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Further studies on hepatitis C virus NS5B RNA-dependent RNA polymerase inhibitors toward improved replicon cell activities: Benzimidazole and structurally related compounds bearing the 2-morpholinophenyl moiety ☆

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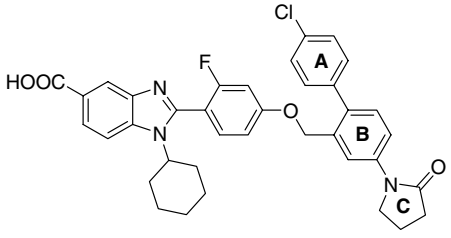
Abstract—Following the discovery of JTK-109 (**1**) as a potent inhibitor of hepatitis C virus NS5B RNA-dependent RNA polymerase, [(a) Hirashima, S.; Suzuki, T.; Ishida, T.; Noji, S.; Yata, S.; Ando, I.; Komatsu, M.; Ikeda, S.; Hashimoto, H. *J. Med. Chem.* **2006**, *49*, 4721. (b) Hashimoto, H.; Mizutani, K.; Yoshida, A. Int. Patent Appl. WO 01/47883, 2001.] further studies toward the improvement of the cellular potency have been performed. A greater than 40-fold improvement was achieved through replacing the biphenyl moiety with a 2-morpholinophenyl group and the benzimidazole ring with the tetracyclic scaffold to afford compound **7** with an excellent replicon potency (EC_{50} = 7.6 nM).

Hepatitis C virus (HCV) is a common pathogen that infects an estimated 170 million people worldwide and that can lead to liver cirrhosis, carcinoma, and hepatic insufficiency.[2] The standard therapy is based on pegylated interferon-α and ribavirin, a treatment course that is not necessarily effective for the patients infected with the most prevalent HCV genotype 1 virus and that is frequently associated with severe adverse events.[3] Therefore, there is an unmet clinical need for novel anti-HCV agents, especially against genotype 1 HCV. Among the encoded HCV non-structural proteins, HCV NS5B RNA-dependent RNA polymerase (RdRp) is an attractive target for drug development because it plays a vital role in HCV viral replication,[4] and because corresponding RdRps are not known in mammalian systems. Many nucleoside and non-nucleoside inhibitors against NS5B have been described.[5]

We have recently reported a potent NS5B inhibitor, JTK-109 (**1**, Fig. 1), with a low nanomolar biochemical potency and a submicromolar cellular potency.[1a] However, a relatively large 'shift in potency between biochemical assay and cellular assay' (EC_{50}/IC_{50} = 19, abbreviated as *shift* in this paper) was observed. Although cellular activity is generally influenced by factors such as membrane permeability, metabolism, and affinity for proteins, the elevated binding of **1** to protein



JTK-109 (**1**)
IC_{50}(NS5B:1b) = 0.017 μM
EC_{50}(replicon) = 0.32 μM
EC_{50}/IC_{50} = 19

Figure 1. Structure of JTK-109 (**1**).

Keywords: HCV; NS5B polymerase; Inhibitor.
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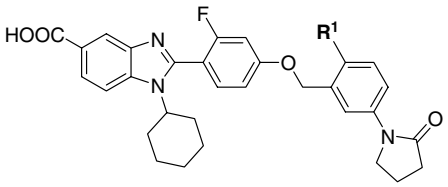
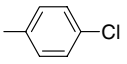
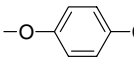
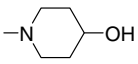
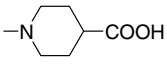
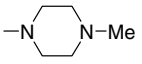
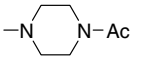
(>99% in 10% FCS employed in the replicon cell assay) was considered as the major factor for the large *shift*. The hydrophobic biphenyl substructure is known to promote the increased binding affinity to a wide range of proteins, especially albumin.[6] Indeed, almost all of the compounds in the previous benzimidazole series bearing substituted biphenyl groups showed greater than 20-fold *shift*s. Thus, replacement of the biphenyl moiety with a non-biphenyl substructure is clearly necessary to reduce the *shift*, which may lead to more potent inhibition in the replicon cells. Another aspect of increasing the cellular potency is the improvement of the biochemical potency, which could be achieved by changing the benzimidazole ring to the other structures.

