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Design and synthesis of 2,3,4,9-tetrahydro-1*H*-carbazole and 1,2,3,4-tetrahydro-cyclopenta[*b*]indole derivatives as non-nucleoside inhibitors of hepatitis C virus NS5B RNA-dependent RNA polymerase

Ariamala Gopalsamy,[a,*] Mengxiao Shi,[a] Gregory Ciszewski,[a] Kaapjoo Park,[a] John W. Ellingboe,[a] Mark Orlowski,[b] Boris Feld[b] and Anita Y. M. Howe[b]

[a]*Chemical and Screening Sciences, Wyeth Research, Pearl River, NY 10965, USA*
[b]*Infectious Diseases, Wyeth Research, Pearl River, NY 10965, USA*

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Abstract—A novel class of HCV NS5B RNA dependent RNA polymerase inhibitors containing 2,3,4,9-tetrahydro-1*H*-carbazole and 1,2,3,4-tetrahydro-cyclopenta[*b*]indole scaffolds were designed and synthesized. Optimization of the aromatic region showed preference for 5,8-disubstitution pattern in both the scaffolds examined while favoring the *n*-propyl moiety for the C-1 position. 1,2,3,4-tetrahydro-cyclopenta[*b*]indole scaffold was slightly more potent than the corresponding 2,3,4,9-tetrahydro-1*H*-carbazole and analogue **36** displayed an IC_{50} of 550 nM against HCV NS5B enzyme.

Hepatitis C virus (HCV) is the major etiological agent of post-transfusion and sporadic non-A, non-B hepatitis.[1] HCV infection is one of the causes for liver cirrhosis and hepatocellular carcinoma leading to liver failure. Current estimates of approximately 2–3% of the word population as HCV carriers represent a significant medical problem with economic burden implications.[2] The present approved therapy involves pegylated interferon-α as a single agent or in combination with the broad-spectrum anti-viral ribavirin.[3] The severe side effects associated with these therapies significantly reduce patient's compliance. This approach is not aimed at any particular viral target. Prevalence of various HCV subtypes underscores the need for direct inhibition of viral targets.

HCV is a positive strand RNA virus and the genome consists of 9600 base pairs that encode several structural and non-structural proteins.[4] NS5B RNA-dependent RNA polymerase is a central enzyme in the viral replication cycle. Small molecule inhibitors of this target have received much attention in the recent past as potential therapeutic agents for treatment of HCV infection.[5] Both allosteric and active site inhibitors of NS5B polymerase have been reported. We recently reported[6a] pyrano[3,4-*b*]indole **1** 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 scaffold modification[6b] as well. As a first step we were interested in identifying the role of the pyran oxygen in **1**. Toward that end 2,3,4,9-tetrahydro-1*H*-carbazole **2** was considered. 1,2,3,4-tetrahydro-cyclopenta[*b*]indole **3** was chosen to shed more light on the ring size requirement for this region of the molecule (Fig. 1).

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[7] of the representative compound **12** for the 2,3,4,9-tetrahydro-1*H*-carbazole scaffold started from 2-allyl cyclohexanone **4**, which upon deprotonation followed by alkylation with

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* Corresponding author. Tel.: +1 845 602 2841; fax: +1 845 602 3045; e-mail: gopalsa@wyeth.com

