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# Synthesis and anti-hepatitis C virus activity of novel ethyl 1*H*-indole-3-carboxylates in vitro

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ABSTRACT

A series of ethyl 1*H*-indole-3-carboxylates **9a$_{1-6}$** and **9b$_{1-2}$** were prepared and evaluated in Huh-7.5 cells. Most of the compounds exhibited anti-hepatitis C virus (HCV) activities at low concentration. The selectivity indices of inhibition on entry and replication of compounds **9a$_2$** (>10; >16.7) and **9b$_1$** (>6.25; >16.7) were higher than those of the other evaluated compounds, including the lead compound Arbidol (ARB, 6; 15). Moreover, the selective index of inhibition on entry of compound **9a$_3$** (>6.25) was higher than that of ARB (6). Of these three initial hits, compound **9a$_2$** was the most potent.



## 1. Introduction

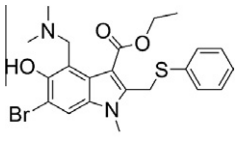
Hepatitis C virus (HCV) infection currently affects approximately 170 million people worldwide and is resolved in only a minority of patients.[1] The chronic viral infection frequently progresses to end-stage liver disease, cirrhosis and in some cases, to the development of hepatocellular carcinoma.[2,3] Therefore, hepatitis C is now the most frequent indication for liver transplantation. There is no therapeutic or prophylactic vaccine available for HCV, and the only effective antiviral therapy, pegylated recombinant interferon α (IFN-α) and ribavirin, produces sustained viral clearance in less than 50% of treated patients.[4] New anti-HCV drugs with novel mechanisms of action are needed.

Ethyl 6-bromo-5-hydroxy-1*H*-indole-3-carboxylate derivatives display a variety of biological effects, such as antiviral effects, immunostimulative effects, and interferon-induced activity. A representative agent is Arbidol (ARB, Fig. 1). Launched in Russia for the prophylaxis and treatment of acute respiratory viral infections, Arbidol acts as an inhibitor of virus entry and membrane fusion.[5–8] ARB is a broad-spectrum antiviral agent that inhibits acute and chronic HCV infection.

The anti-HCV effect appears to be due to the interaction of ARB with membranes and to subsequent ARB-induced membrane alterations. Lipids and membranes are clearly central to the HCV life cycle, where each step is directly related to membrane activity. ARB inhibits steps of the HCV life cycle that are dependent on cell membranes. It displays inhibitory effects on HCV entry, fusion, and replication, thanks to its tropism for membranes. For example, ARB-induced inhibition of HCV nonstructural proteins interactions with endoplasmic reticulum membranes might also contribute to suppression of HCV replication.[9]

The biological properties of ethyl 6-bromo-5-hydroxy-1*H*-indole-3-carboxylates led us to focus on their derivatives as potential anti-HCV agents.

We designed and synthesised several novel ethyl 1*H*-indole-3-carboxylate derivatives in which the hydroxy and bromo groups at the 5- and 6-positions of the indole ring of Arbidol were removed and the amino group was introduced in the 5-position instead of the 4-position. Other modifications were mainly focused on positions 2 and 5 on the indole ring (Fig. 2). We are interested in exploring the effects of these changes on the anti-HCV activity of the resulting compounds.




Arbidol

**Figure 1.** Structure of Arbidol.

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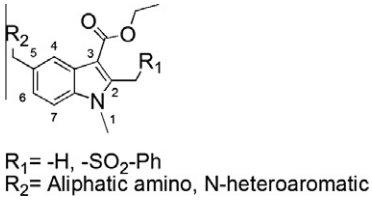



**Figure 2.** General structure of target compounds.

## 2. Results and discussion

### 2.1. Design of derivatives

The general design of ARB analogs was guided by the modular composition of this synthetic product.

ARB antiviral activity toward HCV is due probably to a direct effect of ARB on virus-cell membrane interactions where ARB intercalates into membranes and adopts a consistent orientation with the formation of an 'ARB cage'. This lead to excessive stabilization of cell membranes, which become resistant to HCV fusion.

Its tropism for membranes or membrane like environments is due to ARB's indole-derived structure and to its interaction with lipids via other hydrophobic and aromatic moieties on the molecule.