Table 1. NS5B enzyme assay IC_{50} values and replicon cell-based assay EC_{50} values for compounds **2** (A-part variation)



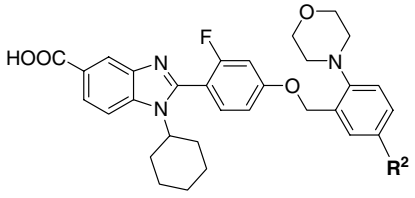
Compound	R^1	NS5B IC_{50}[a] (μM)	Replicon EC_{50}[a] (μM)	EC_{50}/IC_{50}
1		0.017	0.32	19
2a		0.23	3.1	13
2b	OMe	0.41	1.4	3.4
2c		0.15	0.80	5.3
2d		0.15	1.4	9.3
2e		0.18	3.5	19
2f		0.13	1.1	8.5
2g		0.11	0.95	8.6
2h		0.066	0.31	4.7
2i		1.0	>30	>30
2j		0.26	1.3	5.0

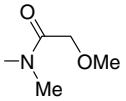
[a] IC_{50} and EC_{50} values in all tables are means of at least two independent determinations, standard deviation ±30%.

Herein, we report our results toward highly potent compounds in the replicon cells through the above strategies. This work led to the identification of compound **7** with a single digit nanomolar potency (EC_{50} = 7.6 nM).

In our search for a non-biphenyl substructure, we initially focused on the A-part of **1** (Table 1).[7] Compounds **2a**, **b** were synthesized to deform the shape of the biphenyl functionality. Insertion of the ether linker between the two phenyl rings (**2a**) and replacement of the aromatic ring with a methoxy group (**2b**) decreased potency 10-fold against NS5B compared to **1**. The biphenyl ether **2a** exhibited no improvement in the *shift*, however, the *shift* of **2b** was enormously reduced (EC_{50}/IC_{50} = 3.4). We therefore speculated that the aromatic property of the A-part has a deleterious effect on the *shift*, while a ring structure directly connected to the B-ring is necessary to maintain the biochemical potency, and hence we implemented replacement of the phenyl ring with an aliphatic ring. Substitution with piperidine (**2c**) yielded a compound with a modest improvement in the biochemical potency over **2a** or **2b**, but still worse than **1**. However, the *shift* (EC_{50}/IC_{50} = 5.3) was much improved. Introduction of either a hydroxyl (**2d**) or carboxylic acid (**2e**) on the piperidine, or replacement with piperazines (**2f**, **g**), gave compounds with equal biochemical potency, but with larger *shift*s than **2c**. On the other hand, the morpholine variant (**2h**) exhibited

Table 2. NS5B enzyme assay IC_{50} values and replicon cell-based assay EC_{50} values for compounds **3** (C-part variation)



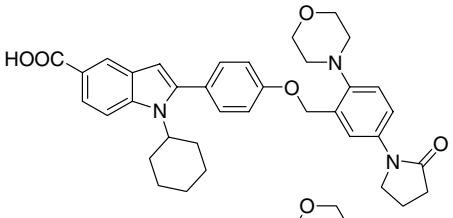
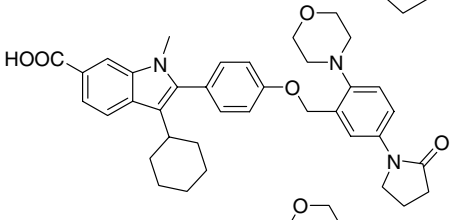
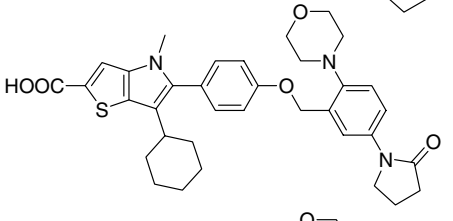
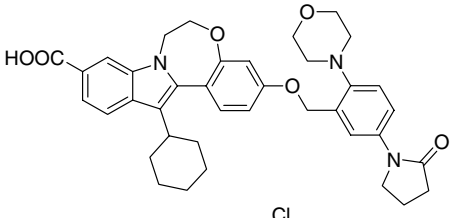
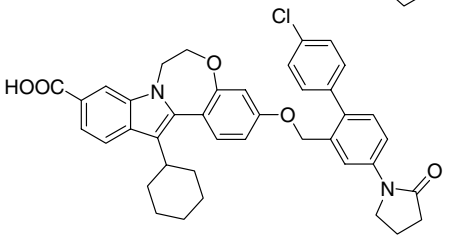
Compound	R^2	NS5B IC_{50} (μM)	Replicon EC_{50} (μM)	EC_{50}/IC_{50}
2h		0.066	0.31	4.7
3a	H	0.13	1.0	7.7
3b	Cl	0.10	1.2	12
3c		0.071	0.55	7.7
3d		0.090	0.58	6.4
3e	NMeAc	0.051	0.37	7.3
3f	NHAc	0.070	0.91	13
3g		0.099	1.2	12
3h	N*i*-PrAc	0.042	0.27	6.4
3i	$CONMe_2$	0.068	0.44	6.5
3j	CONH*i*-Pr	0.048	0.38	7.9

