Letters

Development and Preliminary Optimization of Indole-*N*-Acetamide Inhibitors of Hepatitis C Virus NS5B Polymerase

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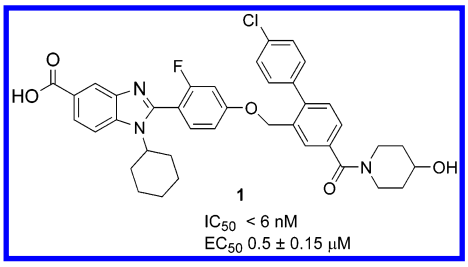
Abstract: Allosteric inhibition of the hepatitis C virus (HCV) NS5B RNA-dependent RNA polymerase enzyme has recently emerged as a viable strategy toward blocking replication of viral RNA in cell-based systems. We report here a novel class of allosteric inhibitor of NS5B that shows potent affinity for the NS5B enzyme and effective inhibition of subgenomic HCV RNA replication in HUH-7 cells. Inhibitors from this class have promising characteristics for further development as anti-HCV agents.

Hepatitis C virus is a major world health problem that affects an estimated 170 million individuals worldwide.[1] The disease is primarily transmitted through contaminated blood products, and its spread has consequently slowed as a result of improvements in blood screening in many countries.[2] Nonetheless, hepatitis C virus (HCV) remains the leading cause of liver transplantation worldwide and results in up to 10 000 deaths annually in the U.S. alone.[3] In the absence of new antiviral therapies to combat the disease, the death rate is expected to triple over the next 2 decades. Current treatments based on interferon-α have a poor rate of success, particularly for genotype-1 infections that predominate in Europe, Japan, and the U.S.[4] These treatments are also poorly tolerated and expensive, adding urgency to the need for novel therapeutic agents to combat HCV.

The genome of HCV is encoded in a (+)-stranded RNA sequence that contains a single open reading frame. Following translation of the HCV polyprotein, the virally encoded NS3 serine protease is released and effects the downstream peptide cleavages that free the viral NS4A, NS4B, NS5A, and NS5B proteins.[5] NS5B has been identified as an RNA-dependent RNA polymerase that is responsible for the synthesis of complementary (−)-stranded HCV RNA.[6] It is this (−)-stranded intermediate that serves as a template during regeneration of the viral genome, a process that is also mediated by NS5B.

Inhibition of NS5B has emerged as an attractive strategy for the development of antivirals to combat HCV. NS5B not only plays a crucial role in the viral replication cascade it also lacks a functional equivalent in mammalian cells, where RNA replication is directed from DNA templates. Effective inhibition of NS5B therefore offers the potential for development of selective and nontoxic anti-HCV agents, and there is intense effort toward this goal within the pharmaceutical industry. In common with other nucleotide polymerizing enzymes, NS5B has been shown[7] to contain three structural domains designated the palm, fingers, and thumb because of a tertiary structure that resembles a right hand.[8] The active site of the enzyme is defined by a conserved region of amino acids in the palm domain, and it has been shown that inhibition of HCV can be achieved through interaction of chain terminating nucleoside ligands at this site.[6,9] Non-nucleoside inhibitors that act as product-like isosteres of pyrophosphate also bind at the active site and show low-nanomolar potency against the isolated NS5B enzyme.[10] Several allosteric sites on NS5B have also been identified, and these are located in the thumb domain of the enzyme, 30 Å or more away from the catalytic center.[6,11,12] Benzimidazole inhibitors such as **1** were recently shown to interact at



1

IC_{50} < 6 nM

EC_{50} 0.5 ± 0.15 μM

one such allosteric site that lies close to a conserved amino acid, proline 495, on the surface of the thumb domain.[13] Compounds such as **1** are potent inhibitors of the isolated NS5B enzyme and are efficient at blocking the replication of subgenomic HCV RNA in a cell-based setting. However, significant issues remain for development of such compounds as drug candidates. **1** elicits a number of off-target activities[14] and its high molecular weight is a further concern with regard to the development of an orally active drug.[15] In this report, we describe the discovery of a series of indol-*N*-acetamides that address these concerns while retaining affinity for the same allosteric site of NS5B. In addition, we describe our preliminary efforts toward optimization of this series, which has resulted in compounds that are equipotent with **1** as inhibitors of HCV replication in a surrogate cell-based assay system.

