Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 5143–5149
Bioorganic & Medicinal Chemistry Letters

Development of carboxylic acid replacements in indole-*N*-acetamide inhibitors of hepatitis C virus NS5B polymerase

Ian Stansfield,* Marco Pompei, Immacolata Conte, Caterina Ercolani, Giovanni Migliaccio, Mark Jairaj, Claudio Giuliano, Michael Rowley and Frank Narjes

IRBM (Merck Research Laboratories Rome), Via Pontina Km 30,600, 00040 Pomezia, Rome, Italy

Received 16 May 2007; revised 28 June 2007; accepted 29 June 2007
Available online 7 July 2007

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 2 series of indole-*N*-acetamides, bearing physicochemically diverse carboxylic acid replacements, which show potent affinity for the NS5B enzyme with reduced potential for formation of glucuronide conjugates. Preliminary optimization of these series furnished compounds that are potent in the blockade of subgenomic HCV RNA replication in HUH-7 cells.

HCV is a major human pathogen associated with chronic hepatitis and liver disease, cirrhosis, hepato-cellular carcinoma, and liver failure.[1] Worldwide, there are an estimated 170 million chronic carriers,[2] whilst in the US alone, 4 million have antibodies to HCV, indicating an on-going or previous infection with the virus. For over 10 years, frontline therapies have been based around interferon-α, commonly dosing in conjunction with ribavirin. Despite progress with such therapies (e.g., introduction of pegylated interferon),[3] sustained viral response (SVR) rates are still typically poor, particularly for genotype-1 infections that predominate in Europe, Japan, and the U.S.[4] In addition, therapy is often accompanied by significant adverse side effects[5]—consequently, there is a pressing need for new and broadly effective therapeutics to combat HCV.[3,6]

HCV is a small, enveloped, single stranded positive RNA virus in the Flaviviridae family. The genome is approximately 10,000 nucleotides and encodes a single polyprotein of about 3000 amino acids. This polyprotein comprises the structural (C, E1, and E2) and non-structural (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) proteins that are required for replication and packaging of viral genomic RNA. NS5B is the viral RNA-dependent RNA polymerase (RdRp). The RdRp activity of NS5B is essential for viral replication[7] and has no functional equivalent in uninfected mammalian cells—thus making the NS5B protein an attractive target for drug discovery.[8]

The NS5B RdRp comprises the palm, fingers, and thumb subdomains common to nucleotide polymerizing enzymes.[9] Inhibition of NS5B can be achieved through interaction at the active site, for which both nucleoside ligands[10,11] and non-nucleoside inhibitors have been described.[11–15] Alternatively, several allosteric inhibitor binding sites on NS5B have been identified distal to the catalytic center.[11,16] Recent reports from our laboratories documented the development of *N*-acetamido-indole-6-carboxylates, such as **1**, as potent inhibitors interacting at one such allosteric site that lies close to a conserved amino acid, proline 495, on the surface of the thumb domain.[17–19] An issue with compounds from this class, however, was that glucuronide conjugates of the carboxylic acid were frequently observed as major circulating metabolites (Fig. 1).

To moderate the formation of glucuronide conjugates in the systemic circulation, viable alternatives to the C6-carboxylate were sought. There are a number of moieties documented in the literature as mimetics for carboxylic acids, with diverse physical attributes and spanning a multi-log unit range of pK_a values. In this

Keywords: Hepatitis C virus; NS5B polymerase; Allosteric inhibition; Indoles; Acid replacements.
* Corresponding author. Tel.: +39 06 91093286; fax: +39 06 91093654; e-mail: ian_stansfield@merck.com

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.06.093

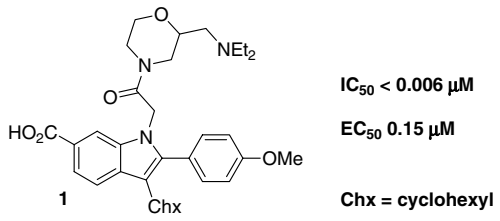


Figure 1. Potent *N*-acetamido-indole-6-carboxylic acid inhibitor of HCV NS5B.