~threefold improved biochemical potency compared to **2c** with a similar *shift* (EC_{50}/IC_{50} = 4.7). This compound was as potent as **1** in the cellular assay, although the biochemical potency was ~fourfold less than **1**. The protein binding of **2h** was evaluated and found to be 90%, suggesting that the decreased *shift* may be attributed to the decreased affinity for proteins. Two structurally related compounds, **2i** and **2j**, were synthesized to confirm the appropriateness of the morpholine group. Lactam **2i** caused a negative effect on the biochemical potency and the *shift*. The non-ring compound **2j** afforded a fourfold decrease in the biochemical potency compared to **2h**, although the *shift* was comparable.

Employing the morpholin-4-yl group as the A-part of the structure, we turned our attention to the C-part of the parent compound. Since our previous structure–activity relationship studies showed that carbonyl/sulfonyl functional groups at the C-part afforded superior biochemical and the cellular potencies,[1a] those substituents (**3c**–**j**) were mainly examined in this series (Table 2). They showed NS5B inhibitory activities similar to **2h** and were slightly more potent than **3a** (no substituent) or **3b** (non-carbonyl substituent). Compared to the previous series, the effects of the carbonyl functional groups were modest. While several compounds (**3e**, **h**–**j**) were as potent as **2h** in the replicon assay, none of them showed increased cellular potencies.

Although the reduction of the *shift* was achieved by replacing the phenyl ring (A-part) with a morpholine ring, we sacrificed a fourfold loss in the biochemical potency. The initial purpose, improvement of the cellular potency, remained. Thus, we attempted to enhance the biochemical potency by changing the benzimidazole ring to the other structures. In several related inhibitors, substitution of indole,[8] thienopyrrole,[9] or tetracyclic scaffolds[10] for the benzimidazole moiety afforded more potent inhibition in the biochemical assay. We incorporated these same structures into compound **2h** and synthesized compounds **4**–**7**. All of them exhibited improved biochemical and cellular potencies compared to **2h** while retaining smaller *shift*s (Table 3). The rank order of the biochemical potencies generally correlated