The indoles reported in Tables 1 and 2 were accessed through elaboration of the bromoindole intermediate **23** (Scheme 1). Installation of the aromatic functionality at the C2 position of **23** was accomplished by Pd-mediated cross-coupling with phenylboronic acid. Routine alkylation of the indole nitrogen atom of **24** with NaH and an alkyl halide gave the methyl esters of **8**−**9** and **12**. The phenylsulfonamide moiety of **10** was incorporated through reaction of **24** and phenylsulfonyl chloride, again using NaH as the base. Direct deprotection of **24** afforded the N-unsubstituted indole **7**,

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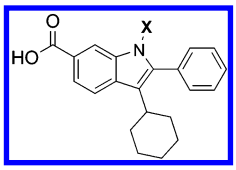
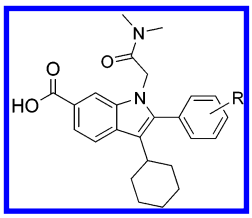
Table 1. NS5B Enzyme Inhibition (IC_{50}) and Cell-Based Efficacy (EC_{50}) for N-Substituted Indole Inhibitors



compd	X	IC_{50} (nM)[a]	EC_{50} (μM)[a]
7	H	48 ± 9	6.7 ± 0.5
8	Me	28 ± 8	6.0 ± 0.5
9	$-CH_2Ph$	37 ± 3	14.2 ± 0.5
10	$-SO_2Ph$	485 ± 180	>50
11	$-CH_2C(O)NHSO_2Me$	45[b]	>50
12	$-CH_2CH_2$-morpholine	111 ± 55	4.6 ± 1.4
13	$-CH_2C(O)NMe_2$	59 ± 6	1.5 ± 0.6
14	$-CH_2C(O)$-morpholine	26 ± 11	0.8 ± 0.3

[a] IC_{50} and EC_{50} values are quoted as the arithmetic mean ± standard deviation for up to 12 independent determinations. [b] $n = 1$.

Table 2. SAR at the C2 Position of **13**: Intrinsic Potency and Cell-Based Activity

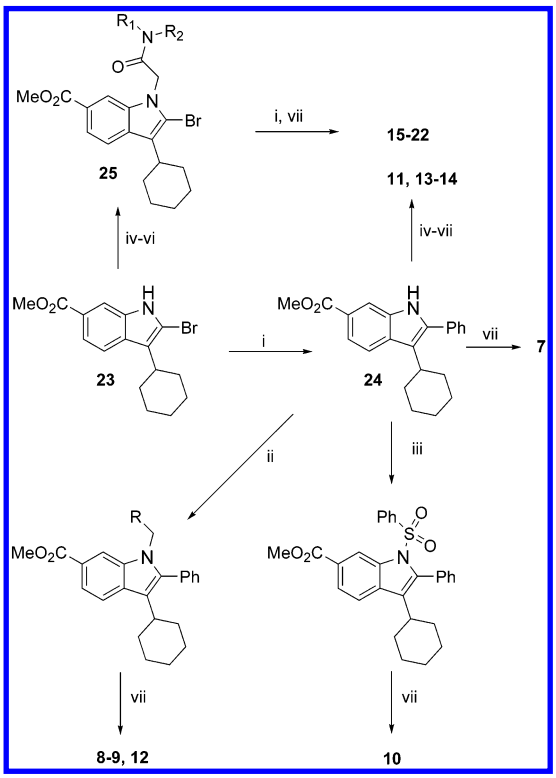


compd	R	IC_{50} (nM)[a]	EC_{50} (μM)[a]
13	H	59 ± 6	1.5 ± 0.6
15	2-Cl	372 ± 72	nd[b]
16	3-Cl	40 ± 30	4.5 ± 0.5
17	4-Cl	15 ± 9	0.9 ± 0.3
18	3-F	18 ± 15	0.6 ± 0.1
19	4-Me	17 ± 2	0.5 ± 0.2
20	4-OMe	18 ± 5	0.5 ± 0.1
21	4-OBn	6 ± 2	2.6 ± 0.6

[a] IC_{50} and EC_{50} values are quoted as the arithmetic mean ± standard deviation up to 12 independent determinations. [b] nd = not determined.

