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Active site inhibitors of HCV NS5B polymerase. The development and pharmacophore of 2-thienyl-5,6-dihydroxypyrimidine-4-carboxylic acid

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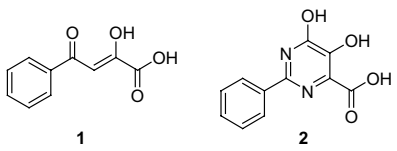
Abstract—5,6-Dihydroxypyrimidine-4-carboxylic acids are a promising series of hepatitis C virus (HCV) NS5B polymerase inhibitors that bind at the active site of the enzyme. Here we report a simple 2-thienyl substituted analogue that shows 10-fold improved activity over the original lead, and which allowed us to further delineate the key elements of the pharmacophore of this class of inhibitor. This work led to the identification of a trifluoromethyl acylsulfonamide group as a viable replacement for the C4 carboxylic acid in this series.

1. Introduction

The front line therapy for HCV, a viral infection that affects around 170 million people worldwide, is interferon-α, often dosed in combination with the nucleoside analogue ribavarin.[1] While progress has been made over the past decade towards the development of improved formulations of these drugs, the treatment remains rather poorly tolerated and its success rate is dependent on the genotype of the HCV infection.[2] As a consequence, HCV continues to be a significant world health burden, and remains the leading cause of liver transplantation in the United States.[3] There is a pressing need for new and broadly active antiviral agents to combat HCV infections.

The NS5B protein of HCV has been shown to be a key component in the viral replication cycle, and has emerged as an attractive target for drug discovery.[4] NS5B is an RNA dependent RNA polymerase that comprises the palm, fingers and thumb sub-domains common to nucleotide polymerising enzymes.[5] Mechanistically, NS5B is thought to mediate viral RNA synthesis through two Mg^{2+} ions that are coordinated in the palm domain and which serve the dual role of positioning/stabilising the pyrophosphate leaving group on the incoming nucleoside tri-phosphate, and activating the 3′-OH of the elongating RNA towards nucleophilic attack.[6]

While several series of allosteric inhibitors of NS5B have been disclosed,[7] to date the only nonnucleoside inhibitors that bind at the active site of the enzyme are a series of keto-acids and a series of dihydroxy pyrimidines (Fig. 1) that were recently reported.[8,9] Compounds from both these classes show biochemical behaviour reminiscent of pyrophosphate isosteres such as Foscarnet, and are thought to be product-like inhibitors of the NS5B enzyme. Notably, the measured activity of these inhibitors is highly dependent on the nature of the metal ion[10] used in the enzyme inhibition assay, suggesting that



1 IC_{50} 5.7 μM 2 IC_{50} 30 μM

Figure 1. Active-site nonnucleoside inhibitors of HCV NS5B polymerase.

Keywords: Hepatitis C virus; HCV; Polymerase.
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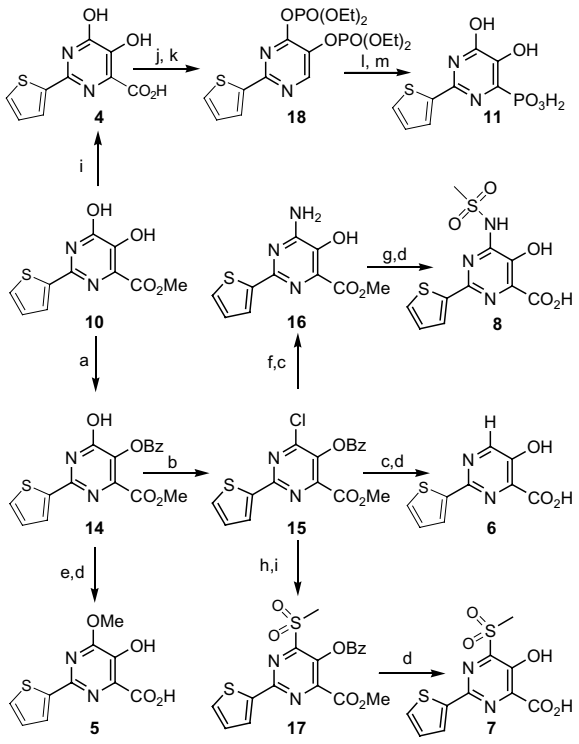
their mechanism of action likely involves chelation to at least one of the two Mg^{2+} ions that are present in the active site of the enzyme.

Despite the rather weak intrinsic potency of **2**, this series appeared attractive as a lead since the potential for undesirable biochemical reactivity of the keto-acid moiety is diminished by virtue of its incorporation into an aromatic scaffold. Here, in a continuation of our work in this area, we describe our preliminary efforts aimed at both improving potency and at delineating the structural features required for activity in this series.