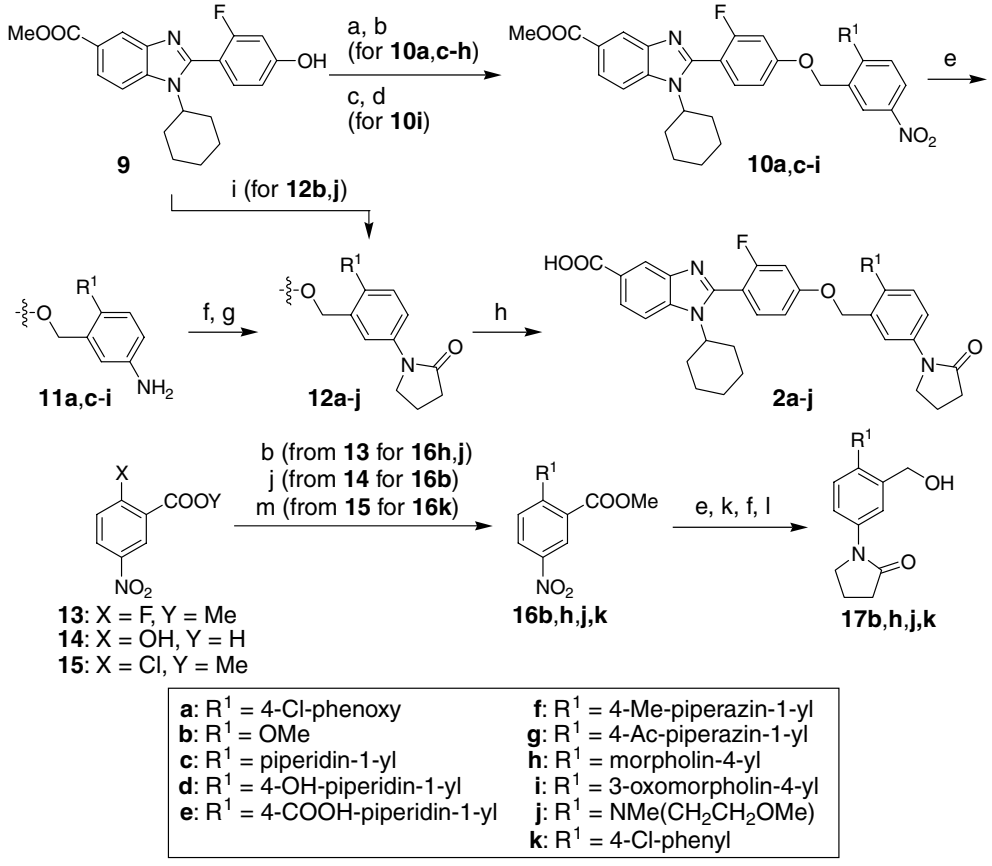
Table 3. NS5B enzyme assay IC_{50} values and replicon cell-based assay EC_{50} values for compounds **4**–**8**

Compound		NS5B IC_{50}(μM)	Replicon EC_{50}(μM)	EC_{50}/IC_{50}
4		0.030	0.21	7.0
5		0.0072	0.052	7.2
6		0.010	0.052	5.2
7		0.0072	0.0076	1.1
8		0.019	0.060	3.1

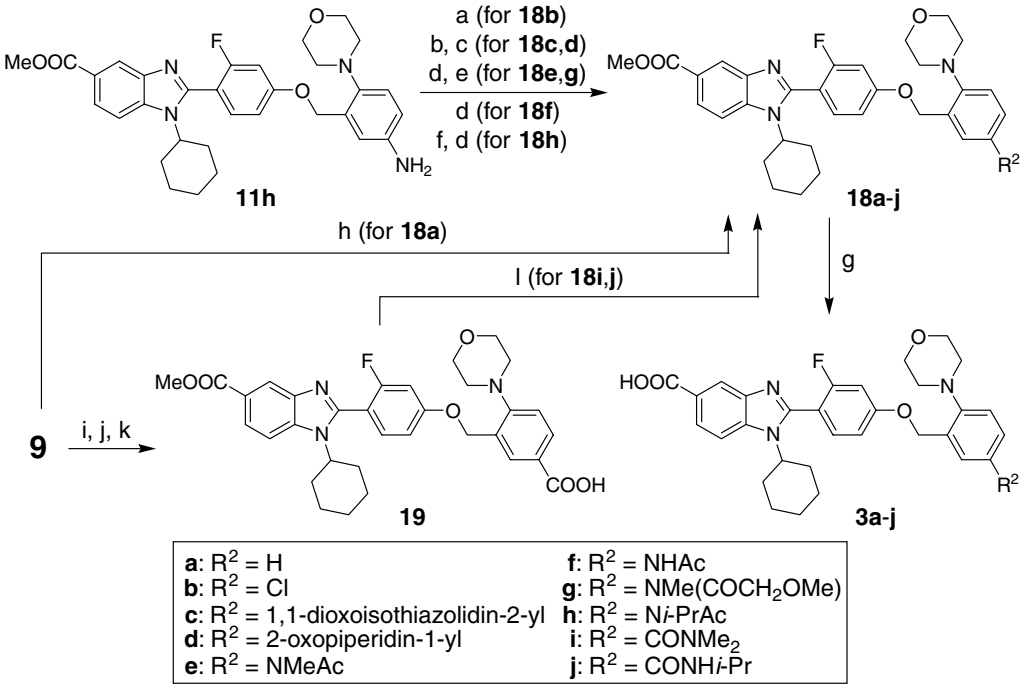
with the order for the scaffolds reported in the literature.[8c,9a,10] The cellular potency increased in parallel with the increased biochemical potency. Notably, the tetracyclic compound **7** showed a significantly improved cellular potency (EC_{50} = 7.6 nM) with no *shift* (EC_{50}/IC_{50} = 1.1). Thus, a 42-fold of improvement in the replicon cellular potency was achieved. To verify the effect of the morpholine group, the biphenyl compound **8** was prepared. A threefold larger *shift* compared with **7** was observed, as was the case in benzimidazole (**1** vs **2h**). In contrast to the case of benzimidazole, the morpholine group did not lose the biochemical potency. The tetracyclic ring also seems to be significantly effective in reducing the *shift*, judging by the fact that the *shifts* of **7** and **8** were five- to sixfold smaller than those of **2h** and **1**, respectively. All compounds in this study showed low cytotoxicity (CC_{50} > 20 μM).