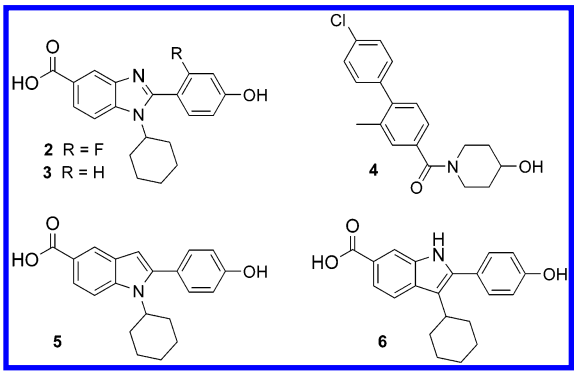
while acetamide functionality on the indole nitrogen of **24** was incorporated using a three-step strategy that allowed modifications in the amide dimension at a late stage of the synthesis. Thus, alkylation of **24** with NaH/*tert*-butyl bromoacetate followed by unmasking of the *tert*-butyl ester and HATU mediated amide formation furnished precursors to **11**, **13**−**14**. Reversal of the order of this chemistry proved convenient for exploration of SAR at the C2 position of the indole, in this case **15**−**22** being obtained through Suzuki-type cross-coupling between an N1 elaborated indole **25** and an arylboronic acid. The final step in all of the above chemistry was removal of the methyl ester functionality at the 6-position of the indole, which could be accomplished through hydrolysis (KOH, dioxane, 50 °C) or dealkylation (BBr_3, CH_2Cl_2). Compounds **5** and **6** were accessed using the chemistry outlined in Scheme 2. Accordingly, indoles **28** and **29** were obtained following concomitant Sonogashira-type cross-coupling/heteroannulation[16] using respectively **26** and **27** as substrate. Alkylation of these indoles with 3-cyclohexenyl bromide allowed isolation of the corresponding C- and N-alkylated products following flash chromatography. Hydrogenation of the C-alkylated product from **28** and the N-alkylated product from **29** was followed by concomitant deprotection of the methyl ester and methyl ether functionalities (BBr_3/CH_2Cl_2) to give the target inhibitors. Benzimidazole inhibitors **2** and **3** were prepared by condensation of methyl 3-amino-4-(cyclohexylamino)benzoate with the appropriate ben-

Scheme 1[a]

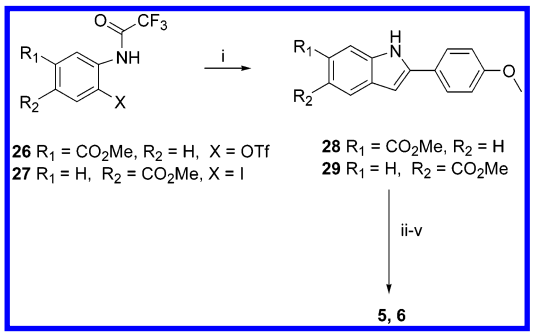


[a] Reagents: (i) $Ar-B(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3; (ii) NaH, $R-CH_2X$, DMF; (iii) NaH, $PhSO_2Cl$; (iv) NaH, $BrCH_2CO_2Bu^t$, DMF; (v) TFA, CH_2Cl_2; (vi) R_1R_2NH, HATU, DIEA; (vii) KOH, dioxane/H_2O or BBr_3, CH_2Cl_2.