2. Synthesis

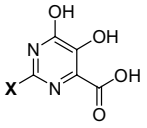
2-Aryl-5,6-dihydroxypyrimidine-4-carboxylic acids were accessed by routine hydrolysis of the corresponding methyl esters that were prepared according to literature precedent.[11] Thus the 2-thienyl pyrimidine **4** (Scheme 1) was obtained from treatment of **10** with LiOH, and an analogous strategy was used in the preparation of the 2-pyridine substituted pyrimidine **3** (Table 1). Compound **10** also served as the starting point for the preparation of pyrimidines **5**–**8** and **11**.

Following regioselective protection of the C5–OH group as a benzoate ester, alkylation of **14** with NaH/MeI



Scheme 1. Reagents and conditions: (a) PhCOCl, pyridine (84%); (b) $POCl_3$ 120° C (90%); (c) H_2(g), 10% Pd/C (92%); (d) LiOH, THF/H_2O 50 °C then reversed phase-HPLC (30–48%); (e) NaH, MeI (42%); (f) NaN_3, DMF 50 °C (98%); (g) $MeSO_2Cl$, pyridine then NaH, DMF 50 °C (two steps, 67%); (h) MeSNa, NMP (68%); (i) H_2O_2, AcOH (21%), LiOH THF/H_2O (85%); (j) 6 N HCl, reflux (75%); (k) $HPO(OEt)_2$, Et_3N (66%); (l) LiTMP, THF, −78 °C (16%); (m) TMSBr 15 equiv CH_2Cl_2, then reversed phase-HPLC (21%).

Table 1. Structural modifications at the C2 position of 5,6-dihydroxypyrimidine-4-carboxylic acid inhibitors of NS5B polymerase



Compound	X	[b]IC_{50} (μM)
2	Ph	30
3	2-Pyridyl	n/a[a]
4	2-Thienyl	2.6

[a] Compounds designated n/a showed less than 30% inhibition of NS5B at an inhibitor concentration of 50 μM.

[b] IC_{50} values in this and subsequent tables are the arithmetic mean of at least two independent measurements.

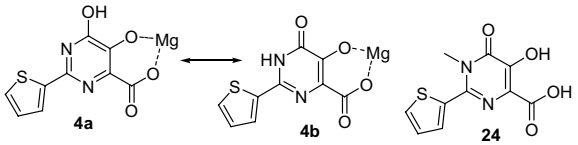
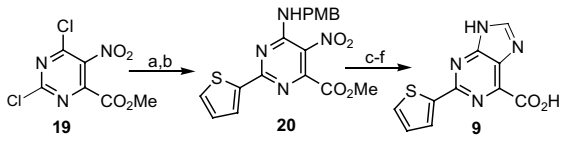


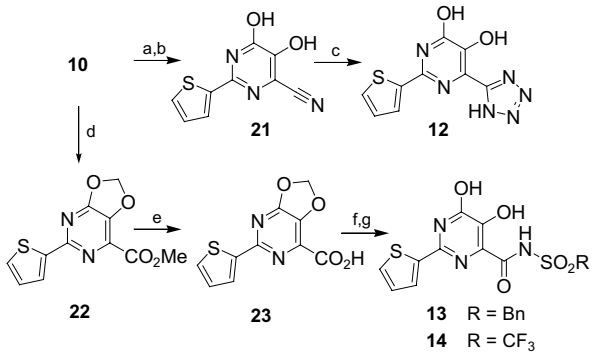
Figure 2. Tautomeric isomers of 2-thienyl pyrimidine **4** and a potential mode of interaction with Mg^{2+} ions in the active site of NS5B.

afforded both **5** and **24** (Fig. 2) after LiOH mediated hydrolysis. Treatment of **14** with $POCl_3$ afforded the 6-chloropyrimidine **15** and hydrogenolytic removal of the Cl atom followed by hydrolysis afforded the deshydroxy compound **6**. Treatment of **15** with sodium azide gave smooth substitution of the 6-Cl atom, albeit with concomitant cleavage of the benzoate ester. Following hydrogenolysis of the azide group to the 6-amino pyrimidine **16** direct elaboration of the amine functionality could not be achieved due to preferential reaction of 5-OH group with electrophilic reagents. The sulfonamide group of **8** was therefore installed by NaH mediated isomerisation of the C5 mesylate formed from **16** and 1 equiv of $MeSO_2Cl$. In contrast, no cleavage of the benzoate protecting group was observed on treatment of **15** with NaSMe, allowing smooth access to **7** by oxidation of the intermediate pyrimidine thioether with H_2O_2 to afford **17**, followed by deprotection of the C5 benzoate and C4 ester groups. The C4 phosphonic acid derivative **11** proved a challenging synthetic target, but could be accessed using a phosphate–phosphonate rearrangement strategy. Decarboxylation of **4** and conversion of the resulting diol to the bis-diethylphosphate derivative **18** provided the required substrate. LiTMP mediated rearrangement proceeded with loss of the C6 phosphate group, and the resulting C4 phosphonate ester, isolated after RP-HPLC purification, was deprotected using TMSBr.