paper, we describe the discovery and initial optimization of 2 such structural classes (oxadiazolones and acyl sulfonamides/sulfonyl ureas) chosen for their disparate pK_a's and physical characteristics—leading to indole *N*-acetamides with reduced potential regarding formation of glucuronide conjugates, that retain intrinsic affinity for the NS5B enzyme and potency as inhibitors of HCV replication in a surrogate cell-based assay.

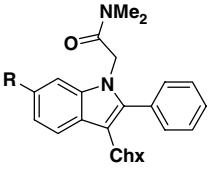
The compounds described herein were assessed for activity (IC_{50}) against the purified ΔC55 NS5B enzyme in the presence of heterogeneous template RNA. Inhibition of replication of subgenomic HCV RNA was measured in HUH-7 cells using a modification of the procedure of Bartenschlager.[20] Unless otherwise stated, cell-based data (EC_{50}) were measured in the presence of 10% fetal calf serum. Pharmacokinetic studies in rats were performed with $n = 3$, and the following dosing parameters were used: iv 3 mg/kg (60% DMSO + 20% PEG400 + 20% H_2O), po 3 mg/kg (PEG400).

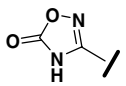
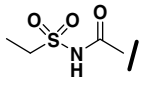
The indoles reported in Tables 1–4 were prepared from the methyl 2-bromo-3-cyclohexylindole-6-carboxylate precursor[17] **2**, as outlined in Scheme 1. Thus, either BBr_3 or hydrolytic cleavage of the methyl ester, formation of the primary amide and dehydration to the nitrile, followed by alkylation with NaH/*tert*-butyl bromoacetate afforded intermediate **3**. Preparation of the amide oxime and a one-pot acylation/cyclization protocol using carbonyldiimidazole generated the oxadiazolone. Pd-mediated cross-coupling with the appropriate arylboronic acid then installed the aromatic functionality at C2 in **4**. Unmasking of the *tert*-butyl ester and TBTU-promoted amide bond formation yielded the final compounds **5**. Clearly, this strategy is well suited to modifications to the acetamide dimension in the final step, however, the synthesis could be readily tailored to fit final diversification steps at alternative positions around the indole. Hence, for example, manipulation at C6 could be performed equally well after diversification at C2 and in the *N*1-acetamide moieties, simply by moving steps in the synthetic sequence. Initial Suzuki coupling on **2** followed by the 3-step alkylation, deprotection, amide bond formation sequence, and final ester cleavage afforded C6 carboxylic acid **6**. The carboxylate moiety could then either be manipulated as before to set up the oxadiazolone ring, or subjected to EDAC mediated coupling with sulfonamides or sulfamides to yield the corresponding acylsulfonamides or sulfonylureas **7**.

As illustrated in Scheme 2, taking bromo derivative **8** through the analogous synthetic sequence allows functionalization at C2 as the final step.

In the field of angiotensin II receptor antagonists, heterocycles such as oxadiazolones have been reported as replacements for carboxylic acid moieties.[21] Although 2 tautomeric forms can be envisaged, studies suggest that generally the non-aromatic oxadiazolone form is

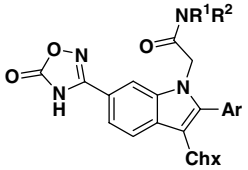
Table 1. HCV NS5B polymerase enzyme inhibition (IC_{50}), cell-based efficacy (EC_{50}), and in vivo rat pharmacokinetic properties for diverse C6 acid moieties