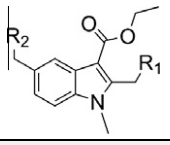
In particular, the indole ring exhibits a preference for membrane interfaces, due to its flat rigid structure and to its aromaticity and establishes cation–π interactions with the positively charged quaternary ammonium lipid headgroups. The *S*-phenyl group could interact with the hydrophobic fatty acid chains of phospholipids inside the bilayer. The amino groups could bind the phosphate moieties of phospholipids, establishing a salt bridge between two adjacent phospholipid molecules as an ion pair complex. At low pH, these interactions increase for the protonation of the amino groups. The ester group could be a substrate for hydrolysis in vivo, leading to intracellular accumulation of the cleaved compound. In light of the above considerations, ARB may be considered a pro-drug which is chemically converted into an active drug by cellular metabolic processes.[9,10]

In order to maintain anti-HCV activity we preserved the groups responsible of Arbidol interaction with membranes: indole ring, *S*-phenyl group, ester group and amino group. In particular, the first series of compounds contains analogs whose structures are closely related to ARB. The indole building block was simplified by elimination of hydroxy and bromo groups at the 5- and 6-positions of the indole ring and by shift of amino group form 4-position to 5-position (Fig. 2) to evaluate the influence of the polar group.

In order to examine whether the nature of the amino group could have an effect on the potential anti-HCV activity, we introduced a variety of amines, including imidazole, pyrrolidine, dimethylamine, 4-methyl piperazine, morpholine and homopiperazine (**$9a_{1-6}$**). Previously, it was demonstrated that the oxidation of sulfide reduced the cellular toxicities and kept up or increased the antiviral activities[5]; in this regard, we introduced phenylsulfonylmethyl groups at the 2-position.

Subsequently, for investigating the influence of changes to the steric environment and the role of phenylsulfonylmethyl group on the antiviral activity, we introduced the methyl group at the 2-position (**$9b_{1-2}$**).

**Table 1**
Structures of compounds **$9a_{1-6}$** and **$9b_{1-2}$**



| Compound | $R_1$ | $R_2$ |
|---|---|---|
| **$9a_1$** | Phenylsulfonyl | Dimethylamino |
| **$9a_2$** | Phenylsulfonyl | Pyrrolidinyl |
| **$9a_3$** | Phenylsulfonyl | 4-Methyl piperazinyl |
| **$9a_4$** | Phenylsulfonyl | Morpholino |
| **$9a_5$** | Phenylsulfonyl | Homopiperazinyl |
| **$9a_6$** | Phenylsulfonyl | Imidazolyl |
| **$9b_1$** | Hydrogen | Dimethylamino |
| **$9b_2$** | Hydrogen | Pyrrolidinyl |

### 2.2. Synthetic approach

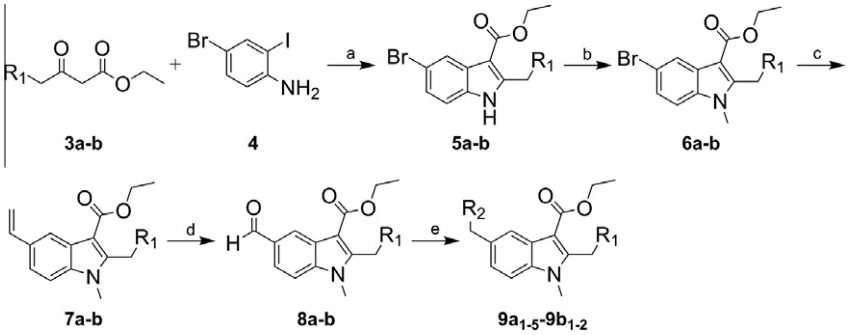
The compounds **$9a_{1-6}$** and **$9b_{1-2}$** were synthesised, and their structures are listed in Table 1.

The synthesis of target compounds **$9a_{1-5}$** and **$9b_{1-2}$** was achieved using a convenient five-step procedure starting from 4-bromo-2-iodoaniline and β-keto esters **3a** and **3b**, respectively, as depicted in Scheme 1.