Chart 1



Scheme 2[a]



[a] Reagents: (i) $Pd(PPh_3)_2Cl_2$, 4-methoxy-1-ethynylbenzene, CuI, tetramethylguanidine, DMF; (ii) NaH, 3-cyclohexenyl bromide, DMF; (iii) flash chromatography; (iv) H_2(g), Pd/C; (v) BBr_3, CH_2Cl_2.

zaldehyde followed by ester hydrolysis.[17] The biphenyl compound **4** was accessed in a routine fashion via Suzuki coupling of methyl 4-bromo-3-methylbenzoate with 2-chloroboronic acid followed by subsequent elaboration of the carboxylic ester.

The compounds described herein were assessed for activity (IC_{50}) against the purified ΔC_{55} NS5B enzyme in the presence of heterogenic template RNA. Inhibition of replication of subgenomic HCV RNA was measured in HUH-7 cells using a modification of the procedure of Bartenschlager.[18] Unless otherwise stated, cell-based data (EC_{50}) were measured in the presence of 10% fetal calf serum. Counterscreening assays were performed in-house or out-sourced and in all cases followed procedures described in the literature. Pharmacokinetic studies in rats and dogs were performed with $n = 3$, and the following dosing parameters were used: rat, iv 3 mg/kg (60% DMSO + 20% PEG400 + 20% H_2O), po 3 mg/kg (PEG400); beagle, iv 1 mg/kg (50% DMSO + 50% PEG400), po 2 mg/kg (PEG400).

The observation that benzimidazole-based compounds such as **1** inhibit the HCV polymerase via an allosteric mechanism of action aroused our interest in the structural features of this class of molecule that are required for interaction with NS5B. Our initial simplistic analysis viewed **1** as an adduct of two lipophilic domains (namely, the trisubstituted benzimidazole and the biphenyl moiety) that are linked through a flexible hydroxymethyl tether. To assess the relative contributions to binding affinity made by each of these lipophilic regions, **2** and **4** were prepared and evaluated. While **4** showed no activity against the NS5B enzyme, the benzimidazole **2** ($IC_{50} = 1.4\ \mu M$) retained micromolar potency. Furthermore, **2** showed no activity against NS5B harboring the P495L mutation, a structural change in the thumb region of NS5B that has previously been characterized as an escape mutant that confers resistance to **1**.[13] Apparently, while the presence of the biphenyl portion of **1** adds much to binding affinity, a pharmacophore involving a central benzimidazole core with pendant aryl functionality at C2, cycloalkyl functionality at N1, and carboxylic acid functionality at C5 is sufficient for allosteric inhibition at the site occupied by **1**.[19]

In view of its low molecular weight and structural simplicity, **2** was judged an attractive lead for further medicinal chemistry efforts, and our early explorations focused on evaluating alternatives to benzimidazole as the central scaffold in the pharmacophore. To evaluate the role of hydrogen bonding between the N3 atom of **2** and a hydrogen bond donor on the NS5B enzyme, indoles **5** and **6** were prepared. Rewardingly, the *N*-cyclohexylindole **5** ($IC_{50} = 340$ nM) proved 10-fold more potent than **3** ($IC_{50} = 3.0\ \mu M$) while a further order of magnitude improvement in activity was measured for the isomeric *C*-cyclohexyl compound **6** ($IC_{50} = 48$ nM). N-Methylation of the indole nitrogen of **6** was tolerated (Table 1, **8**), indicating that the improved activity of **6** over **3** does not come from replacement of hydrogen bond acceptor functionality adjacent to the C2 aromatic ring in the latter compound with hydrogen bond donor functionality. Presumably, the improved potency of **5** and **6** stems from replacement of a polar heteroatom in the central core with a carbon atom, favoring interaction at a lipophilic site on the enzyme.[20] This effect is more pronounced for replacement of the N1 nitrogen atom of **3** that accommodates the lipophilic cyclohexyl substituent which likely interacts at a highly hydrophobic region of the enzyme.

