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Further SAR Studies on Novel Small Molecule Inhibitors of the Hepatitis C (HCV) NS5B Polymerase

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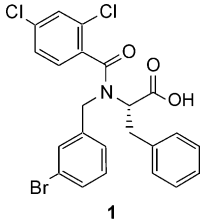
Abstract—Herein, we describe the structure–activity relationship (SAR) of *N,N*-disubstituted phenylalanine series of NS5B polymerase inhibitors of hepatitis C. The NS5B polymerase inhibitory activity of the most active compound exhibited an IC_{50} of 2.7 μM.

It is estimated that 170 million people worldwide are infected with Hepatitis C virus (HCV) including 4 million in the United States. Within 20 years of infection, about 4–5% of them will develop cirrhosis and hepatocellular carcinoma, often resulting in death.[1] The recommended current treatment, interferon α (IFN-α, 2a or 2b or a polyethylene glycol conjugate) in combination with ribavirin provides a sustained viral response in only 54–56% of the treated patients. However, for patients infected with HCV genotype 1a/b, the predominant genotype found in the USA, Japan and parts of Europe, the response is at best 42–46% and, furthermore, treatment is expensive and side effects can be severe.[2] The lack of an effective and well-tolerated treatment has therefore spurred intense research efforts to develop affordable, oral and novel anti-HCV agents.

HCV is a 9.6-kb positive strand RNA virus of the Flaviviridae, genus *hepacivirus* which encodes a 3011–3033 amino acid polyprotein variable in several genotypes.[3] This polyprotein is further processed into various structural (core, E1 and E2, p7) and non-structural (NS2, NS3, NS4A, NS4B, NS5A and NS5B) viral proteins by host and viral proteases.[4] The NS3 chymotrypsin-like protease and the NS5B RNA dependent RNA polymerase (RdRp) are probably the most studied targets for anti-HCV therapy as they are crucial for viral replication.[5] Despite intense research effort on NS3 protease, NS5B polymerase inhibitors started appearing recently in the patent literature.[6] Only, NS5B polymerase inhibitor (JTK-002/JTK-003) from Japan Tobacco Inc.[7] has been reported to be undergoing Phase II clinical trials.

We have recently reported the identification of a novel class of HCV NS5B polymerase inhibitors (e.g., **1**).[8] These *N,N*-disubstituted phenylalanine analogues displayed not only potent inhibition of HCV polymerase but also selectivity over human polymerases.[8] Furthermore, X-ray crystal structures of these inhibitors bound to NS5B polymerase enzyme revealed an allosteric site in the thumb region[8c] about 30 Å from the catalytic site and about 10 Å from the recently identified rGTP binding site.[9] As part of ongoing research on Hepatitis C programme,[8a] herein we disclose the detailed structure–activity relationship (SAR) of the benzamide portion of **1**.



1

The test compounds (e.g., **1**) were synthesized according to Scheme 1. Reductive amination of the C-protected L-phenylalanine with *m*-bromobenzaldehyde using $ZnCl_2$ and $NaCNBH_3$ in MeOH at room temperature gave the

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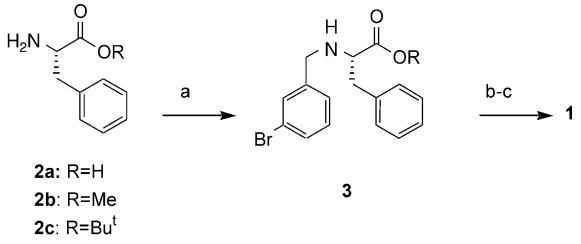
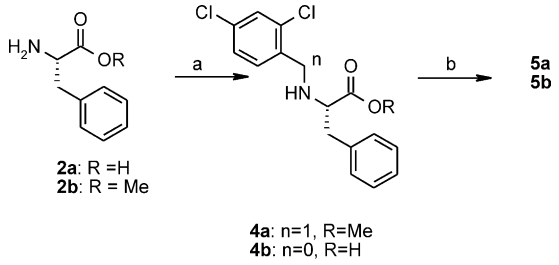
secondary amine in 70–80% yield. Acylation of the secondary amine was carried out using acid chloride with *N*-methylmorpholine (NMM) in CH_2Cl_2 in the presence of a catalytic amount of DMAP to provide the amides in 70–90% yields. Finally, deprotection of methyl esters with LiOH in dioxane and *tert*-butyl esters with TFA/CH_2Cl_2 (1:2, v/v) generated the test compounds in 80–90% yields.[10]

CuI catalyzed C–N coupling[11] of **2a** and 2,4-dichlorobromobenzene in DMF followed by selective *N*-alkylation of the resultant amino acid **4b** with *m*-bromobenzyl bromide gave compound **5b** (Scheme 2, Table 2). Reaction of 2,4-dichlorophenyl-isocyanate and **3** in DCE at 80 °C followed by hydrolysis of the resultant ester gave the desired urea (entry 19). Sulfonamide (entry 20) was synthesized from compound **3** and the corresponding sulfonyl chloride as described in Scheme 1.

As shown in Table 1, removal of the two chlorine atoms resulted in a significant loss of inhibitory activity (entry 2). A single chloro substituent (entries 3–5) or 2,4,6-trisubstitution is marginally tolerated (entry 7). There is, however, a clear preference for substituents both at *ortho* and *para* positions (entry 1). We have also examined other 2,4-disubstiuted benzamide analogues (entries 8–10). It appears that the only substitution tolerated at the para position is either chloro or methyl.

