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Design and synthesis of 3,4-dihydro-1*H*-[1]-benzothieno[2,3-*c*]pyran and 3,4-dihydro-1*H*-pyrano[3,4-*b*]benzofuran derivatives as non-nucleoside inhibitors of HCV NS5B RNA dependent RNA polymerase

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Abstract—A novel class of HCV NS5B RNA dependent RNA polymerase inhibitors containing 3,4-dihydro-1*H*-[1]-benzothieno[2,3-*c*]pyran and 3,4-dihydro-1*H*-pyrano[3,4-*b*]benzofuran scaffolds were designed and synthesized. Optimization of the alkyl substituent in the pyran ring showed preference for an *n*-propyl group, while 5,8-disubstitution pattern is preferred for the aromatic region. Analog **19** displayed potent activity with an IC_{50} of 50 nM against HCV NS5B enzyme and was selective over a panel of polymerases.

Hepatitis C virus (HCV) infection is one of the causes for liver cirrhosis and hepatocellular carcinoma leading to liver failure. Current estimates of approximately 170 million people worldwide as HCV carriers represent a significant medical problem with economic burden implications.[1] Currently approved therapy involves pegylated interferon-α as a single agent[2] or in combination with the broad spectrum anti-viral ribavirin.[2] Although somewhat effective, patient compliance is compromised due to severe side effects attributed to each of the agents. Furthermore, the current therapeutic approach is not aimed at any particular viral target. Lack of specificity of current therapies against the known HCV subtypes underscores the need for direct inhibition of viral targets in an effort to significantly improve patient outcomes.

HCV is a positive strand RNA virus and the genome consists of 9600 base pairs that encode several structural and non-structural proteins.[3] Inhibitors of NS5B RNA dependent RNA polymerase have received much attention recently, as potential therapeutic agents for treatment of HCV infection.[4] Both allosteric and active site inhibitors of NS5B polymerase have been reported (Fig. 1). We recently reported[5] pyrano[3,4-*b*]indole **5** as a potent (IC_{50} = 0.33 μM) and selective inhibitor of NS5B polymerase. While the preliminary structure–activity relationship was explored retaining the pyrano[3,4-*b*]indole intact, we were interested in expanding our optimization efforts to some other scaffolds like 3,4-dihydro-1*H*-[1]-benzothieno[2,3-*c*]pyran **6** and 3,4-dihydro-1*H*-pyrano[3,4-*b*]benzofuran **7** to see if these two different heterocyclic middle rings, which are devoid of the hydrogen bond donor capability, are tolerated (Fig. 2).

In this communication, we report the synthesis of these scaffolds and explore the structure–activity relationship requirements for these new scaffolds.

As shown in Scheme 1, the synthesis of the representative compound **19** for the 3,4-dihydro-1*H*-[1]-benzothieno[2,3-*c*]pyran scaffold, started from 1-bromo-2-fluoro-4-methyl-benzene **8**, which upon nitration followed by

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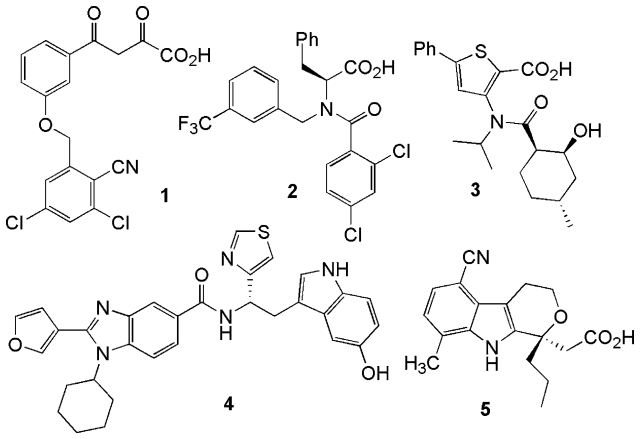


Figure 1. Small molecule inhibitors of HCV NS5B polymerase.

