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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 1859–1863

# Benzimidazole inhibitors of hepatitis C virus NS5B polymerase: Identification of 2-[(4-diarylmethoxy)phenyl]-benzimidazole

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Received 29 November 2005; revised 29 December 2005; accepted 4 January 2006
Available online 7 February 2006

**Abstract**—A series of 1-cycloalkyl-2-phenyl-1*H*-benzimidazole-5-carboxylic acid derivatives was synthesized and evaluated for inhibitory activity against HCV NS5B RNA-dependent RNA polymerase (RdRp). A SAR study was performed and led to identify the 2-[(4-diarylmethoxy)phenyl]-benzimidazoles as potent inhibitors. They inhibit subgenomic HCV RNA replication in the replicon cells at low micromolar concentrations ($EC_{50}$ as low as 1.1 μM). They are selective against DNA polymerases ($IC_{50}$ > 10 μM) and exhibit low cytotoxicity.


Hepatitis C is an infectious disease caused by hepatitis C virus (HCV).[1] It is estimated that more than 170 million people in the world are chronically infected.[2] About 20% of the chronically infected people develop cirrhosis. Moreover, most hepatocellular carcinomas are caused by HCV infection. The currently available drug for HCV is a cytokine, IFN-α.[3–5] But the problems of IFN-based therapy are low efficacy and side effects. Even in the recent combination therapy of pegylated IFN-α and ribavirin, the sustained viral response rate is under 50% in the patients infected with the most widespread genotype 1 virus. In spite of the low efficacy, adverse events are seen in almost all patients. Therefore, there is urgent and strong medical demand for the development of new, effective and well-tolerated anti-HCV drugs.

HCV is an enveloped single strand RNA virus in the Flaviviridae family and encodes a polyprotein chain of about 3000 amino acids, which is processed into structural and non-structural (NS) proteins.[3] NS5B RdRp, one of the NS proteins, plays a central role in the replication of HCV and therefore is an attractive target for drug development.[3,6] Many groups have ongoing research programs to find inhibitors of NS5B. Several classes of nucleoside and non-nucleoside inhibitors have been reported recently.[3,6–9] We discovered a new class of benzimidazoles.[7,8] In this paper, we report the synthesis and SAR studies of a series of 1-cycloalkyl-2-phenyl-1*H*-benzimidazole 5-carboxylic acids as NS5B inhibitors and also report anti-HCV activity of the 2-[(4-diarylmethoxy)phenyl]-benzimidazoles in a cell-based assay.

We screened our in-house compound collection to identify NS5B (genotype 1b) inhibitors and found a benzimidazole derivative **1** (Fig. 1) as one of the hit compounds (47% inhibition at 10 μM). Initial structural optimization of the amide part of **1** led to a more potent carboxylic acid **2** ($IC_{50}$ = 3.2 μM). We selected this compound **2** for further SAR studies and structural optimization.

The lead compound **2** and its cyclohexyl or cycloheptyl analogues **13** and **14** were synthesized as shown in Scheme 1. Ethyl 4-fluoro-3-nitrobenzoate, **3**, was reacted

*Keywords*: HCV; NS5B polymerase; Inhibitor; Benzimidazole.
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doi:10.1016/j.bmcl.2006.01.032