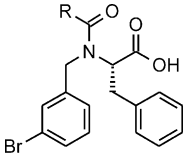


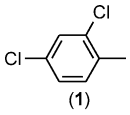
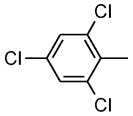
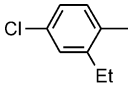
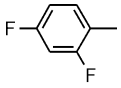
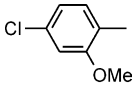
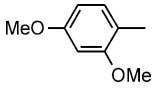
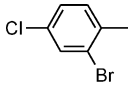
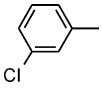
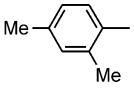
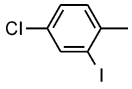
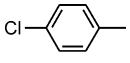
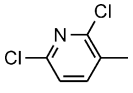
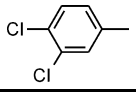
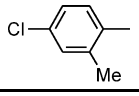
Scheme 1. Reagents and conditions: (a) *m*-bromobenzaldehyde, $NaCNBH_3$, THF, rt, 4 h; (b) acid chloride, NMM, cat DMAP, CH_2Cl_2, rt, 16 h; (c) for R = Me, LiOH, H_2O, 1,4-dioxane, rt; for R = Bu^t, TFA–CH_2Cl_2, rt, 4 h.



Scheme 2. Reagents and conditions: For **5a**: (a) **2b**, 2,4-dichlorobenzaldehyde, MS 4 Å, CH_2Cl_2, rt, 16 h, and $NaBH_4$, MeOH, 0 °C, 3 h, 50%; (b) (i) Cs_2CO_3, *m*-bromobenzylbromide, DMF, rt, 16 h, 45 °C, 5 h, 45%; (ii) LiOH, THF–MeOH–H_2O (3:2:1, v/v), rt, 2 h, 74%; For **5b**: (a) **2a**, 2,4-dichlorobromobenzene, CuI, K_2CO_3, DMF, 110 °C, 17 h, 32%; (b) NaH, *m*-bromobenzylbromide, THF, 0 °C, rt, 24%.

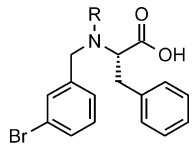
Table 1. Effect of benzamide substituents on NS5B polymerase activity



Entry	R	Poly IC_{50}[a]	Entry	R	Poly IC_{50}[a]	Entry	R	Poly IC_{50}[a]
1	 (1)	3.5	7		13	13[12]		5.6
2		>50	8		43	14		11
3		31	9		10.7	15		5.0
4		30	10		5.9	16		2.7
5		10.6	11		31			
6		30	12		3.7			

[a]The inhibitory effect (IC_{50}, μM) of test compounds on polymerization activity was performed using a Flashplate scintillation proximity assay.[8a,13]

Table 2. Effect of nitrogen-functionalized group on NS5B polymerase



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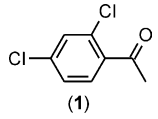
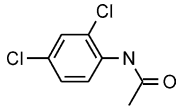
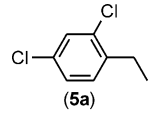
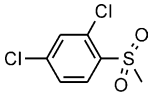
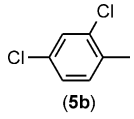
Entry	R	Poly IC_{50}[a]	Entry	R	Poly IC_{50}[a]
1	 (**1**)	3.5	19		31
17	 (**5a**)	26	20		31
18	 (**5b**)	15			

[a]The inhibitory effect (IC_{50}, μM) of test compounds on polymerization activity was performed using a Flashplate scintillation proximity assay.[8a,13]

For example, significant loss of activity was observed for small substituents, such as fluoro (entry 8), while approximately 2- to 3-fold-reduced potency were observed for dimethoxy and dimethyl benzamides, respectively (entries 9 and 10). A basic nitrogen in the dichlorobenzamide ring is also detrimental to activity (8-fold loss, entry 11).

Based on these observations it appeared that the *para* substituent is important for activity while having an extra substituent at the *ortho* position enhances potency. Thus, apparent additive effect of an extra *ortho* substituent on the polymerase activity was examined next (entries 12–16). Polymerase inhibitory activity was either slightly decreased or maintained with a variety of functional groups at the *ortho* position (entries 12–16). The *ortho* position seems to be quite tolerant since moderately bulky groups (Cl, Me, Br, and I) increase the potency (entries 1 and 12–16).

The effect of a non-amidic link to phenylalanine nitrogen on NS5B polymerase activity was also examined (Table 2). Removal of the amide functionality resulted in an 8-fold loss of activity (entry 17). Direct linkage of the 2,4-dichlorophenyl moiety also diminishes the activity (entry 18). The activity was considerably decreased by replacing the 2,4-dichlorobenzamide with other functional groups such as urea and sulfonamide (entries 19 and 20). The mechanism by which this compound inhibits NS5B polymerase is being actively examined. Further optimization of **1** is in progress and will be reported in due course.

A SAR study on the benzamide portion of a newly identified anti-NS5B polymerase lead is described. A hydrophobic chlorine atom at the *para* position of the benzamide is a requisite for activity. Having an extra hydrophobic atom (Cl, Me, Br, I) at the *ortho* position enhances potency whereas substitution at the *meta* position diminishes the activity. Of all the *N*-functionalities examined, the amide was found optimal.

Acknowledgements

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