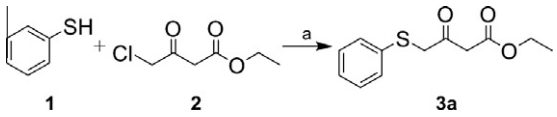
Moreover, the thioether **3a** was prepared via etherification of ethyl 4-chloroacetoacetate **2** with thiophenol **1** (Scheme 2).[11,12]

The compound **3a** and the commercially available compound **3b** were treated with 4-bromo-2-iodoaniline **4** in a copper-catalyzed Ullmann-type coupling reaction to give the key intermediates **5a–b**. The N-alkylation of compounds **5a–b** with iodomethane using the procedure of Kikugawa afforded in high yields the compounds **6a–b**.

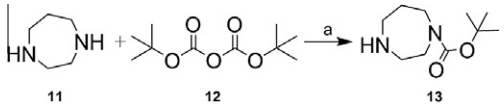
These compounds were subsequently subjected to a Suzuki–Miyaura cross-coupling reaction with potassium vinyltrifluoroborate, affording the intermediates **7a–b** in good yields. The combination of osmium tetroxide and sodium periodate (Lemieux–Johnson oxidation) efficiently achieved both the oxidative cleavage of the vinyl group to an aldehyde group and the oxidation of the sulfide group of compound **7a** to sulfonyl group, thereby yielding the compounds **8a–b**.




**Scheme 1.** Synthesis of target compounds. Reagents and conditions: (a) CuI, BINOL, $Cs_2CO_3$, DMSO, 50 °C, 5–7 h, 48–56%; (b) $ICH_3$, KOH, DMF, 0 °C, 1–2 h, 92–97%; (c) potassium vinyltrifluoroborate, 6 mol% $PdCl_2$, 18 mol % $PPh_3$, $Cs_2CO_3$, THF/$H_2O$ 9:1; 85 °C, 20–22 h, 80–86%; (d) $OsO_4$, 2,6-lutidine, dioxane, $NaIO_4$ in $H_2O$, rt, 20 min, 90–95%; (e) aliphatic amine, acetic acid, THF, $NaB(OAc)_3H$, MW, 100–130 °C, 20 min, 75–95%.



**Scheme 2.** Synthesis of ethyl 3-oxo-4-(phenylthio)butanoate **3a**. Reagents and condition: (a) KOH, methanol, rt, 94%.



**Scheme 3.** Synthesis of *N*-Boc homopiperazine **13**. Reagent and condition: (a) dichloromethane, 0 °C, 1 h, 88%.

Finally, we synthesised the target compounds $\mathbf{9a_{1-5}}$ and $\mathbf{9b_{1-2}}$ via a rapid microwave-assisted reductive amination procedure that involves the formation of an imine and subsequent reduction with $NaB(OAc)_3H$ in a one-pot reaction. To produce compound $\mathbf{9a_5}$, we used *N*-Boc protected homopiperazine, which was synthesised according to Scheme 3.

Imidazole could not be introduced into the 5-position by direct reductive amination with **8a**. Instead, it was realized through a two-step sequence beginning with the reduction of the aldehyde group of compound **8a** to an alcohol group to obtain the intermediate **10a**. This was followed by treatment of **10a** with imidazole and 1,1-carbonyldiimidazole in acetonitrile to generate the derivative $\mathbf{9a_6}$ in good yield (Scheme 4).

### 2.3. Anti-HCV analysis

All of the target compounds $\mathbf{9a_{1-6}}$, $\mathbf{9b_{1-2}}$ and Arbidol were tested in vitro in Huh-7.5 cells for cytotoxicity and anti-HCV activity, specifically, the ability to inhibit the entry and replication of HCV. The properties of these compounds are summarized in Table 2.

Several compounds exhibited inhibitory effects on HCV comparable to Arbidol. Compound $\mathbf{9a_2}$ showed the most potent in vitro anti-HCV activity. Its $IC_{50}$ (5 and 3 μM) values in both entry and replication assays were highly comparable to those of Arbidol (2 and 1 μM, respectively). Interestingly, compound $\mathbf{9a_2}$ did not display cytotoxicity at 50 μM concentration, while at 100 μM concentration an inhibition of cell viability of 95% and 80% on entry and replication, respectively, was detected. Therefore compound $\mathbf{9a_2}$ selectivity indices (>10, >16.7) were higher than those of Arbidol (6, 15).