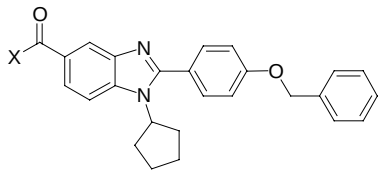


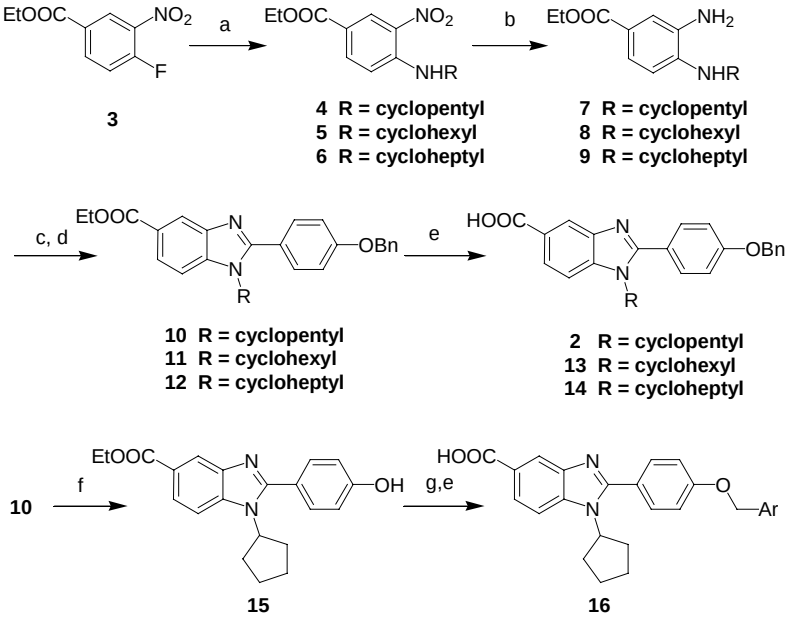

**Figure 1.**

with appropriate cycloalkylamine to give the corresponding anilines **4**–**6**. The nitro group was hydrogenated to afford diaminobenzenes **7**–**9** which were converted to the benzimidazoles by acylation with 4-benzyloxybenzoyl chloride and subsequent heating in AcOH.[10] Hydrolysis of ester gave the benzimidazole 5-carboxylic acids **2**, **13** and **14**. Benzimidazoles **16** bearing a substituent on the benzyl group of compound **2** were prepared from compound **10** (Scheme 1). The benzyl group was removed by hydrogenolysis to give a phenol **15**. Alkylation of the phenol and subsequent hydrolysis of the ester group afforded compound **16**.

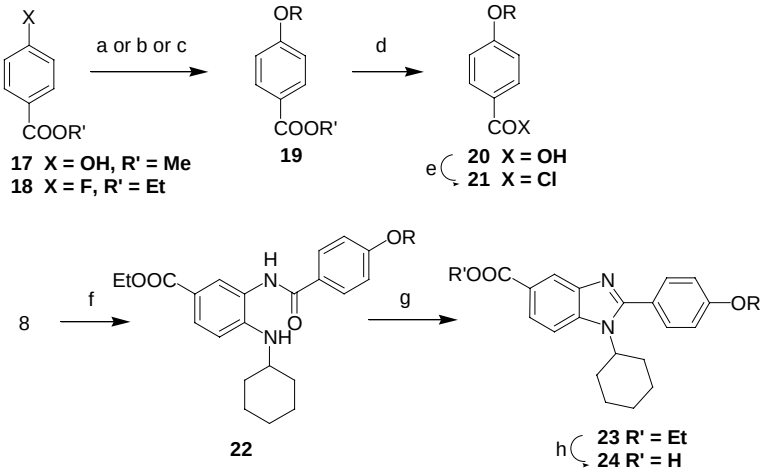
Benzimidazoles **24** were prepared by essentially the same method as employed in the synthesis of the benzyloxy version **13** (Scheme 2). Two biphenyl compounds, **25** and **26**, were prepared from the corresponding bromide in two steps by Suzuki coupling[11] and following hydrolysis as shown in Scheme 3. The diarylmethyl ethers, **33** and **34**, were synthesized by using the imidate coupling route from the diamine **8** as shown in Scheme 4. The diamine **8** was reacted with imidate **29** or **30** to give the benzimidazole **31** or **32**, respectively.[12] The imidates were prepared from the corresponding 4-hydroxybenzonitrile. Alkylation of the phenol group of **31** and **32** with various diarylmethylhalides and subsequent hydrolysis of the ester group afforded **33** and **34**, respectively. All the compounds assayed in this article were characterized by $^1$H NMR, MS and elemental analysis.

