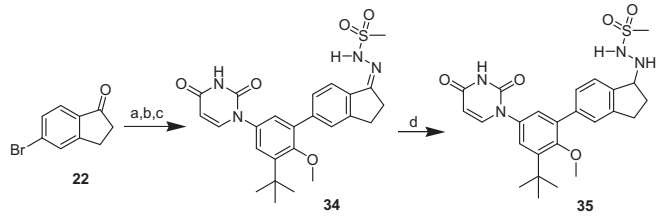
Scheme 4. Reagents and conditions: (a) hexane-2,5-dione, PPTS, benzene, Dean–tark trap, reflux, 16 h, 84%; (b) bis(pinacolato)diboron, KOAc, bis(di-*tert*-butyl(hydroxyl)phosphino)palladium(II) dichloride, toluene, reflux, 72 h, 73–74%; (c) compound **3**, Pd(dppf)$_2$Cl$_2$–CH_2Cl_2, EtOH, toluene, 1 M aq Na_2CO_3, 100 °C, microwave, 1 h, 65–68%; (d) TFA, water, 80 °C, 2.5 h, 98%; (e) $MeSO_2Cl$, pyridine, CH_2Cl_2, 25 °C, 16 h, 4%; (f) methyl mercaptoacetate, Et_3N, DMSO, 75 °C, 2 h, 100%; (g) $LiAlH_4$, diethyl ether, 5 °C, 1 h, 83%; (h) NBS, TPP, CH_2Cl_2, 25 °C, 2 h, 89%; (i) *N*-(4-methoxybenzyl)methanesulfonamide, 1.0 M aq NaOH, EtOH, reflux, 1 hr, 33%; (j) TFA, CH_2Cl_2, 25 °C, 4 h, 44%.



Scheme 5. Reagents and conditions: (a) $MeOCH_2PPh_3^+Cl^-$, THF, KOtBu, −20 to 25 °C, 3 h, 93%; (b) BBr_3, −78 °C, 4 h, 45%; (c) 4-methoxybenzylamine, decaborane, MeOH, 25 °C, 1 h, 57%; (d) $MeSO_2Cl$, pyridine, 25 °C, 1 h, 46–51%; (e) bis(pinacolato)diboron, KOAc, $Pd(dppf)_2Cl_2$–CH_2Cl_2, dioxane, 95 °C, 8 h, 66–83%; (f) $Pd(dppf)_2Cl_2$–CH_2Cl_2, EtOH, toluene, 1 M aq Na_2CO_3, 100 °C, microwave, 1 h, 47–85%; (g) TFA, 25 °C, 1 h, 95%; (h) 1,3-dithiane, THF, −30 to −15 °C, *n*BuLi, then **22**, 0 °C, 18 h; (i) pTSA, reflux, benzene, 1 h; (j) concd HCl, AcOH, reflux, 3 h, 58% for three-steps; (k) MeOH, 4 N HCl in dioxane, reflux, 8 h, 77%; (l) LiHMDS, THF, MeI, −78 to 25 °C, 96%; (m) Me_3SiOK, THF, reflux, 3 h, 99%; (n) oxalyl chloride, DMF, hexanes, 25 °C, 1 h, then concd NH_4OH, acetone, 0–25 °C, 1 h, 100%; (o) BH_3–Me_2S, THF, reflux, 2 h, 68%; (p) $(Boc)_2O$, THF, Na_2CO_3, H_2O, 25 °C, 18 h, 94%; (q) 4 N HCl in dioxane, 25 °C, 18 h, 96%.



Scheme 6. Reagents and conditions: (a) *N*-methyl-morpholine *N*-oxide, TMSCN, CH_2Cl_2, 25 °C, 72 h, 86%; (b) $LiAlH_4$, Et_2O, 0–25 °C, 3 h, then NH_4OH, 70%; (c) 6 N aq HCl, MeOH, 70 °C, 3 h, 92%; (d) $MeSO_2Cl$, $(iPr)_2NEt$, CH_2Cl_2, 25 °C, 10 h, 56–83%; (e) bis(pinacolato)diboron, KOAc, $Pd(dppf)_2Cl_2$–CH_2Cl_2, dioxane, 95 °C, 8 h, 65–87%; (f) compound **3**, $Pd_2(dba)_3$, 1,3,5,7-tetramethyl-2,4,8-trioxa-6-phospha-6-phenyl-adamantane (Cytec [97739-46-3]), THF, H_2O, 1 M aq K_3PO_4, 50 °C, 2.5 h, 24–84%; (g) LiHMDS, THF, then MeI, 25 °C, 2 h, 57%; (h) LDA, THF, diethylcyanophosphonate, −10 to 25 °C, 3 h, then BF_3–OEt_2, −78 °C to rt, 16 h, 69%; (i) anhydrous $CeCl_3$, THF, MeLi–LiBr, −78 to −20 °C, 24 h, 20%.