The analogs $\mathbf{9a_3}$ and $\mathbf{9b_1}$ also exhibited significant efficacy against HCV (structures were listed in Fig. 3). Their $IC_{50}$ values on entry were 8 and 8 μM and on replication were 4 and 3 μM, respectively, which were higher than the positive control Arbidol. Also in these cases, the selectivity indices of $\mathbf{9a_3}$ and $\mathbf{9b_1}$ on HCV entry (>6.3) and on HCV replication (>12.5, >16.7) were higher than or comparable to those of ARB.

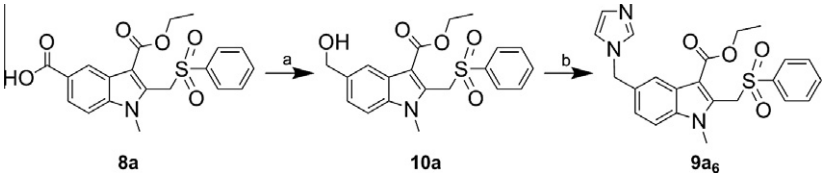
Compounds $\mathbf{9a_{1-6}}$, with the same groups in position 1–3 but different ammines in 5-position, showed an $IC_{50}$, on entry and replication, in the range from 3 to 21 μM concentration. These results suggested that the different amines introduced at the 5-position had a relatively minor influence on the antiviral activities. Moreover, the imidazolyl group in compound $\mathbf{9a_6}$ seemed to shift the selectivity toward inhibition of HCV entry, to reduce the potency against HCV replication and to enhance the cytotoxicity.

Compounds $\mathbf{9b_1}$ and $\mathbf{9b_2}$ exhibited strong anti-HCV effects. This revealed that the elimination of the phenylsulfonyl moiety preserved the anti-HCV activity.

These preliminary results demonstrated that the removal of the 6-bromo and 5-hydroxy groups from the indole ring of Arbidol did not have an influence on its anti-HCV efficacy. This implies that these groups might not be the antiviral pharmacophores.

## 3. Conclusion

In summary, we synthesised a series of new ethyl 1*H*-indole-3-carboxylate derivatives and examined their anti-HCV activities and



**Scheme 4.** Synthesis of compound $\mathbf{9a_6}$. Reagents and conditions: (a) $NaBH_4$ in methanol, THF, rt, 1 h, 91%; (b) imidazole, 1,1-carbonyldiimidazole, acetonitrile, reflux, 16 h, 67%.

**Table 2**
Anti-HCV activity and cytotoxicity of target compounds in vitro

| Compound | HCV entry (genotype 2a) | | | HCV replication (genotype 2a) | | |
|---|---|---|---|---|---|---|
| | $IC_{50}$[a] (μM) | $TC_{50}$[b] (μM) | SI[c] | $IC_{50}$[a] (μM) | $TC_{50}$[b] (μM) | SI[c] |
| $\mathbf{9a_1}$ | 19 | >50 | >2.6 | 10 | >50 | >5 |
| $\mathbf{9a_2}$ | 5 | >50<br>N.A. (50)[d]<br>95% Inh. (100)[e] | >10 | 3 | >50<br>N.A. (50)[d]<br>80% Inh. (100)[e] | >16.7 |
| $\mathbf{9a_3}$ | 8 | >50 | >6.3 | 4 | >50 | >12.5 |
| $\mathbf{9a_4}$ | 14 | >50 | >3.6 | 4 | >50 | >12.5 |
| $\mathbf{9a_5}$ | 21 | >50 | >2.4 | 12 | >50 | >4.2 |
| $\mathbf{9a_6}$ | 5 | 23 | 4.6 | 15 | 18 | 1.2 |
| $\mathbf{9b_1}$ | 8 | >50 | >6.3 | 3 | >50 | >16.7 |
| $\mathbf{9b_2}$ | 13 | >50 | >3.8 | 5 | >50 | >10 |
| Arbidol | 2 | 12 | 6 | 1 | 15 | 15 |

[a] $IC_{50}$ is 50% inhibitory concentration in Huh-7.5 cells.
[b] $TC_{50}$ is 50% cytotoxic concentration.
[c] Selectivity index (SI: $TC_{50}/IC_{50}$).
[d] No cytotoxic activity at 50 μM concentration.
[e] % of inhibition at 100 μM concentration.