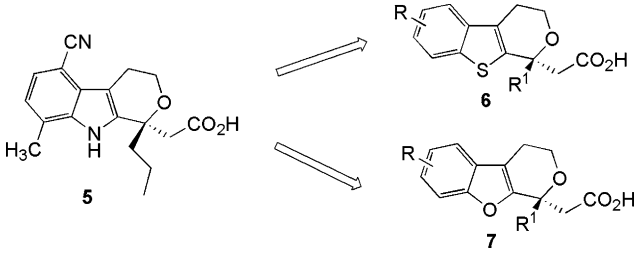
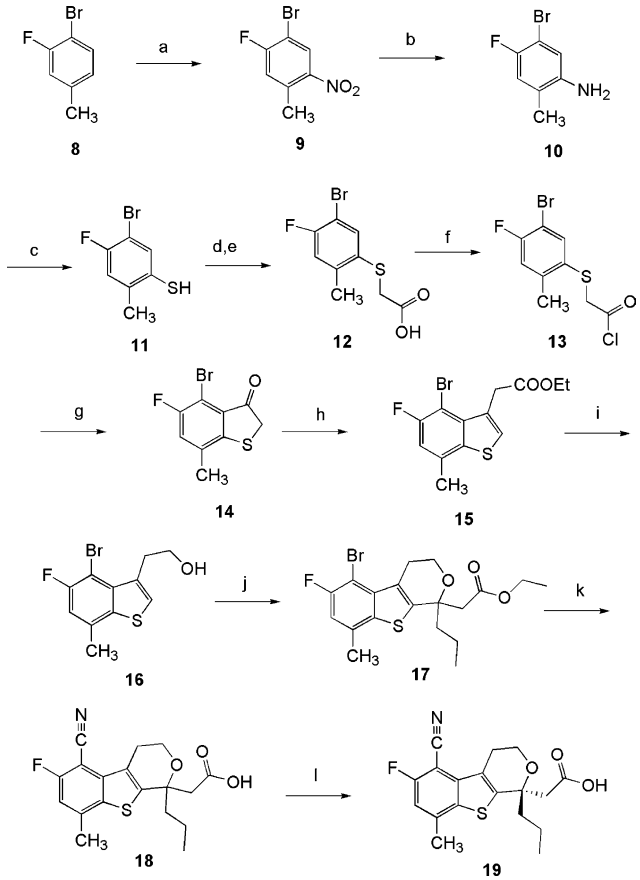


Figure 2. Scaffold substitution for pyrano[3,4-*b*]indole.

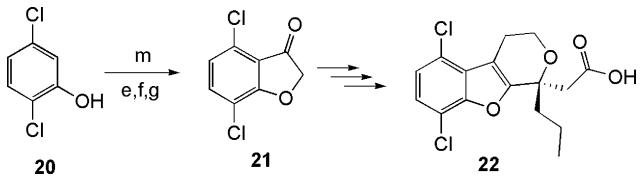
reduction afforded aniline **10**. The amino group was converted to the thiol **11** by diazotization followed by reaction with potassium ethyl xanthate. Alkylation of the thiol followed by deprotection gave the acetic acid derivative **12**, which was converted to the acid chloride **13** and subjected to Friedel–Crafts cyclization to give the 3-benzothiophenone **14**. Wittig reaction of the ketone and subsequent reduction of the ester afforded the alcohol **16**. Treatment of the alcohol **16** with β-ketoester in the presence of Lewis acid gave the required tricyclic scaffold **17**. The aromatic bromo substituent was converted to cyano under microwave condition and the ester was hydrolyzed to give the racemic acid **18** of interest. Further separation of the required enantiomer was accomplished by chiral HPLC methods.[6]

The synthesis of 3,4-dihydro-1*H*-pyrano[3,4-*b*]benzofuran analogs **7** was carried out in an analogous manner following Scheme 1 where the thiol **11** was replaced with the appropriately substituted phenol **20** (Scheme 2). Although, the formation of benzofuran **21** by the cyclization of intermediate phenoxy acetyl chloride proceeded in sub-optimal yield (15–20%), subsequent steps followed the same trend observed for the 3,4-dihydro-1*H*-[1]-benzothieno[2,3-*c*]pyran scaffold to give the final tricyclic target **22**.