The purine **9** was prepared from the commercial dichloropyrimidine **19** (Scheme 2). Displacement of the C6 Cl atom with *p*-methoxybenzylamine followed by Stille cross coupling with tri-*n*-butyl(2-thienyl) tin gave **20**. Following PMB deprotection with TFA, the nitro group was reduced (Zn/HCO_2H) and the purine ring system



Scheme 2. Reagents and conditions: (a) PMB–NH_2, $NaHCO_3$ (99%); (b) 2-thienyl-$SnBu_3$, $Pd(PPh_3)_2Cl_2$, DMF, 90 °C (68%); (c) TFA 60 °C (99%); (d) Zn, HCO_2H (94%); (e) $(EtO)_3CH$, conc. HCl (26%); (f) LiOH, THF/H_2O 50 °C then reversed phase-HPLC (62%).



Scheme 3. Reagents and conditions: (a) NH_3/MeOH (2 N), 50 °C (92%); (b) $(CF_3CO)_2O$, $CHCl_3/CH_2Cl_2$ (1:2) (71%); (c) NaN_3, $Et_3N{\cdot}HCl$, xylene/DMF (1:1), reflux, then reversed phase-HPLC (25%); (d) CH_2Br_2, KF, DMF, 120 °C (99%); (e) NaOH, H_2O, dioxane, 1.5 min, 20 °C (75%); (f) H_2NSO_2R, DMAP, EDCI, CH_2Cl_2 (95%); (g) NaOH, H_2O, dioxane, then reversed phase-HPLC (23%).

was generated by reaction of the diamine with triethyl orthoformate. Finally, the carboxylic acid was unmasked by hydrolysis with LiOH.

Compounds **12**–**14** were prepared as outlined in Scheme 3. Tetrazole **12** was obtained from **21** via dipolar cycloaddition with sodium azide. The required nitrile was obtained by dehydration of the primary amide formed through reaction of **10** with NH_3/MeOH. In contrast, attempts to form acylsulfonamides **13**–**14** either from **10** or from the unprotected pyrimidine 4-carboxylic acid **4** proved unsatisfactory. Protection of the diol moiety in **10** as the cyclic methylene acetal **22** increased the reactivity of the C4 methyl ester (rendering it highly labile towards basic hydrolysis) and the corresponding carboxylic acid **23** underwent smooth reaction with sulfonamides under standard peptide coupling conditions.

Surprisingly, removal of the acetal functionality required basic conditions, presumably occurring through nucleophilic attack of hydroxide at either the C5 or C6 position of the pyrimidine. All yields quoted in the synthetic schemes are of isolated material, and are unoptimised.

3. Results and discussion

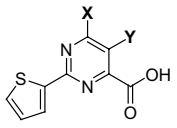
In order to quantify the effect of structural changes at the dihydroxypyrimidine carboxylic acid core, we initially sought a structurally simple lead that showed improved activity over **2**. An early breakthrough came during an evaluation of heterocyclic replacements for the C2 phenyl ring of **2**. While six-membered heterocycles at this position (e.g., pyridine **3**, Table 1) were significantly less active than the corresponding phenyl compound, five-membered heterocycles such as the thiophene **4** (IC_{50} 2.6 μM) were an order of magnitude more potent. This finding was unexpected[12] given that a thiophene ring often serves as a phenyl isostere, but nonetheless provided us with a starting point from which to probe changes to the pyrimidine core of the inhibitor.

5,6-Dihydroxypyrimidines such as **4** may exist in several tautomeric forms, though quantum mechanical calculations[12] suggest that the aromatic form (**4a**, Fig. 2) and amide form **4b** are favoured in the ground state. We hypothesised that either of these isomers might interact with an active site Mg^{2+} ion as a neutral bidentate ligand forming a six-membered chelate between the C4 carboxylic acid and the C5 phenol group. In this binding orientation, the C6 phenol/keto group of the inhibitor would not directly interact with the active site metal ions, leading us to focus our initial exploration of the pharmacophore of this series on the C6 region of the inhibitor.