The compounds evaluated in this study were synthesized as described in Schemes 1–3 . The syntheses of **2a**–**j** were started from the phenol **9**,[1b] which was converted to **10** in two different ways: (i) for **10a**, **c**–**h**, alkylation by Mitsunobu reaction with 2-fluoro-5-nitrobenzylalcohol, and subsequent amination with the corresponding amines or alkoxylation with 4-chlorophenol; (ii) for **10i**, alkylation with 2-bromo-5-nitrobenzylbromide and subsequent Pd-mediated amination. The nitro group of **10** was reduced with iron to give the aniline **11**. Acylation with 4-chlorobutyryl chloride and the subsequent intramolecular alkylation afforded the lactams **12a**, **c**–**i**. Compounds **12b**, **j** were directly prepared from **9** by Mitsunobu coupling with **17b**, **j**. The benzylalcohols **17** were derived from the esters **16** in four steps: reduction of the nitro group with iron; $LiAlH_4$ reduction of the methyl ester; acylation of the aniline with 4-chlorobutyryl chloride; and cyclization to form the lactam ring. Compounds **16** were synthesized from **13**, **14**, or **15** by either amination (**16h**, **j**), alkylation (**16b**), or Suzuki coupling (**16k**). Finally, hydrolysis of the methyl esters of **12** gave **2**.

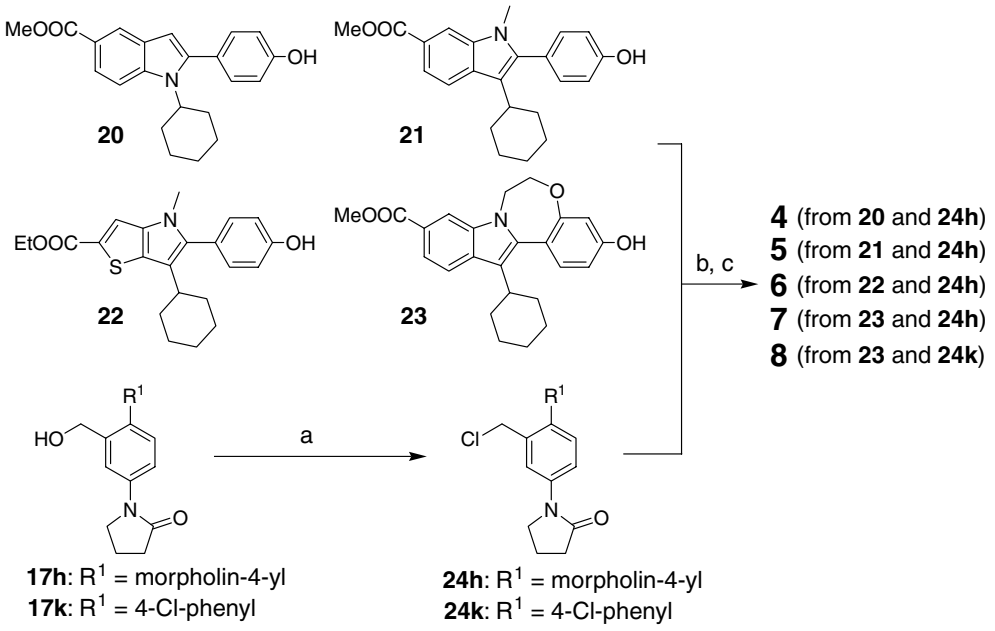
As shown in Scheme 2, **3a**–**j** were synthesized from either aniline **11h** or the phenol **9** via **18**. The aniline **11h** was converted to **18b**–**h** in five different ways: (i) for **18b**, replacement of the aniline to the chloride by Sandmeyer reaction; (ii) for **18c**, **d**, acylation/sulfonylation and subsequent cyclization; (iii) for **18e**, **g**, acylation with the corresponding acylchlorides and subsequent *N*-alkylation with MeI; (iv) for **18f**, acetylation; (v) for **18h**, reductive amination with acetone in the presence of $NaBH(OAc)_3$ and subsequent acetylation. Compound **18a** was directly prepared from the phenol **9** by Mitsunobu reaction. Compounds **18i**, **j** were derived from **9** via the carboxylic acid **19**, which was synthesized in three-step sequence: alkylation of the phenol; Pd-medi-