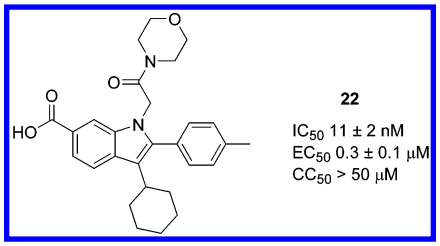
Appraisal of substituents on the N1 atom of **7** (Table 1) clearly indicated that electron-donating groups could be accommodated by the enzyme (**8**−**9**), while electron-withdrawing groups (**10**) were detrimental to activity. However, while electron-donating substituents such as the benzyl group in **9** were tolerated, no positive improvement in potency was achieved, and in fact, substituents of this type were detrimental to cell-based activity. This suggested that substituents of N1 projected away from the enzyme surface and N-substitution emerged as an attractive route to modulate the overall physicochemical profile of the inhibitor without negatively impacting binding efficiency. Strategies to lower the lipophilicity of the inhibitors featured strongly in the choices of substituent introduced and were fundamental in improving cell-based potency. Polar functionality could be incorporated into the N1 substituent at positions remote from the indole ring with **11** and **12** both retaining activity in the nanomolar range and highlighting the tolerance for acidic and basic functionality in this region. However, the neutral *N*-acetamide side chain of **13** emerged as especially profitable with regard to cell-based activity, resulting in a 5-fold improvement over the corresponding N-unsubstituted indole **7**. Significant space was apparently available to the amide at N1, the morpholine analogue **14** providing a further modest gain in potency in the enzyme and cell-based assays.

Indole-*N*-acetamide **13** had an attractive overall profile, showing no off-target activities in counterscreening assays against mammalian and viral polymerases (human DNA polymerases α, β, and γ; poliovirus RNA polymerase) and no cytotoxicity at 50 μM. Furthermore, the compound was clean against a panel of over 150 biological targets in counterscreens rund in the MDS Pharma Services Laboratories.[14] Despite having substantially lower molecular weight than **1** (404 vs 682), **13** showed only 3-fold weaker inhibition of HCV subgenomic RNA replication in our cell-based screen. The two compounds were again shown to bind at the same allosteric site by experiments with NS5B incorporating the P495L mutation, against which **13** lost around 3 orders of magnitude in potency ($IC_{50} = 32\ \mu M$).

Pharmacokinetic studies indicated that **13** was well absorbed in rat, where oral bioavailability was 45 ± 4%. The plasma half-life and plasma clearance were 4.5 ± 1.8 h and 32 mL min^{-1} kg^{-1}, respectively. In vitro studies in rat liver microsomes revealed that the compound was turned over as a result of glucuronidation of the C6 carboxylic acid. The probable involvement of a glucuronide of **13** in mediating the plasma clearance of the parent compound was a concern because of the potentially reactive nature of glucuronide conjugates of carboxylic acids.[21] Consequently, a ^3H-labeled sample of **13** was prepared and administered to rats with a 20 mg/kg dose of unlabeled compound. Very low levels of irreversibly bound compound were measured in liver homogenates (<5 pmol/mg of protein), suggesting that covalent labeling of endogenous proteins is not an inherent liability for this series. Interestingly, the clearance for **13** was significantly lower in dog (3 ± 0.3 mL min^{-1} kg^{-1}) where the compound showed full oral bioavailability and a half-life of 4.8 ± 1 h.