In practice, as highlighted in Table 2, compound **4** proved extremely sensitive to structural changes at C6. Capping the 6-OH group as a methyl ether (compound **5**) resulted in a 20-fold loss in potency while the deshydroxy compound **6** showed no activity against the polymerase enzyme. The introduction of electron withdrawing functionality was explored as a means of increasing the acidity of the C5 OH group, thereby potentiating its interaction with metal ions at the active site. The drop in potency observed for sulfone **7** was typical for substitutions of this type, indicating that changes in pK_a at the C5 phenol group are significantly less important than stereoelectronic effects at C6.

Replacement of the C6 OH group of **4a** with alternative hydrogen-bond donor functionality was also unsuccessful. Thus both sulfonamide **8** and the purine **9** (that

Table 2. Structural modifications at the C6 position of 5,6-dihydroxypyrimidine-4-carboxylic acid inhibitors of NS5B polymerase



Compound	X	Y	IC_{50} (μM)
4	OH	OH	2.6
5	OMe	OH	50
6	H	OH	n/a[a]
7	SO_2Me	OH	49
8	$NHSO_2Me$	OH	n/a
9	–NHCH=N–		n/a

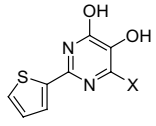
[a] Compounds designated n/a showed less than 30% inhibition of NS5B at an inhibitor concentration of 50 μM.

retains both a hydrogen bond donor at C6 and a nitrogen lone pair orientated in similar fashion to the C5 O–Mg bond in the proposed chelate) were devoid of activity. Assuming that metal-binding does indeed involve a six-membered chelate as depicted in Figure 2, these results, together with the observation that *N*-methylpyrimidones[9] such as **24** retain activity (IC_{50} 4.2 μM) suggest that pyrimidines may interact with the enzyme as the keto-tautomer **4b** rather than in the enol form **4a**, and that hydrogen bond acceptor functionality is required at C6.

The effects of structural changes at the C4 carboxylic acid group are summarised in Table 3. A charged group at this position was essential; carboxylic esters (compound **10**) showed no inhibition of the polymerase enzyme. Surprisingly, charged acid isosteres such as the phosphonic acid **11**, the tetrazole **12**, and the acylsulfonamide derivative **13** were also inactive. While this may reflect an inability of these groups to satisfy the geometric requirements for interaction with Mg^{2+} at the active site, the electronic nature of the acidic group is also likely to be important. Changes in pK_a at C4 likely affect the Mg^{2+} interaction of the C4 acidic group itself and may also have an impact on the pK_a/metal interaction of the C5 (or C6) phenolic OH groups. To further explore the role of pK_a at C4 the trifluoromethyl acylsulfonamide derivative **13** was prepared, and provided the first structure in which modification of the β,γ-dihydroxy carboxylic acid motif did not seriously compromise potency.

The improved potency of **14** (IC_{50} 6.2 μM) relative to **13** (IC_{50} > 50 μM) most likely stems from its lower acylsulfonamide pK_a[13] rather than alleviation of an unfavourable steric interaction (for the benzyl group of **13**) since methyl acylsulfonamides were inactive in a closely related series.[14] Compound **13** retained the metal dependent inhibition profile[9] shown by the corresponding dihydroxypyrimidine carboxylic acid **4**, suggesting that the binding modes of these compounds are similar. This was confirmed by a kinetic competition study[15] in which the IC_{50} of **4** was measured in the presence of three fixed concentrations of **14**. The expected linear dose-dependent shift in the inhibition curve of **4** (Dixon plot) was indeed observed.

Table 3. Structural modifications at the C4 position in the dihydroxypyrimidine series



Compound	X	IC_{50} (μM)
4	COOH	2.6
10	COOMe	n/a[a]
11	PO_3H_2	n/a
12	Tetrazole	n/a
13	$CONHSO_2Bn$	n/a
14	$CONHSO_2CF_3$	6.2

[a] Compounds designated n/a showed less than 30% inhibition of NS5B at an inhibitor concentration of 50 μM.

In conclusion, we have described a series of 2-thiophene substituted 5,6-dihydroxypyrimidine-4-carboxylic acids that show a 10-fold improvement in activity over the original lead compound in this series **2**. Tight SAR around the pyrimidine ring was delineated, and structural changes at the C6 OH group were not permitted. In contrast, a trifluoromethyl acyl sulfonamide replacement for the C4 carboxylic acid group of **4** was tolerated, providing an alternative structurally related series for future development.

Acknowledgement

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