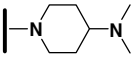
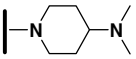
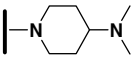
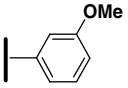
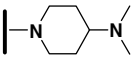
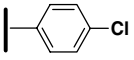
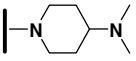
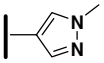
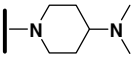
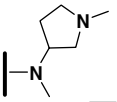
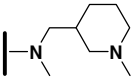
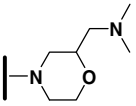


Compound	R	IC_{50}/EC_{50}[a] (μM)	F_{rat} (%)[b,c]/ $t_{1/2}$ (h)[d]	Cl (ml/min/kg)[e]/ AUC (μM h)[f]
9		0.086/**1.5**	45/5	32/2
10		0.068/**4.4**	20/13	12/2
11		0.046/**2.1**	24/2	51/1

[a] IC_{50}/EC_{50} values are means from at least 2 experiments.
[b] Compounds were dosed as trifluoroacetate salts. po/iv 3 mg/kg body weight; $n = 3$. Vehicle iv 60% DMSO/20% PEG400/20% H_2O, po PEG400.
[c] Oral bioavailability.
[d] Terminal phase plasma halflife following iv administration.
[e] Plasma clearance.
[f] After oral dosing.

Table 2. C6 hydroxyoxadiazole SAR: intrinsic potency and cell-based activity



Compound	Ar	NR^1R^2	IC_{50}[a] (μM)	EC_{50}[a] (μM)	hPPB (% free)
10		NMe_2	0.068	4.4	0.1
12		NMe_2	0.78	20	—
13			0.059	1.3	—
14			0.053	2.4	—
15			0.3	17	—
16			0.067	3.7	—
17			0.017	1.9	—
18			0.17	>50	9.4
19			0.075	1.60	—
20			0.047	1.1	2.1
21			0.026	1.1	—

[a] IC_{50}/EC_{50} values are means from at least 2 experiments.

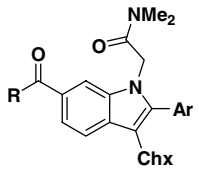
predominant over the hydroxyoxadiazole.[22] As a consequence, the pK_a is significantly elevated (and so the % of the neutral form at physiological pH) with respect to carboxylic acids. In contrast, simple acyl sulfonamides/sulfonyl ureas have pK_a values that approach more closely that of the parent carboxylic acid, but offer a handle from which to explore structural space on the enzyme surface. (Measured values: **9** pK_a = 4.8; **10** pK_a = 6.1; **23** pK_a = 5.2.)[23]

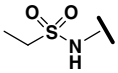
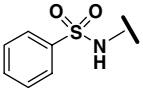
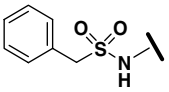
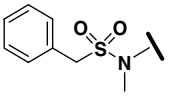
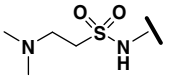
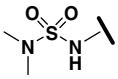
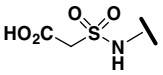
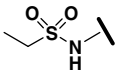
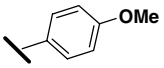
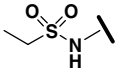
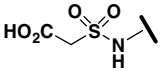
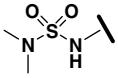
Pleasingly, despite diverse physical properties, both oxadiazolone **10** and acylsulfonamide **11** display excellent intrinsic potency on the isolated enzyme, being if anything marginally more potent than the parent carboxylic acid **9**, with comparable activity in the cell based assay (Table 1). Furthermore, as prototypical molecules for each class, they both show acceptable oral bioavailability and systemic exposure in rat.

Most significantly, in vitro studies with **10** and **11** in rat liver microsomes demonstrated that both these series potentially offer a means by which to radically reduce the extent of glucuronidative metabolism in the indole *N*-acetamide class (Fig. 2). In vivo, elevated clearance for **11** was not associated with the formation of conjugates, but rather a greater first pass effect due to oxidative metabolism and biliary excretion.