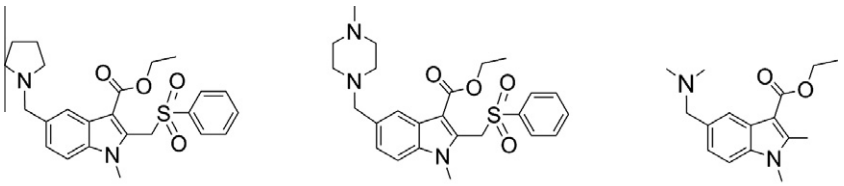



**Figure 3.** Chemical structures of typical compounds that showed significant anti-HCV activity.

cytotoxicities in Huh-7.5 cells. According to the above results, the following conclusions can be drawn:

1. The elimination of the phenylsulfonyl group at the 2-position seemed to preserve the antiviral activities.
2. Compounds with different amines introduced at the 5-position did not show a wide variation in anti-HCV effects.
3. The 6-bromo and 5-hydroxy groups might not be the pharmacophores responsible for the anti-HCV effects.

Despite several changes made to the Arbidol structure in our derivatives, the antiviral activity was maintained, and the cytotoxicity was reduced.

These encouraging results on HCV entry and replication reveal some promising leads in the search for new and efficient anti-HCV therapies.

## 4. Experimental

### 4.1. Chemistry

Microwave experiments were performed in a CEM Discover monomode reactor (CEM Corp., Matthews, NC). All reactions were conducted in a specially adapted cylindrical Pyrex vessel. All reagents were analytical grade and purchased from Sigma–Aldrich (Milano, Italy). Flash chromatography was performed on Carlo Erba silica gel 60 (230–400 mesh; Carlo Erba, Milan, Italy). TLC was carried out using plates coated with silica gel 60F 254 nm purchased from Merck (Darmstadt, Germany). Melting points were determined in open capillary tubes on an Electrothermal 9100 apparatus and are uncorrected. $^1$H NMR spectra were registered on a Bruker AC 300. Chemical shifts are reported in ppm.

All target compounds were assessed for purity by analytical high performance liquid chromatography (HPLC) using an Agilent 1100 series instrument equipped with a UV detector monitoring at 254 nm, HP Chem Station software, and a Waters C18 RP-column (5 μm, 300 mm × 3.9 mm). All target compounds were found to have >95% purity.

Mass spectra (MS) were taken in ESI mode on a Finnigan LCQ Deca *ion trap* instrument. Microanalyses were carried out on a Carlo Erba 1106 elemental analyzer.

Compound **3a** was synthesised in accordance with the literature procedures.[11,12]

#### 4.1.1. General procedure for the synthesis of compounds 5a–b

A mixture of 4-bromo-2-iodoaniline (20 mmol), ethyl acetoacetate **3b** or 3-oxo-4 (phenylthio)butanoate **3a** (22 mmol), CuI (2 mmol), BINOL (4 mmol) and $Cs_2CO_3$ (20 mmol) in DMSO (35 mL) was stirred at 50 °C for 5–7 h under an atmosphere of nitrogen. After this time, the mixture was partitioned between ethyl acetate and saturated $NH_4Cl$. The organic layer was washed with brine, dried over sodium sulfate and concentrated in vacuo. The residue was purified by flash chromatography over silica gel to give 5a–**b**.

**4.1.1.1. Ethyl 5-bromo-2-((phenylthio)methyl)-1*H*-indole-3-carboxylate (5a).** Elution with *n*-hexane/EtOAc (95:5) afforded **5a** (56%) as yellow powder; mp: 142–144 °C; $^1$H NMR ($CDCl_3$): $\delta$ 1.47 (t, $J$ = 7.2 Hz, 3H, –OCH$_2$C*H*$_3$); 4.42 (q, $J$ = 7.2 Hz, 2H, –OC*H*$_2$CH$_3$); 4.80 (s, 2H, –C*H*$_2$-sulfonyl–); 7.18 (d, 1H, –Φ*H*); 7.20–7.5 (m, 6H, –Φ*H* and –Ph*H*); 8.20 (s, 1H, –Φ*H*); 8.8 (s, 1H, –N*H*); MS: *m/z* 390.0, 392.0 [MH$^+$].