The benzimidazole 5-carboxylic acids synthesized in this study were evaluated for their ability to inhibit HCV NS5B RdRp activity. While there are several genotypes in HCV, our assay employed genotype 1 enzymes. All compounds were examined against a genotype 1b enzyme lacking C-terminal 47 residues (1b $NS5B_{544}$),[13] and the results are summarized in Tables 1–4. The compounds in Table 4 were also evaluated for another subtype 1a enzyme (1a $NS5B_{544}$), and we examined their ability to inhibit the replication of subgenomic HCV RNA in a replicon cell system using a Huh-5-2 cells (a Huh-7 derived cell line which possesses a 1b HCV replicon containing the luciferase reporter gene).[14]

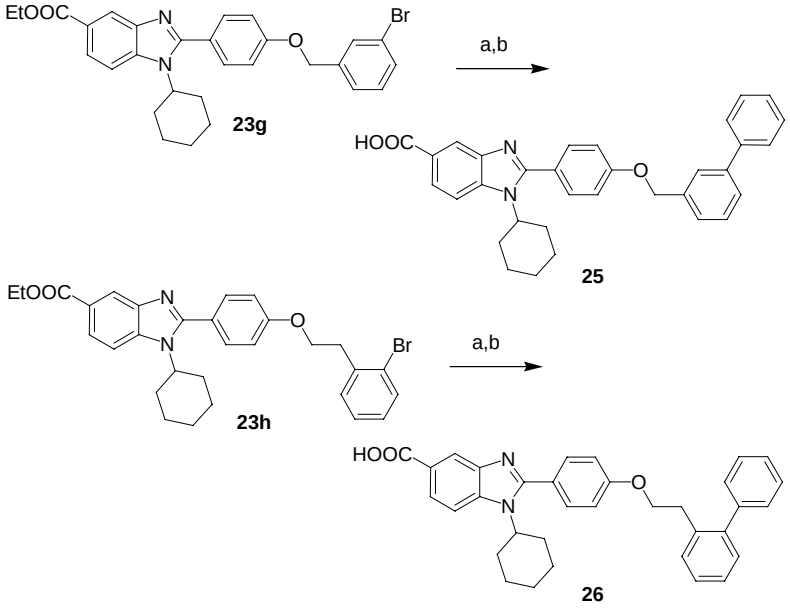
We first examined the cyclopentyl ring part and the effect of substituents on the benzyl moiety of the lead compound **2**. As seen in Table 1, an expansion of the ring size to cyclohexyl (**13**) showed 2-fold increased activity. Further ring expansion to a seven-membered ring (**14**) retained the activity. It seems that a six-membered ring optimally fits to a hydrophobic pocket.[15] Introduction of the lipophilic substituents such as Me, $CF_3$, *t*-Bu and Cl (**16a**–**c**,**e**) at the *para* position slightly increased the potency, while an ionic substituent COOH (**16d**) decreased activity (Table 2). Changing the benzene ring to 4-pyridine (**16g**) could not improve the activity. Changing the position of Cl group (**16h** and **i**) or introduction of another Cl atom (**16j** and **k**) did not afford further increase in the potency. Thus, an attempt to increase the activity by introducing a substituent on the benzyl moiety brought an unsatisfactory result. Therefore, we next tried to seek a more attractive structure for optimization instead of the benzyl group.




**Scheme 1.** Reagents and conditions: (a) $RNH_2$, $K_2CO_3$, DMSO, rt; (b) $H_2$ (1 atom), Pd/C, EtOH; (c) 4-benzyloxybenzoyl chloride, cat. DMAP, pyridine–$CH_2Cl_2$, rt; (d) AcOH, reflux; (e) 4 N aq NaOH, EtOH–THF, reflux; (f) $H_2$ (1 atom), Pd/C, AcOH; (g) $ArCH_2Br$, $K_2CO_3$, DMF, 85 °C.