Having shown that it was possible to address glucuronidation without compromising significantly intrinsic potency, attention then focused on SAR to optimize cell-based activity (Table 2). In the oxadiazolone class,

Table 3. C6 acylsulfonamide SAR: intrinsic potency and cell-based activity



Compound	R	Ar	IC_{50}[a] (μM)	EC_{50}[a] Standard/(no serum) (μM)	hPPB (% free)
11		Ph	0.046	2.1/(0.52)	0.8
22		Ph	0.011	3.9/(0.13)	0.3
23		Ph	0.031	5.0/(0.15)	<0.1
24		Ph	0.64	NA[b]	—
25		Ph	0.031	5.4/(2.0)	1.9
26		Ph	0.009	0.71	0.8
27		Ph	0.029	1.6	—
28			0.011	1.5	—
29			0.022	1.5	0.8
30			0.019	0.56	1.0
31			0.014	0.38	1.1

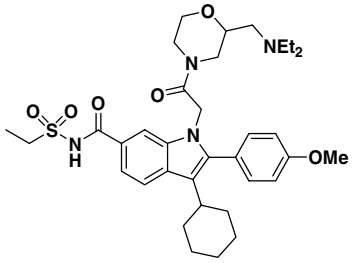
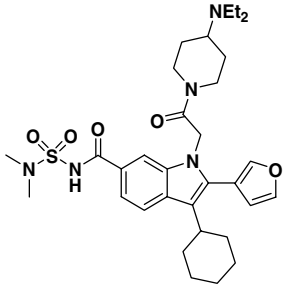
[a] IC_{50}/EC_{50} values are means from at least 2 experiments.
[b] NA, not active.

a consequence of the tautomeric preference and charge delocalization upon ionization is a greater lipophilicity for this moiety over a carboxylic acid. In light of this, the possibility to introduce polarity in other regions of the structure would be highly desirable as a means of reducing the overall lipophilicity and improving parameters such as plasma protein binding (PPB).

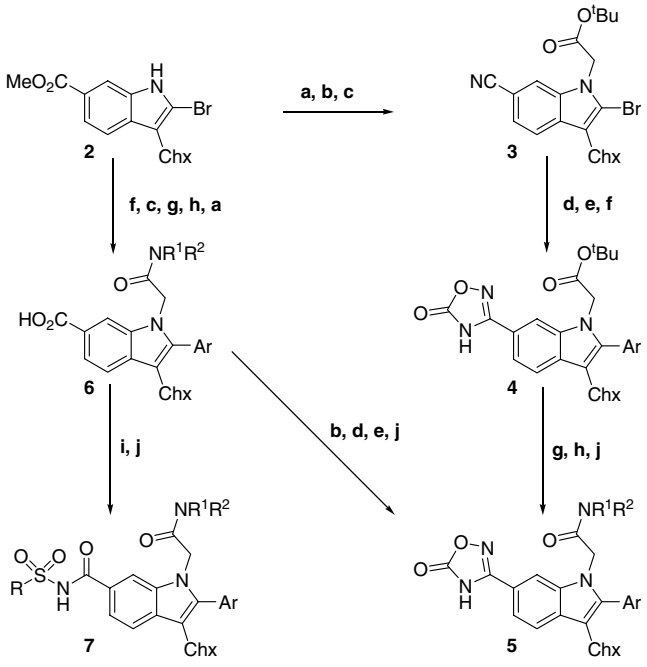
Regrettably, polar heterocycles at C2 (e.g., **12**) proved largely incompatible with the primarily lipophilic interactions of this element and the enzyme surface. In contrast, introducing a basic centre in the acetamide sidechain (e.g., **13**) to afford formally zwitterionic species gave a moderate enhancement in cell-based potency.