Our initial efforts were focused on extrapolating the SAR that we had observed in the case of pyrano[3,4-*b*]indole to 3,4-dihydro-1*H*-[1]-benzothieno[2,3-*c*]pyran scaffold. To this end, we started our optimization with the pyran ring of the molecule. As seen from Table 1, with changing the alkyl group on the C-1 carbon from



Scheme 1. Reagents and conditions: (a) HNO_3, H_2SO_4, 0 °C, 92%; (b) $SnCl_2$, EtOAc, 63%; (c) 1—$NaNO_2$, H_2O, HCl; 2—potassium ethyl xanthate; 3–KOH, EtOH, 92%; (d) $BrCH_2CO_2t$-Bu, pyridine, 0 °C; (e) TFA, CH_2Cl_2, 45% over two steps; (f) $SOCl_2$, 90 °C; (g) $AlCl_3$, chlorobenzene, 52% over two steps; (h) $Ph_3P{=}CHCO_2Et$, toluene, 120 °C, 39%; (i) $LiALH_4$, THF, 0 °C, 83%; (j) $CH_3(CH_2)_2COCH_2CO_2Et$, BF_3-Et_2O, CH_2Cl_2, 75%; (k) 1—CuCN, NMP, microwave, 220 °C, 15 min; 2—1 N NaOH, EtOH, THF, 61% over two steps; (l) chiral prep HPLC.[6]

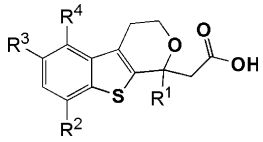


Scheme 2. Reagents and conditions: (m) $BrCH_2CO_2t$-Bu, K_2CO_3, DMF, rt, 12 h.

methyl to ethyl to *n*-propyl **23**–**25**, the polymerase inhibition was dramatically improved. However, going to longer *n*-butyl group **26** was not favorable indicating that the *n*-propyl group is the ideal group for this region of the molecule.

Moving to the aromatic substitutions, a simple unsubstituted aromatic system **27**, even with the optimal substitution of *n*-propyl in the C-1 position, was completely devoid of any activity indicating the need for aromatic substitutions for the polymerase activity. Addition of groups like 6-fluoro and 8-methyl (e.g., **28**) did not

Table 1. HCV NS5B inhibitory activity of 3,4-dihydro-1*H*-[1]-benzothieno[2,3-*c*]pyran derivatives

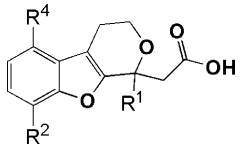


Compound	R^1	R^2	R^3	R^4	Stereo[8]	IC_{50} (μM)[9a]
23	Me	Cl	H	Cl	—	17.6
24	Et	Cl	H	Cl	—	1.9
25	*n*-Pr	Cl	H	Cl	—	0.14
26	*n*-Bu	Cl	H	Cl	—	0.75
27	*n*-Pr	H	H	H	—	>20
28	*n*-Pr	Me	F	H	—	>20
29	*n*-Pr	Me	H	Br	—	0.19
30	*n*-Pr	Me	H	CN	—	0.32
31	*n*-Pr	Me	H	CN	*R*	0.17
32	*n*-Pr	Me	H	CN	*S*	2.85
18	*n*-Pr	Me	F	CN	—	0.13
19	*n*-Pr	Me	F	CN	*R*	0.05
33	*n*-Pr	Me	F	CN	*S*	5.36

satisfy the requirements. However, a 5,8-disubstitution pattern was found to be regaining substantial amount of potency as in the case of bromo and cyano derivatives **29** and **30**, respectively. A trisubstituted pattern was well tolerated and showed a slight enhancement in potency. As observed in the case of pyrano[3,4-*b*]indole analogs, one of the enantiomers was more potent than the other enantiomer as exemplified by the 2 pairs separated **31**, **32** and **19**, **33**.[7] It is probably the *R* enantiomer that shows activity analogous to the pyrano[3,4-*b*]indole series.[8]

Now focusing on the second scaffold of interest, 3,4-dihydro-1*H*-pyrano[3,4-*b*]benzofuran, we synthesized the proof-of-concept analogs with 5,8-dichloro substituents in the aromatic region since that pattern showed good enzyme potency with the 3,4-dihydro-1*H*-[1]-benzothieno[2,3-*c*]pyran scaffold. It was interesting to see that compound **22** (Table 2) was much less potent, when compared to compound **25**. However, the structure–activity relationship observed for the C-1 alkyl substituents showed the same trend with *n*-propyl being better than smaller ethyl **36** or methyl **35** analogs and the activity decreased with longer *n*-butyl **37** analog. The aromatic substituents were deemed necessary irrespective of the scaffold employed since compound **34** was inactive.