**4.1.1.2. Ethyl 5-bromo-2-methyl-1*H*-indole-3-carboxylate (5b).** Elution with toluene/EtOAc (95:5) afforded **5b** (48%) as gray-green powder; mp: 163–165 °C; $^1$H NMR ($CDCl_3$): $\delta$ 1.5 (t, $J$ = 7.2 Hz, 3H, –OCH$_2$C*H*$_3$); 2.75 (s, 3H, –C*H*$_3$); 4.45 (q, $J$ = 7.2 Hz, 2H, –OC*H*$_2$CH$_3$); 7.2 (d, 1H, –Φ*H*); 7.35 (dd, $J$ = 8.55, 1.75 Hz, 1H, –Φ*H*); 8.28 (s, 1H, –Φ*H*); 8.38 (s, 1H, –N*H*); MS: *m/z* 282.1, 284.1 [MH$^+$].

#### 4.1.2. General procedure for the synthesis of compounds 6a–b

To a stirred solution of compound **5a** or **5b** (9 mmol) in DMF (40 mL) at 0 °C was added freshly powdered potassium hydroxide (10 mmol). After stirring the mixture for 30 min at 0 °C, methyl iodide (13.5 mmol) was added dropwise with vigorous stirring. After 1 h, the reaction mixture was diluted with diethyl ether and washed with saturated NaCl solution. The organic layer was dried ($Na_2SO_4$), and the solvent was removed in vacuo to yield **6a–b**.

**4.1.2.1. Ethyl 5-bromo-1-methyl-2-((phenylthio)methyl)-1*H*-indole-3-carboxylate (6a).** White powder (97%); mp: 109–111 °C; $^1$H NMR ($CDCl_3$): $\delta$ 1.45 (t, $J$ = 7.2 Hz, 3H, –OCH$_2$C*H*$_3$); 3.70 (s, 3H, –NC*H*$_3$); 4.35 (q, $J$ = 7.2 Hz, 2H, –OC*H*$_2$CH$_3$); 4.78 (s, 2H, –C*H*$_2$-sulfonyl–); 7.18 (d, 1H, –Φ*H*); 7.20–7.5 (m, 6H, –Φ*H* and –Ph*H*); 8.23 (s, 1H, –Φ*H*); MS: *m/z* 404.0, 406.0 [MH$^+$].

**4.1.2.2. Ethyl 5-bromo-1,2-dimethyl-1*H*-indole-3-carboxylate (6b).** Brown powder (92%); mp: 98–100 °C; $^1$H NMR ($CDCl_3$): $\delta$ 1.5 (t, $J$ = 7.2 Hz, 3H, –OCH$_2$C*H*$_3$); 2.83 (s, 3H, –C*H*$_3$); 3.77 (s, 3H, –NC*H*$_3$); 4.45 (q, $J$ = 7.2 Hz, 2H, –OC*H*$_2$CH$_3$); 7.2 (d, 1H, –Φ*H*); 7.35 (dd, $J$ = 8.55, 1.75 Hz, 1H, –Φ*H*); 8.28 (s, 1H, –Φ*H*); MS: *m/z* 296.1, 298.1 [MH$^+$].

#### 4.1.3. General procedure for the synthesis of compounds 7a–b

A solution of potassium vinyltrifluoroborate (8 mmol), $PdCl_2$ (0.2 mmol), $PPh_3$ (0.6 mmol), $Cs_2CO_3$ (9.6 mmol), and compound **6a** or **6b** (3.2 mmol) in THF/$H_2O$ (9:1) (24 mL) was heated at 85 °C and stirred for 20–22 h. Then, the reaction mixture was cooled to room temperature and diluted with $H_2O$, followed by extraction with dichloromethane. The solvent was removed in vacuo, and the crude product was purified by silica gel chromatography (*n*-hexane/EtOAc, 9:1) to give compounds **7a–b**.