Using zwitterionic indole oxadiazolone **13** as a starting point, SAR around the C2 phenyl was explored. Substitution in this series showed that although small moieties in the *ortho* position such as F (**14**) were tolerated, bulkier substituents (**15**) proved deleterious—presumably a steric clash between the C2 and *N*1 elements perturbing their orientations and compromising interactions with the enzyme. Electron donating functionality at the *meta* position was acceptable (**16**), whilst the *para*-position could accommodate substitution with a modest gain in enzyme affinity (**17**). Once more, polar electron deficient heteroaromatics at C2 proved incompatible with enzyme affinity (**18**). C2/*N*1-acetamide moiety optimization, using amines from commercial sources and in-house synthesis, afforded potent enzyme inhibitors spanning

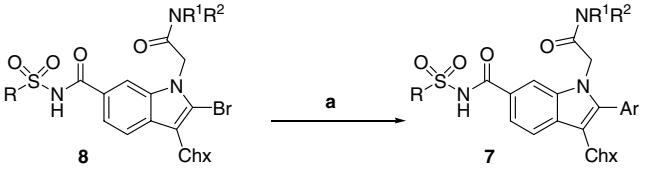
Table 4. Optimized C6 acylsulfonamide and acylsulfonylurea: intrinsic potency and cell-based activity

Compound	Structure	IC_{50}[a] (μM)	EC_{50}[a] (μM)
32		0.009	0.34
33		0.004	0.12

[a] IC_{50}/EC_{50} values are means from at least 2 experiments.



Scheme 1. Reagents and conditions: (a) BBr_3, CH_2Cl_2, or NaOH, dioxane, H_2O, 60 °C, 75–100%; (b) (i) $(BOC)_2O$, H_4NHCO_3, pyridine, DMF, 24 h; (ii) $(CF_3CO)_2O$, Et_3N, CH_2Cl_2, $CHCl_3$, 0 °C, 3 h, 75%; (c) NaH, *tert*-butyl bromoacetate, DMF, 60 °C, 95%; (d) $H_2NOH{\cdot}HCl$, $^iPr_2NEt$, MeOH, 48 h, 80%; (e) carbonyldiimidazole, dioxane, 70 °C, 40%; (f) $ArB(OH)_2$, $Pd(PPh_3)_2Cl_2$, Na_2CO_3 (aq), 1,4-dioxane, reflux, 75–85%; (g) CF_3CO_2H, CH_2Cl_2, 2 h, 100%; (h) TBTU, $^iPr_2NEt$, HNR^1R^2, CH_2Cl_2, 16 h, 50–75%; (i) EDCI, DMAP, RSO_2NH_2, 35–70%; (j) RP-HPLC, 50–85%.



Scheme 2. Reagents and condition: (a) $ArB(OH)_2$, $Pd(PPh_3)_2Cl_2$, Na_2CO_3 (aq), 1,4-dioxane, reflux, 75–85%.

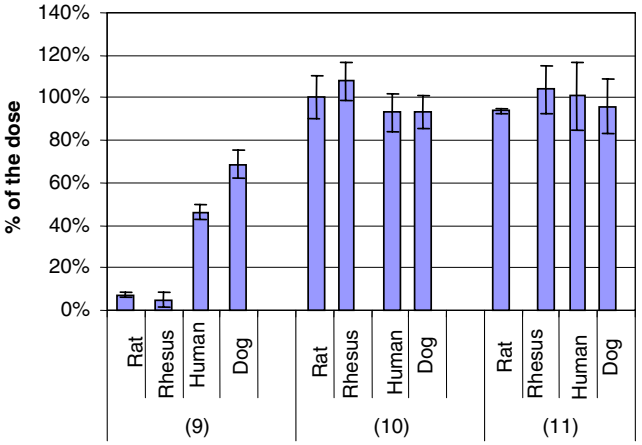


Figure 2. Turnover in liver microsomes from diverse species in the presence of UDPGA (% dose remaining at 1 h).

a 20–80 nM activity range. Although it was possible to improve physical properties (as judged by hPPB) without compromising intrinsic potency (e.g., **20**), it proved difficult to break into sub-μM levels of cell based activity. This would suggest that plasma protein binding may not be the key determinant restricting cell-based activity for this series. Potentially log D (which for the oxadiazolone series generally remains around a log unit higher than for the analogous examples in the C6 carboxylic acid class), coupled with the higher H-bond acceptor

count, may contribute to limiting the free intracellular levels of compound attainable. In accord with this hypothesis, there was minimal impact on cell-based activity when the assay was performed in the absence of serum proteins. (e.g., compound **19** EC_{50} (no serum) 1.0 vs 1.6 μM in standard conditions).