Table 2. HCV NS5B inhibitory activity of 3,4-dihydro-1*H*-pyrano[3,4-*b*]benzofuran derivatives



Compound	R^1	R^2	R^4	IC_{50} (μM)[9a]
34	*n*-Pr	H	H	>20
35	Me	Cl	Cl	>20
36	Et	Cl	Cl	16.3
22	*n*-Pr	Cl	Cl	1.49
37	*n*-Bu	Cl	Cl	4.46

The most potent inhibitor **19** from this SAR study was selected for further characterization. Compound **19** showed no inhibitory activity against a panel of human polymerases[9b] including mitochondrial DNA polymerase gamma, and other unrelated viral polymerases up to 50 μM, demonstrating its specificity for the HCV polymerase. It was found to be selective against helicase (IC_{50} = >75 μM) as well as HIV reverse transcriptase (IC_{50} = >100 μM). Compound **19** was not cytotoxic in rapidly dividing and stationary Vero and Huh7 cells as measured by a standard MTS metabolic assay (IC_{50} = >75 μM). Moreover, a single administration to Huh7 cells containing the HCV sub-genomic replicon for 3 days resulted in a dose-dependent reduction of the steady-state levels of viral RNA and protein (EC_{50} = 3.2 μM for HCV RNA).

In conclusion, we have explored 3,4-dihydro-1*H*-[1]-benzothieno[2,3-*c*]pyran and 3,4-dihydro-1*H*-pyrano[3,4-*b*]benzofuran derivatives as a novel class of HCV NS5B RNA dependent RNA polymerase inhibitors. The structure–activity requirement for this class of inhibitors seems to track very well with the pyrano[3,4-*b*]indole series. While 3,4-dihydro-1*H*-[1]-benzothieno[2,3-*c*]pyran scaffold displayed the same level of potency as that of pyrano[3,4-*b*]indoles, 3,4-dihydro-1*H*-pyrano[3,4-*b*]benzofurans were found to be much less potent. This can probably be attributed to the electronics of the central rings rather than steric, since the pyrrole and thiophene rings have a comparable degree of aromaticity.[10] Hence, 3,4-dihydro-1*H*-[1]-benzothieno[2,3-*c*]pyran scaffold presents itself as a novel chemotype specific for HCV polymerase with replicon activity for further exploration.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2005.08.114.

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6. HPLC Conditions: CHIRAL PACK-AD (250X20 mm) column, 10% isopropyl alcohol in heptane (0.1% TFA) eluant.
7. The enantiomeric excess (ee) of the separated enantiomers was in the range of 89–100%.
8. Stereochemical assignment for the isomers is tentative based on the earlier work carried out on pyrano[3,4-*b*]indoles (see Ref. 5).
9. (a) The recombinant C-terminally truncated NS5B enzyme used in the assay was derived from genotype 1b, BK strain. Inhibitors were pre-incubated with the enzyme for 15 min followed by an addition of an RNA template, NTPs, and [α-^{32}P]GTP. The reaction was carried out at room temperature for 2 h. Product RNA containing incorporated radioactive nucleotides was collected by filtration and the amount of radioactivity was quantified using a scintillation counter. The IC_{50} values reported are mean values for more than two independent measurements. Each assay plate contained at least one proprietary compound that has demonstrated in vitro and in vivo activity as positive inhibitor control; (b) Howe, A. Y. M.; Feld, B.; Bloom, J.; Gopalsamy, A.; Krisnamurthy, G.; Chunduru, S.; Young, D.; O'Connell, J. F. *Antimicrobial Agents Chemotherapy* **2004**, *48*, 4813.
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