**4.1.3.1. Ethyl 1-methyl-2-((phenylthio)methyl)-5-vinyl-1*H*-indole-3-carboxylate (7a).** Yellow powder (97%); mp: 103–105 °C; $^1$H NMR ($CDCl_3$): $\delta$ 1.42 (t, $J$ = 7.2 Hz, 3H, –OCH$_2$C*H*$_3$); 3.70 (s, 3H, –NC*H*$_3$); 4.35 (q, $J$ = 7.2 Hz, 2H, –OC*H*$_2$CH$_3$); 4.78 (s, 2H, –C*H*$_2$-sulfonyl–); 5.23 (d, $J$ = 10.96 Hz, 1H, C*H*$_2$=CH–); 5.7 (d, $J$ = 11.54 Hz, 1H,

$CH_2$=CH–); 6.87 (dd, $J$ = 17.87, 10.74 Hz, 1H, $CH_2$=C*H*–); 7.25 (d, 1H, –Φ*H*); 7.35–7.5 (m, 6H, –Φ*H* and –Ph*H*); 8.20 (s, 1H, –Φ*H*); MS: *m/z* 352.0 [$MH^+$].

**4.1.3.2. Ethyl 1,2-dimethyl-5-vinyl-1*H*-indole-3-carboxylate (7b).** Yellow powder (92%); mp: 51–53 °C; $^1$H NMR ($CDCl_3$): $\delta$ 1.5 (t, $J$ = 7.2 Hz, 3H, –$OCH_2CH_3$); 2.83 (s, 3H, –$CH_3$); 3.77 (s, 3H, –$NCH_3$); 4.45 (q, $J$ = 7.2 Hz, 2H, –$OCH_2CH_3$); 5.23 (d, $J$ = 11.18 Hz, 1H, $CH_2$=CH–); 5.7 (d, $J$ = 17.54, 1H, $CH_2$=CH–); 6.87 (dd, $J$ = 17.10, 10.74 Hz, 1H, $CH_2$=C*H*–); 7.25 (d, 1H, –Φ*H*); 7.45 (dd, $J$ = 8.55, 1.75 Hz, 1H, –Φ*H*); 8.20 (s, 1H, –Φ*H*); MS: *m/z* 244.0 [$MH^+$].

**4.1.4. General procedure for the synthesis of compounds 8a–b**

$OsO_4$ (0.1 mmol) was added to a stirred mixture of **7a** or **7b** (1 mmol) and 2,6-lutidine (2 mmol) in dioxane (26 mL). The mixture turned from colorless to black in 1 min. Sodium periodate (4 mmol) in water (6 mL, warmed to dissolve) was added. A gray precipitate was immediately formed. The mixture was stirred for 20 min, then extracted with water and dichloromethane. The organic layers were combined, dried, filtered and concentrated. The residue was purified by flash chromatography over silica gel (*n*-hexane/EtOAc, 7:3) to yield **8a–b**.

**4.1.4.1. Ethyl 5-formyl-1-methyl-2-((phenylsulfonyl)methyl)-1*H*-indole-3-carboxylate (8a).** Gray-white powder (95%); mp: 136–138 °C; $^1$H NMR ($CDCl_3$): $\delta$ 1.3 (t, $J$ = 7.2 Hz, 3H, –$OCH_2CH_3$); 4.03 (s, 3H, –$NCH_3$); 4.15 (q, $J$ = 7.2 Hz, 2H, –$OCH_2CH_3$); 5.33 (s, 2H, –$CH_2$-sulfonyl–); 7.45–7.75 (m, 6H, –Φ*H* and –Ph*H*); 7.95 (d, 1H, –Φ*H*); 8.56 (s, 1H, –Φ*H*); 10.12 (s, 1H, –CO*H*); MS: *m/z* 386.1 [$MH^+$].