Concomitant with the oxadiazolones, the acylsulfonamide series was explored. In this case, pK_a and log D can approach more closely that of the parent carboxylic acid class, and it proved easier to rationalize variations in cell-based activity in terms of substituent impact on log D, PPB, and/or cell permeability rather than an inherent limitation of the structural class.

Studies initially probed the sulfonamide/sulfonyl urea fragment (Table 3). It was found that methylation of the acidic NH proved highly detrimental to potency, reflecting the importance of this structural element (e.g., **24**). Although bulky functionality at C6 was tolerated, it was not necessary for optimal enzyme affinity and cell-based potency, with simple acylsulfonylurea **26** affording a 10-fold boost in intrinsic enzyme affinity over the carboxylic acid **9** along with sub-μM cell-based activity. For overly lipophilic molecules (e.g., **22**, **23**), plasma protein binding became limiting for cell-based activity, as can be seen from the shifts between no serum and standard conditions. Polar/charged moieties could reduce PPB, but not always did this improve free intracellular compound concentration (e.g., **25** shows reduced serum shift with respect to **23**, but raised EC_{50}(no serum)/IC_{50} ratio). Intriguingly, a di-acid replacement for the C6 carboxylic acid **27** did not limit cell-based activity. SAR at C2 identified electron-rich heteroaromatics, such as furan, as being optimal for enzyme affinity, and gratifyingly gave further improvements in cell-based activity (e.g., **30**, **31**).

At this stage, introduction of a basic charged center to the acetamide moiety to give formally zwitterionic acylsulfonamides and sulfonyl ureas gave a significant boost in both intrinsic and cell-based potency (Table 4). *N*1/C2 optimization afforded compounds (e.g., **32** and **33**) possessing intrinsic potency and cell-based activity to now rival the related C6 carboxylic acid series.[18] Importantly, no cytotoxicity was observed for compounds of this type, the CC_{50} values for compounds **32**, **33** being above 50 μM.

In summary, we have described here the development of 2 series of indole-*N*-acetamides, bearing physicochemically diverse replacements for the C6 CO_2H, that are potent allosteric inhibitors of the HCV NS5B enzyme with reduced potential regarding formation of glucuronide conjugates. Preliminary optimization of these series furnished compounds that are non-cytotoxic and equipotent with their carboxylic acid counterparts—showing 100 nM potency in the blockade of subgenomic HCV RNA replication in HUH-7 cells.

Acknowledgments

We thank Sergio Altamura and Licia Tomei for enzymology; Fabrizio Fiore and Odalys Gonzalez-Paz for PK analysis; Marina Taliani for metabolism studies; Fabio Bonelli, Alan Bishop, Maria Verdirame, and Steven Thomas for analytical work and Nadia Gennari, Monica Bisbocci, and Ottavia Cecchetti for biological testing. This work was funded in part by a grant from the MIUR.