The results in Table 1 highlight that the chemical structure of an electron-donating substituent on the

indole nitrogen has little impact on enzyme affinity. In contrast, the nature of the aryl substituent at the C2 position of the indole proved crucial for intrinsic potency. Substituents at the ortho position of the phenyl ring were detrimental to activity (**15**, Table 2), but gains could be made through introduction of functionality at the meta or especially para positions. Small, lipophilic substituents proved optimal in this regard, with **17**−**21** showing between a 3- and 10-fold improvement over the unsubstituted 2-phenylindole **13**. Improved cell-based activity was also achieved, albeit at times to a lesser extent. **21** highlights an extreme case, with poorer activity than **13** being observed in the cell despite 10-fold higher enzyme affinity. A likely explanation is that introduction of lipophilic functionality causes higher binding to serum proteins present in the assay media, the consequence being a reduced intracellular concentration of the inhibitor. In line with this hypothesis, compounds in this series showed potent inhibition of HCV replication when the cell-based assay was performed in the absence of serum proteins (e.g., **17**, EC_{50} = 0.13 μM; **21** showed 85% inhibition at 0.3 μM). It is apparent that a challenge for future work in this area is the alignment of high enzyme affinity with efficacy in a physiologically relevant cell-based assay system. Nonetheless, application of the SAR described in Table 2 in the more active morpholineacetamide series provided compounds such as **22** (EC_{50} = 0.3 μM) that already show an improvement in inhibition of HCV RNA replication over **1** (EC_{50} = 0.5 μM) despite lower molecular weight and reduced structural complexity. Importantly, no cytotoxicity was observed for compounds of this type, the CC_{50} values for compounds **13**−**22** being above 50 μM.



We have described here the development of a series of indole-N-acetamides that are potent allosteric inhibitors of the HCV NS5B enzyme. Preliminary optimization of this series furnished compounds that show submicromolar blockade of subgenomic HCV RNA replication in HUH-7 cells. These results, together with the encouraging profile shown by the initial lead **13** in *in vivo* pharmacokinetic studies and *in vitro* counterscreens, highlight the potential of this series for the development of new anti-HCV agents.