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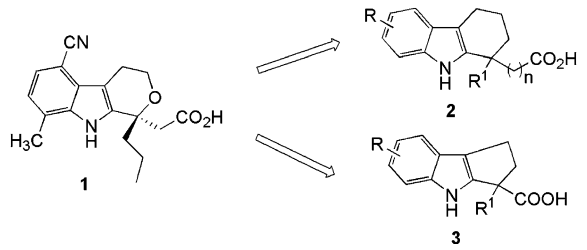
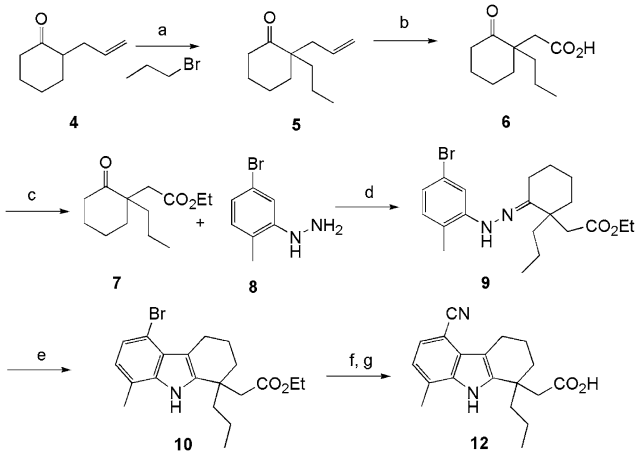


Figure 1. Scaffold substitution for pyrano[3,4-*b*]indole.



Scheme 1. Reagents and conditions: (a) NaH, toluene, 100 °C, 6 h, 68%; (b) RuO_2–$NaIO_4$, CCl_4–acetone, 0 °C to rt, 3 h, 77%; (c) EtI, Na_2CO_3, 8 h, 66%; (d) NaOAC, MeOH, 60 °C, overnight, 62%; (e) BF_3–Et_2O, HOAc, 100 °C, 2 h, 58%; (f) CuCN, NMP, microwave, 220 °C, 15 min, 70%; (g) 1 N NaOH, EtOH, THF, 8 h, 72%.

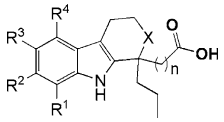
bromopropane afforded **5**. Ruthenium oxide–sodium metaperiodate oxidation of the allyl group gave the acid **6**. The acid was converted to the ester **7**, which was condensed with the required phenyl hydrazine **8** to give the hydrazone **9**. Lewis acid-catalyzed cyclization of **9** afforded the required tricyclic carbazole scaffold **10**. The aromatic bromo substituent was converted to cyano group under microwave condition and the ester was hydrolyzed to give the acid **12** of interest. The synthesis of 1,2,3,4-tetrahydro-cyclopenta[*b*]indole **3** was carried out in an analogous manner following Scheme 1.

Our initial efforts were focused on extrapolating the SAR that we had observed in the case of pyrano[3,4-*b*]indole to 2,3,4,9-tetrahydro-1*H*-carbazole scaffold. From our earlier SAR work around the pyranoindole molecule, C-1 *n*-propyl group was found to be ideal with some flexibility for the substituents in the aromatic region. Based on this knowledge we synthesized carbazole analog **13** incorporating the exact aromatic substituents as that of the pyranoindole **1**. The compound was found to be slightly less potent than the pyranoindole **1**, although it was tested as racemic mixture. Extending the study to other carbazoles with varying aromatic substituents did not improve the potency. However, the structure–activity correlation observed in the pyranoindole series was found to be extendable to the carbazole series.

A simple unsubstituted aromatic system **14**, 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. Mono chloro analog **15** or various difluoro analogs **16**–**18** did not satisfy the requirements. However, as observed previously for the pyranoindole series, a 5,8-disubstitution pattern was found to be regaining substantial amount of potency, with preference for a halogen or cyano group (analogs **13**, **20**, and **21** in Table 1). We also investigated the requirement for the acetic acid moiety in this series. On a direct comparison of analog **13** with analog **23**, where the acetic acid was replaced with formic acid, a 7-fold loss in potency was observed. However, by placing the appropriate 5,8-aromatic substituents equivalent potency could be regained even with formic acid (analog **26**) as the C-1 substituent.