References and notes

1. Wong, T.; Lee, S. S. *CMAJ* **2006**, *174*, 649.
2. WHO. Hepatitis C Fact Sheet No. 164. Rev. October 2000.
3. Hayashi, N.; Takehara, T. *J. Gastroenterol.* **2006**, *41*, 17.
4. Dymock, B. W. *Emerg. Drugs* **2001**, *6*, 13.
5. Fried, M. W. *Hepatology* **2002**, *36*, S237.
6. Davis, G. L.; Lindsay, K. L. *Lancet Infect. Dis.* **2005**, *5*, 524.
7. Appel, N.; Schaller, T.; Penin, F.; Bartenschlager, R. *J. Biol. Chem.* **2006**, *281*, 9833.
8. Walker, M. P.; Hong, Z. *Curr. Opin. Pharmacol.* **2002**, *2*, 534.
9. Bressanelli, S.; Tomei, L.; Roussel, A.; Incitti, I.; Vitale, R. L.; Mathieu, M.; De Francesco, R.; Rey, F. A. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 13034.
10. 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. *J. Biol. Chem.* **2003**, *278*, 11979.
11. Beaulieu, P. L.; Tsantrizos, Y. S. *Curr. Opin. Invest. Drugs* **2004**, *5*, 838.
12. Summa, V.; Petrocchi, A.; Pace, P.; Matassa, V. G.; De Francesco, R.; Altamura, S.; Tomei, L.; Koch, U.; Neuner, P. *J. Med. Chem.* **2004**, *47*, 14.
13. Stansfield, I.; Avolio, S.; Colarusso, S.; Gennari, N.; Narjes, F.; Pacini, B.; Ponzi, S.; Harper, S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5085.
14. Crescenzi, B.; Poma, M.; Ontoria, J. M.; Marchetti, A.; Nizi, E.; Matassa, V. G.; Gardelli, C. *Lett. Drug Des. Discov.* **2005**, *2*, 451.
15. Ponzi, S.; Giuliano, C.; Donghi, M.; Poma, M.; Matassa, V. G.; Stansfield, I. *Lett. Drug Des. Discov.* **2005**, *2*, 456.
16. Koch, U.; Narjes, F. *Infect. Disord.: Drug Targets* **2006**, *6*, 31.
17. Harper, S.; Pacini, B.; Avolio, S.; Di Filippo, M.; Migliaccio, G.; Laufer, R.; De Francesco, R.; Rowley, M.; Narjes, F. *J. Med. Chem.* **2005**, *48*, 1314.
18. Harper, S.; Avolio, S.; Pacini, B.; Di Filippo, M.; Altamura, S.; Tomei, L.; Paonessa, G.; Di Marco, S.; Carfi, A.; Giuliano, C.; Padron, J.; Bonelli, F.; Migliaccio, G.; De Francesco, R.; Laufer, R.; Rowley, M.; Narjes, F. *J. Med. Chem.* **2005**, *48*, 4547.
19. A parallel evolution of allosteric indole inhibitors of NS5B has recently been described: Beaulieu, P. L.; Gillard, J.; Bykowski, D.; Brochu, C.; Dansereau, N.; Duceppe, J-S.; Hache, B.; Jakalian, A.; Lagace, L.; LaPlante, S.; McKercher, G.; Moreau, E.; Perreault, S.; Stammers, T.; Thauvette, L.; Warrington, J.; Kukolj, G. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4987.
20. Lohmann, V.; Korner, F.; Koch, J.; Henan, U.; Theilmann, L.; Bartenschlager, R. *Science* **1999**, *285*, 110.

21. Kohara, Y.; Imamiya, E.; Kubo, K.; Wada, T.; Inada, Y.; Naka, T. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1903.
22. Katritzsky, A. R.; Wallis, B.; Brownlee, R. T. C.; Topsom, R. D. *Tetrahedron* **1965**, *21*, 1681.
23. *pKa determination*. Data acquired using Sirius Profiler SGA (Sirius Analytical, Forest Row, East Sussex, UK) and built-in software. A solution of compound in DMSO 10 mmol, 20 μL) was diluted to 2 mL using deionized water and infused at 0.25 mL/min into a 1 mL/min pH gradient. Changes in UV spectrum were recorded with the diode array detector during 2 min run with pH changing from 2 to 12 and used to calculate apparent p*K*a of the compound.