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Supporting Information Available: Protocols for biological assays, synthetic procedures, and spectral data for **5**−**22**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Cohen, J. Hepatitis C. *Science* **1999**, *285*, 26.
(2) EASL International Consensus Conference on Hepatitis C, Consensus Statement. *J. Hepatol*. **1999**, *30*, 956.
(3) Steedman, S. A.; Younossi, Z. M. Hepatitis C: An Update on the Silent Epidemic. *J. Clin. Gastroenterol*. **2000**, *30*, 125.
(4) Dymock, B. W. Emerging Therapies for Hepatitis C Virus Infection. *Emerging Drugs* **2001**, *6*, 13.
(5) Steinkuhler, C.; Koch, U.; Narjes, F.; Matassa, V. G. Hepatitis C Virus Serine Protease Inhibitors: Current Progress and Future Challenges. *Curr. Med. Chem*. **2001**, *8*, 919.
(6) Beaulieu, P. L.; Tsantrizos, Y. S. Inhibitors of the HCV NS5B Polymerase: New Hope for the Treatment of Hepatitis C Infections. *Curr. Opin. Invest. Drugs* **2004**, *5*, 838.
(7) Lesburg, C. A.; Cable, M. B.; Ferrari, E.; Hong, Z.; Mannarino, A. F.; Weber, P. C. Crystal Structure of the RNA-Dependent RNA Polymerase from Hepatitis C Virus Reveals a Fully Encircled Active Site. *Nat. Struct. Biol*. **1999**, *6*, 937.
(8) Ollis, D. L.; Brick, P.; Hamlin, R.; Xuong, N. G.; Steitz, T. A. Structure of a Large Fragment of *Escherichia coli* DNA Polymerase I complexed with dTMP. *Nature* **1985**, *330*, 675.
(9) Carroll, S. S.; Tomassini, J. E.; Bosserman, M.; Getty, K.; Stahlhut, M. W.; Eldrup, A. B.; Bhat, B.; Hall, D.; Simcoe, A. L.; LaFemina, R.; Rutkowski, C. A.; Bohdan, W.; Yang, Z.; Migliaccio, G.; De Francesco, R.; Kuo, L. C.; MacCoss, M.; Olsen, D. B. Inhibition of Hepatitis C Virus RNA Replication by 2′-Modified Nucleoside Analogs. *J. Biol. Chem*. **2003**, *278*, 11979.
(10) Summa, V.; Petrocchi, A.; Pace, P.; Matassa, V. G.; De Francesco, R.; Altamura, S.; Tomei, L.; Koch, U.; Neuner, P. Discovery of α,γ-Diketo Acids as Potent Selective and Reversible Inhibitors of Hepatitis C Virus NS5B RNA-Dependent RNA Polymerase. *J. Med. Chem*. **2004**, *47*, 14.
(11) Wang, M.; Ng, K. K. S.; Cherney, M. M.; Chan, L.; Yannopoulos, C. G.; Bedard, J.; Morin, N.; Nguyen-Ba, N.; Alaoui-Ismaili, M.; Bethell, R. C.; James, M. G. N. Non-nucleoside Analogue Inhibitors Bind to an Allosteric Site on HCV NS5B Polymerase. *J. Biol. Chem*. **2003**, *278*, 9489.
(12) Nguyen, T. T.; Gates, A. T.; Gutshall, L. L.; Johnston, V. K.; Gu, B.; Duffy, K. J.; Sarisky, R. T. Resistance Profile of a Hepatitis C Virus RNA-Dependent RNA Polymerase Benzothiadiazine Inhibitor. *Antimicrob. Agents Chemother*. **2003**, *47*, 3525.
(13) Tomei, L.; Altamura, S.; Bartholomew, L.; Biroccio, A.; Ceccacci, A.; Pacini, L.; Narjes, F.; Gennari, N.; Bisbocci, M.; Incitti, I.; Orsatti, L.; Harper, S.; Stansfield, I.; Rowley, M.; De Francesco, R.; Migliaccio, G. Mechanism of Action and Antiviral Activity of Benzimidazole-Based Allosteric Inhibitors of the Hepatitis C Virus RNA-Dependent RNA Polymerase. *J. Virol*. **2003**, *77*, 13225.
(14) MDS Pharma Services Taiwan Ltd., Pharmacology Laboratories, 158 Li-The Road, Peitou, Taipei, Taiwan 112, R.O.C.
(15) Lipinski, C. P.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Settings. *Adv. Drug Delivery. Rev*. **1997**, *23*, 3.
(16) Battistuzzi, G.; Cacchi, S.; Fabrizi, G. The Aminopalladation/Reductive Elimination Domino Reaction in the Construction of Functionalised Indole Rings. *Eur. J. Org. Chem*. **2002**, 2671.
(17) For a representative synthesis of benzimidazoles such as **2**, see the following: Beaulieu, P. L.; Hache, B.; Von Moos, E. A. Practical Oxone-Mediated, High-Throughput, Solution-Phase Synthesis of Benzimidazoles from 1,2-Phenylenediamines and Aldehydes and Its Application to Preparative Scale Synthesis. *Synthesis* **2003**, *11*, 1683.
(18) Lohmann, V.; Korner, F.; Koch, J.; Henan, U.; Theilmann, L.; Bartenschlager, R. Replication of Subgenomic Hepatitis C Virus RNA's in a Hepatoma Cell Line. *Science* **1999**, *285*, 110.
(19) Allosteric inhibitors of NS5B based on benzimidazoles such as **2** have recently been disclosed: Beaulieu, P. L.; Bos, M.; Bousquet, Y.; Fazal, G.; Gauthier, J.; Gillard, J.; Goulet, S.; LaPlante, S.; Poupart, M.-A.; Lefebvre, S.; McKercher, G.; Pellerin, C.; Austel, V.; Kukolj, G. Non-nucleoside Inhibitors of the Hepatitis C Virus NS5B Polymerase: Discovery and Preliminary SAR of Benzimidazole Derivatives. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 119.
(20) The log *P* of an indole is typically 0.5−1 log unit higher than that of the corresponding benzimidazole.
(21) Ritter, J. K. Roles of Glucuronidation and UDP-glucuronosyltransferases in Xenobiotic Bioactivation Reactions. *Chem.-Biol. Interact*. **2000**, *129*, 171.

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