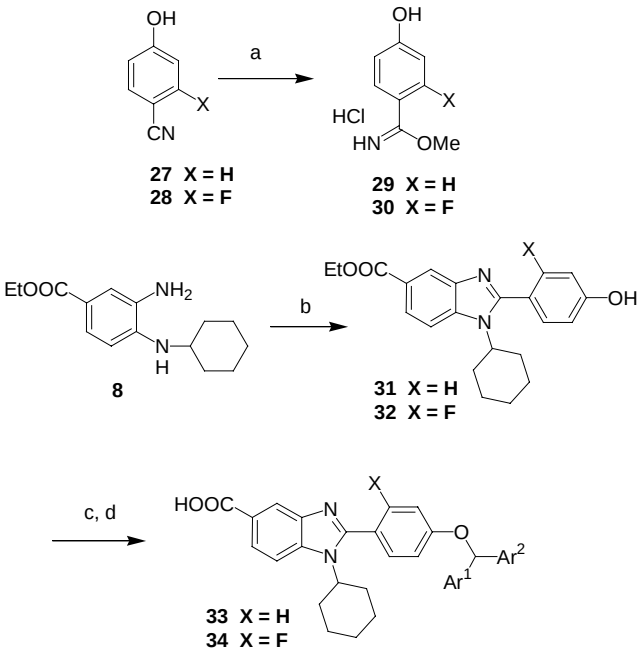

**Scheme 2.** Reagents and conditions: (a) ROH, $PPh_3$, DIAD, THF, 0 °C then rt; (b) RX, NaH, DMF, 0 °C then rt; (c) ROH, $K_2CO_3$, DMSO, 130 °C; (d) 4 N aq NaOH, MeOH–THF, reflux; (e) $(COCl)_2$, $CH_2Cl_2$, rt; (f) **21**, pyridine, rt; (g) AcOH, reflux; (h) 4 N aq NaOH, EtOH–THF, reflux.




**Scheme 3.** Reagents and conditions: (a) $PhB(OH)_2$, cat. $Pd(PPh_3)_4$, $NaHCO_3$, DME–$H_2O$, reflux; (b) 4 N aq NaOH, EtOH–THF, reflux.

Several types of ether were examined, and the results are shown in Table 3. In this series, cyclohexyl group was employed. Elongation of the alkyl chain from benzyl to phenylpropyl, phenylpentyl or phenoxyethyl (**24a**–**c**) did not give much influence on the potency. Although the three-dimensional structure of the binding site of the compounds investigated in this article is not known, we estimated that there is enough space around the benzyl moiety and we might be able to use the space to increase the affinity. So, we added another benzene ring on the benzyl part of **13**. Biphenylmethyl (**24e** and **25**) and diphenylmethyl (**33a**) derivatives showed 2- to 4-fold increase in potency. Lengthening the methylene chain of **24e** to that in **26** showed a similar potency, while a shorter one in **24f** gave a 2-fold decrease. Diphenylethyl **24d** slightly reduced the potency compared to diphenylmethyl, **33a**. With these results, we selected the diphenylmethyl type of compound **33a** for further study.[16]

A series of diarylmethyl derivatives **33** and **34** listed in Table 4 was synthesized and evaluated for activity against the 1b and 1a NS5B enzymes. In general, these derivatives are potent inhibitors for both enzymes and more potent against the 1b subtype. SARs against both enzymes seem to be roughly correlated, although SAR for 1a enzyme is more flat. Introduction of substituents such as Me, Cl and F (**33b**–**e**) on the diphenylmethyl part slightly decreased the potency. We considered that introduction of a substituent on the central phenyl ring connected to the benzimidazole ring might have an influence on the potency. Introduction of a fluorine atom (**34a** and **b**) achieved a 2- to 6-fold increase in potency against the 1b compared with the corresponding parent compound (**33a** and **d**), while no or a slight increase was seen against 1a. At this point, these compounds were tested in the replicon assay (Table 5). They efficiently blocked the replication of subgenomic HCV RNA at