Scheme 1. Reagents and conditions: (a) 2-fluoro-5-nitrobenzylalcohol, DIAD, PPh_3, THF, rt; (b) amines or 4-chlorophenol, K_2CO_3, DMSO, 80 °C; (c) 2-bromo-5-nitrobenzylbromide, K_2CO_3, DMF, 80 °C; (d) morpholin-3-one, $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, 1,4-dioxane, reflux; (e) Fe, NH_4Cl, THF–EtOH–H_2O, reflux; (f) 4-chlorobutyryl chloride, AcONa, AcOH, THF, rt; (g) K_2CO_3, DMF, 65 °C; (h) 4 N aq. NaOH, THF–MeOH–H_2O, 60 °C; (i) **17**, DIAD, PPh_3, THF, rt; (j) MeI, K_2CO_3, acetone, reflux; (k) $LiAlH_4$, THF, 0 °C; (l) KOH, EtOH–H_2O, 80 °C; (m) 4-Cl-PhB$(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, DME–H_2O, reflux.



Scheme 2. Reagents and conditions: (a) *tert*-BuONO, $CuCl_2$, MeCN, 0 °C then rt; (b) $Cl(CH_2)_4COCl$ or $Cl(CH_2)_3SO_2Cl$, AcONa, AcOH, THF, rt; (c) K_2CO_3, DMF, 60 °C; (d) AcCl or $MeOCH_2COCl$, Et_3N, $CHCl_3$, 0 °C then rt; (e) NaH, MeI, DMF, 0 °C then rt; (f) acetone, $NaBH(OAc)_3$, THF, rt; (g) 4 N aq. NaOH, THF–MeOH–H_2O, 60 °C; (h) 2-morpholin-4-ylbenzylalcohol, DIAD, PPh_3, THF, rt; (i) 2-bromo-5-*tert*-butoxycarbonylbenzylbromide, K_2CO_3, 60 °C; (j) morpholine, $Pd_2(dba)_3$, BINAP, Cs_2CO_3, toluene, 100 °C; (k) TFA, $CHCl_3$, rt; (l) amines, PyBop®, Et_3N, DMF, rt.



Scheme 3. Reagents and conditions: (a) $SOCl_2$, Et_3N, $CHCl_3$, 0 °C; (b) phenol (**20**, **21**, **22**, or **23**), K_2CO_3, DMF, 60 °C; (c) 4 N aq. NaOH, THF–MeOH–H_2O, 60 °C.

ated amination; and deprotection of the *tert*-butyl group. Coupling between **19** and the corresponding amines using PyBop® provided **18i**, **j**. The esters **18** were hydrolyzed to give **3**.

As shown in Scheme 3, compounds **4**–**7** and **8** were synthesized from benzylalcohols **17h** or **17k**, respectively, in three steps: conversion of the hydroxyl to the chloride (**24h**, **k**); coupling with the phenols (**20**,[8c] **21**,[8d] **22**,[9b] and **23**[10]); and subsequent hydrolysis of the methyl esters.

In conclusion, we have achieved the improvement of the replicon cellular potency (42-fold in comparison with **1**) through reducing the shift between the biochemical and the cellular potencies and improving the biochemical potency. Reduction of the *shift* was attained by replacing

the biphenyl moiety with a 2-morpholinophenyl ring and the benzimidazole ring with the tetracyclic scaffold. A further increase in biochemical potency occurred by substituting the tetracyclic scaffold for the benzimidazole ring.

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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.2007.03.027.

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