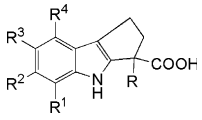
The second scaffold of interest, 1,2,3,4-tetrahydro-cyclopenta[*b*]indoles, was evaluated by using the most favorable aromatic substitutions for the C-5 and C-8 positions in the formic acid derivative as the basis for modification. The analogs explored are shown in Table 2. Comparing direct analogs **23** and **27** from both the scaffolds of interest, it was clear that there is an improved potency with 1,2,3,4-tetrahydro-cyclopenta[*b*]indole. This was further validated with analog **36**, which exhibited submicromolar potency compared to the carbazole analog **26**. However, the overall structure–activity relationship revealed by both scaffolds tracked well with that of the parent pyranoindole scaffold. Of the biased small set of C-1 alkyl groups investigated (*n*-propyl, *n*-butyl, and allyl), good tolerance was shown for these groups in this region. The most potent inhibitor **36** from this SAR study was selected for further characterization of its ability to inhibit hepatitis C virus replicon[9b] in human liver cell. Although the compound

Table 1. HCV NS5B inhibitory activity of 2,3,4,9-tetrahydro-1*H*-carbazole derivatives



Compound	R^1	R^2	R^3	R^4	X	*n*	IC_{50} (μM)[8,9]
1	Me	H	H	CN	O	1	0.57
13	Me	H	H	CN	CH_2	1	2.1
14	H	H	H	H	CH_2	1	>33
15	H	H	Cl	H	CH_2	1	>33
16	H	F	H	F	CH_2	1	30
17	F	F	H	H	CH_2	1	>33
18	F	H	H	F	CH_2	1	>33
19	Cl	H	Br	H	CH_2	1	>33
20	F	H	H	CN	CH_2	1	2.0
21	CN	H	H	CN	CH_2	1	2.3
22	$CONH_2$	H	H	CN	CH_2	1	>33
23	Me	H	H	CN	CH_2	0	15
24	F	H	H	CN	CH_2	0	10
25	Cl	H	H	CF_3	CH_2	0	5.8
26	Cl	H	H	Cl	CH_2	0	3.2

Table 2. HCV NS5B inhibitory activity of 1,2,3,4-tetrahydro-cyclopenta[*b*]indole derivatives



Compound	R	R^1	R^3	R^4	IC_{50} (μM)[8,9]
27	*n*-Pr	Me	H	CN	4.7
28	*n*-Butyl	Me	H	CN	4.6
29	Allyl	Me	H	CN	11.0
30	*n*-Pr	F	H	CN	8.1
31	*n*-Butyl	F	H	CN	5.4
32	Allyl	F	H	CN	14.5
33	*n*-Pr	Me	F	CN	1.8
34	*n*-Butyl	Me	F	CN	1.5
35	Allyl	Me	F	CN	6.3
36	*n*-Pr	Cl	H	Cl	0.55
37	*n*-Butyl	Cl	H	Cl	1.9
38	Allyl	Cl	H	Cl	1.2

was found to be permeable (PAMPA: 6.48×10^{-6} cm/s at pH 7.4), it showed modest activity ($IC_{50} = 27$ μM) in this assay, it showed a narrow therapeutic window with measurable cytotoxicity in the standard MTS metabolic assay ($IC_{50} = 43$ μM).

In conclusion, we have explored 2,3,4,9-tetrahydro-1*H*-carbazole and 1,2,3,4-tetrahydro-cyclopenta[*b*]-indole 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. 1,2,3,4-tetrahydro-cyclopenta[*b*]-indole scaffold is slightly more potent than the corresponding 2,3,4,9-tetrahydro-1*H*-carbazole and is a novel chemo type for HCV polymerase for further exploration.

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8. Compounds were purified by HPLC and the purity was >95%. Compounds were tested as racemic mixtures.
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; (b) Howe, A. Y. M.; Feld, B.; Bloom, J.; Gopalsamy, A.; Krishnamurthy, G.; Chunduru, S.; Young, D.; O'Connell, J. F. *Antimicrob. Agent Chemother.* **2004**, *48*, 4813.