**Scheme 4.** Reagents and conditions: (a) AcCl, MeOH, 0 °C then rt; (b) **29** or **30**, MeOH, reflux; (c) $Ar_1Ar_2CHBr$, $K_2CO_3$, DMF, 80 °C; (d) 4 N aq NaOH, EtOH–THF, reflux.

**Table 1.** In vitro 1b NS5B enzyme inhibition activity of 1-cycloalkyl-2-(4-benzyloxy)phenylbenzimidazole-5-carboxylic acid derivatives

| Compound | R | 1b $IC_{50}$[a] (μM) |
|---|---|---|
| **2** | Cyclopentyl | 3.2 ± 0.75 |
| **13** | Cyclohexyl | 1.4 ± 0.63 |
| **14** | Cycloheptyl | 1.8 ± 0.34 |

[a] Values are means ± SD determined from three separate experiments.

**Table 2.** In vitro 1b NS5B inhibitory activity of 1-cyclopentyl derivatives **16**

| Compound | Ar | 1b $IC_{50}$[a] (μM) |
|---|---|---|
| **2** | Ph | 3.2 ± 0.75 |
| **16a** | 4-Me-Ph | 1.6 ± 0.18 |
| **16b** | 4-$CF_3$-Ph | 1.8 ± 0.54 |
| **16c** | 4-*t*-Bu-Ph | 1.5 ± 0.42 |
| **16d** | 4-(COOH)-Ph | 6.7 ± 0.15 |
| **16e** | 4-Cl-Ph | 1.2 ± 0.37 |
| **16f** | 4-OMe-Ph | 2.1 ± 0.98 |
| **16g** | 4-Pyridyl | 4.2 ± 1.0 |
| **16h** | 2-Cl-Ph | 2.3 ± 0.78 |
| **16i** | 3-Cl-Ph | 1.6 ± 0.26 |
| **16j** | 3,5-Cl-Ph | 1.7 ± 0.22 |
| **16k** | 3,4-Cl-Ph | 1.3 ± 0.19 |

[a] Values are means ± SD determined from three separate experiments.

low micromolar concentrations and showed weak cytotoxicity. Although the potency against the 1b enzyme improved (**34a** and **b**), the cellular potency was not as expected, suggesting that the highly lipophilic property of these molecules had to be changed. Introduction of a polar substituent, *N*,*N*-dimethylamido (**34c**) or replacement of the benzene ring with pyridine (**34d** and **e**), showed 3- to 10-fold reduced potency against

**Table 3.** In vitro 1b NS5B inhibitory activity of **24**–**26** and **33a**

| Compound | R | 1b $IC_{50}$[a] (μM) |
|---|---|---|
| **13** | Bn | 1.4 ± 0.63 |
| **24a** | $Ph(CH_2)_3$– | 0.80 ± 0.28 |
| **24b** | $Ph(CH_2)_5$– | 1.0 ± 0.31 |
| **24c** | $PhO(CH_2)_2$– | 1.2 ± 0.43 |
| **24d** | $Ph_2CHCH_2$– | 0.98 ± 0.21 |
| **24e** | (2-Ph)$PhCH_2$– | 0.30 ± 0.091 |
| **24f** | (2-Ph)Ph– | 0.58 ± 0.097 |
| **25** | (3-Ph)$PhCH_2$– | 0.39 ± 0.10 |
| **26** | (2-Ph)$PhCH_2CH_2$– | 0.34 ± 0.040 |
| **33a** | $Ph_2CH$– | 0.59 ± 0.12 |

[a] Values are means ± SD determined from three separate experiments.