Scheme 7. Reagents and conditions: (a) bis(pinacolato)diboron, KOAc, $Pd(dppf)_2Cl_2$–CH_2Cl_2, dioxane, 80 °C, 8 hrs, 65%; (b) compound **3**, $Pd(dppf)_2Cl_2$–CH_2Cl_2, EtOH, toluene, 1 M aq Na_2CO_3, 100 °C, microwave, 1 h, 61%; (c) methanesulfonylhydrazide, THF, MeOH, 60 °C, 24 hrs, 66%; (d) $NaBH_3CN$, aq HCl, MeOH, THF, 25 °C, 1 hr, 58%.

poor initial results we forged ahead to construct the related benzofuran (**10**) and benzothiophene (**13**) inhibitors. These two analogs displayed improved HCV inhibition results, with the benzothiophene (**13**) analog being better than the amide **1** in the enzyme inhibition assay as well as inhibiting the HCV replicon in Huh7 hepatocytes (containing 5% fetal calf serum). Unfortunately, **13** displayed greater affinity for plasma proteins than **1**, consequently reducing its potency when 40% human plasma was added to the replicon assay. Surprisingly the effect of moving the sulfonamide functionality to a different attachment point on the heterobicyclic ring (2 vs 3 position) as demonstrated in comparing analogs **13** versus **21** was dramatic. The latter, 2-substituted methylsulfonamide (**21**), was much weaker in the cell culture replicon assay, as was the 2-substituted thiazole (**17**). We also explored the possibility of removing all of the heteroatoms from the bicyclic unit. Thus indane inhibitor **25** was synthesized and ultimately demonstrated a very favorable HCV inhibition profile. Inhibitor **25** is slightly more potent than amide **1**, and turned out to be one of the most potent analogs in this series against genotype 1b, even in the replicon experiment containing 40% human plasma. With this success, we constructed a number of closely related analogs to indane inhibitor **25**. This synthetic activity resulted in the methyl substituted indane analog **28**, and the three related indenes (**31**, **32** and **33**). Of these analogs the indene inhibitor **31** is the best, displaying favorable potency profile similar to the potencies of indane inhibitor **25**.

Table 1
Potency of aryl uracil analogs

Compd #	HCV polymerase IC_{50} (nM)[a]		HCV replication EC_{50} (nM)[a,b]			PAMPA Effective Permeability
	Genotype 1a	Genotype 1b	Genotype 1a	Genotype 1b	Genotype 1b with 40% HP	P_e ($\times 10^{-6}$) cm/s
1	11	22	51	27	18	0.22
6	540	510	>1000	>1000	—	—
10	9	21	240	22	260	—
13	5	10	27	3	46	0.81
17	19	45	>1000	>1000	—	1.40
21	180	245	>1000	>1000	>10000	0.81
25	8	18	20	3	13	8.93
28	16	41	480	89	560	—
31	6	11	14	3	22	5.43
32	7	20	4	100	50	—
33	185	215	1000	>1000	>1000	4.06
34	3	3	2	1	1	0.47
35	33	47	140	>100	950	0.24

[a] IC_{50} and EC_{50} values are means of at least two independent determinations, with a maximum twofold value variation.
[b] Assay run with 5% fetal calf serum except for last column, in which 40% human plasma was added.

Table 2
Single IV and oral dose pharmacokinetics of select aryl uracil analogs in rat

Compd	IV dose (mg/kg)	IV $t^{1/2}$ (h)	IV CLp (L/h/kg)	Oral dose[a] (mg/kg)	Oral $t^{1/2}$ (h)	Oral C_{max} (μg/mL)	Oral T_{max} (h)	Oral $AUC_{0-\infty}$ (μg·h/mL)	F (%)
1	5	1.1	1.53	5	1.0	0.03	0.33	0.5	1.4
13	5	3.6	0.27	5	3.2	1.41	0.50	9.3	41
25	5	1.8	0.45	5	2.3	2.36	0.33	13.5	119
28	5	2.3	0.61	5	2.4	1.01	0.25	5.1	62
31	5	1.9	0.77	5	3.0	0.66	6.17	7.2	86
34	5	2.8	1.19	5	3.0	0.12	2.50	0.88	18

[a] Compounds dosed as a solution.

The related hydrazone inhibitor **34** also shows outstanding potency against both genotypes. Unfortunately this analog (**34**) was subsequently found to be unstable in vivo, resulting in cleavage of the hydrazone functionality. In an effort to stabilize this hydrazone analog (**34**) from hydrolysis, the hydrazine analog **35** was made from **34**. Even though **35** is isosterically matched with **25**, the potencies of both analogs were surprisingly different, with **35** suffering a dramatic loss in the replicon experiments. Finally, we examined the pharmacokinetic characteristic of the most potent analogs in rats. As shown in Table 2, we were surprised to see that the potent HCV inhibitors **13**, **25**, **28**, **31** and **34** all demonstrated much improved oral pharmacokinetic parameters in rat when compared to amide **1**.

In summary, we discovered a number of aryl uracil bicyclic HCV NS5B polymerase inhibitors that have both excellent in vitro and cell culture replicon potencies. We also discovered that the intervening scaffolding between the *tert*-butyl aryl uracil and the methane sulfonamide seemed to play an important role in the improvement of PK parameters in the rat upon oral dosing.

Disclosures

This research was sponsored by Abbvie. Abbvie contributed to the research, interpretation of data, writing, reviewing, and approving the publication.

A.C.K., J.T.R., D.A.D., P.L.D., D.L., R.W., G.K., H.B.L., J.M.B., Y.L., W.M.K., K.L.L., A.M., K.D.S. and C.J.M. are all employees of Abbvie.

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