**Table 4.** In vitro 1b NS5B and 1a NS5B inhibitory activity of **33** and **34**

| Compound | $Ar^1$, $Ar^2$ | 1b $IC_{50}$[a] (μM) | 1a $IC_{50}$[a] (μM) |
|---|---|---|---|
| **33a** | Ph | 0.59 ± 0.12 | 1.3 ± 0.05 |
| **33b** | 4-Me-Ph | 1.2 ± 0.31 | 3.1 ± 0.29 |
| **33c** | 4-Cl-Ph | 0.93 ± 0.33 | 2.4 ± 0.19 |
| **33d** | 4-F-Ph | 0.80 ± 0.17 | 1.7 ± 0.40 |
| **33e** | 3-F-Ph | 0.76 ± 0.18 | 1.9 ± 0.08 |
| **34a** | Ph | 0.096 ± 0.040 | 0.8 ± 0.14 |
| **34b** | 4-F-Ph | 0.30 ± 0.066 | 0.84 ± 0.11 |
| **34c** | 4-($CONMe_2$)-Ph | 0.64 ± 0.030 | 0.95 ± 0.35 |
| **34d** | 3-Pyridyl | 1.1 ± 0.25 | 2.2 ± 0.35 |
| **34e** | 3-Pyridyl, Ph | 0.27 ± 0.0069 | 0.80 ± 0.12 |

[a] Values are means ± SD determined from three separate experiments.

**Table 5.** HCV RNA replication inhibitory activity and cytotoxicity on replicon cell of **33** and **34**

| Compound | Replicon[a] $EC_{50}$[c] (μM) | Cytotoxicity[b] $CC_{50}$[c] (μM) |
|---|---|---|
| **33a** | 2.6 ± 0.7 | 31.9 ± 0.6 |
| **33b** | 3.7 ± 0.9 | 23.5 ± 1.0 |
| **33c** | 3.6 ± 1.1 | 23.7 ± 1.4 |
| **33d** | 2.2 ± 0.6 | 27.7 ± 2.7 |
| **33e** | 2.8 ± 0.9 | 24.4 ± 2.3 |
| **34a** | 1.9 ± 0.5 | 27.3 ± 1.1 |
| **34b** | 1.6 ± 0.4 | 27.2 ± 1.3 |
| **34c** | 1.2 ± 0.2 | >50 |
| **34d** | 2.6 ± 0.1 | >50 |
| **34e** | 1.1 ± 0.3 | >50 |

[a] Compounds were incubated in Huh-5-2 cell culture for 48 h.
[b] MTT assay on parallel samples at the same time.
[c] Values are means ± SD determined from three separate experiments.

the 1b enzyme compared to **34a**. However, **34c** and **e** exhibited a higher cellular potency ($EC_{50}$ of 1.2 and 1.1 μM, respectively). In addition, these compounds showed no cytotoxicity up to 50 μM. Compounds **33** and **34** were selective against DNA polymerases α and β ($IC_{50}$ > 10 μM).

In conclusion, we have synthesized a new class of benzimidazole compounds that are potent inhibitors of HCV NS5B RdRp. Preliminary optimization of this series led to the identification of the diarylmethoxy derivatives **33** and **34**. They efficiently blocked the replication of subgenomic HCV RNA in the replicon cells. Further SAR

studies including the effect of substituents other than F atom on the central phenyl ring are on the way and will be reported in due course.

## Acknowledgments

We thank Mr. Mitsumasa Takahashi and Mr. Eita Nagao for analytical support. We are grateful to Mr. Yasushi Niwa and Mr. Masakazu Komatsu for support running the biological assays.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.01.032.

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15. The same observation was seen in the case reported by Boehringer Ingelheim Ltd. See Ref. 8b.
16. The biphenyl type of compounds **24e** is also attractive for further study. The results will be reported in due course.