**4.1.4.2. Ethyl 5-formyl-1,2-dimethyl-1*H*-indole-3-carboxylate (8b).** Beige powder (92%); mp: 118–120 °C; $^1$H NMR ($CDCl_3$): $\delta$ 1.5 (t, $J$ = 7.2 Hz, 3H, –$OCH_2CH_3$); 2.83 (s, 3H, –$CH_3$); 3.77 (s, 3H, –$NCH_3$); 4.45 (q, $J$ = 7.2 Hz, 2H, –$OCH_2CH_3$); 7.45 (d, 1H, –Φ*H*); 7.88 (dd, $J$ = 8.55, 1.75 Hz, 1H, –Φ*H*); 8.67 (s, 1H, –Φ*H*); 10.12 (s, 1H, –CO*H*); MS: *m/z* 246.1 [$MH^+$].

**4.1.5. General procedure for the synthesis of compounds $9a_{1-2}$**

Compound **8a** (0.04 g, 0.1 mmol), dimethylamine or pyrrolidine (0.12 mmol), acetic acid (0.06 mL, 1.02 mmol) and $NaB(OAc)_3H$ (0.05 g, 0.21 mmol) were, in that order, added to dry THF (4 mL). The mixture was irradiated with microwaves for 10 min at 130 °C. Additional amine (0.12 mol) and $NaB(OAc)_3H$ (0.05 g, 0.21 mmol) were added, and the mixture was irradiated at 130 °C for 5 min. This gave 100% conversion to product. The reaction mixture was filtered and concentrated. The residue was dissolved in methanol (1.5 mL) and concd HCl (0.5 mL) and irradiated using microwaves at 100 °C for 5 min. The mixture was filtered and purified by flash chromatography over silica gel (benzene/methanol, 9:1) to give the desired compounds **$9a_{1-2}$**.

**4.1.5.1. Ethyl 5-((dimethylamino)methyl)-1-methyl-2-((phenylsulfonyl)methyl)-1*H*-indole-3-carboxylate ($9a_1$).** Sandy powder (0.033 g, 78%); mp: 205–208 °C; $^1$H NMR ($CDCl_3$): $\delta$ 1.45 (t, $J$ = 7.2 Hz, 3H, –$OCH_2CH_3$); 2.45 (s, 6H, –$N(CH_3)_2$); 3.82 (s, 2H, –$CH_2$N<); 3.95 (s, 3H, –$NCH_3$); 4.05 (q, $J$ = 7.2 Hz, 2H, –$OCH_2CH_3$); 5.25 (s, 2H, –$CH_2$-sulfonyl–); 7.3–7.75 (m, 7H, –Φ*H* and –Ph*H*); 8.0 (s, 1H, –Φ*H*); MS: *m/z* 415.0 [$MH^+$].

**4.1.5.2. Ethyl 1-methyl-2-((phenylsulfonyl)methyl)-5-((pyrrolidin-1yl)methyl)-1*H*-indole-3-carboxylate ($9a_2$).** Beige powder (0.039 g, 87%); mp: 118–120 °C; $^1$H NMR ($CDCl_3$): $\delta$ 1.3 (t, $J$ = 7.2 Hz, 3H, –$OCH_2CH_3$); 1.87 (s, 4H, –pyrrolidinyl *H*); 2.73 (s, 4H, –pyrrolidinyl *H*); 3.82 (s, 2H, –$CH_2$N<); 3.95 (s, 3H, –$NCH_3$); 4.05 (q, $J$ = 7.2 Hz, 2H, –$OCH_2CH_3$); 5.25 (s, 2H, –$CH_2$-sulfonyl–); 7.3–7.75 (m, 7H, –Φ*H* and –Ph*H*); 8.0 (s, 1H, –Φ*H*); MS: *m/z* 441.0 [$MH^+$].

**4.1.6. Synthesis of *N*-Boc homopiperazine 13**

To a solution of dichloromethane (45 mL) and homopiperazine **11** (1.84 g, 18 mmol), maintained at 0 °C using an ice bath, was added dropwise a di-*tert*-butyldicarbonate solution **12** (2 g, 9 mmol in 18 mL dichloromethane). The mixture was stirred for an additional 1 h then was filtered. The filtrate was concentrated to dryness. Water (27 mL) was added to the resulting oil, and the mixture was filtered. The filtrate was saturated with potassium carbonate and extracted with diethyl ether (3 × 13 mL). The solvent was dried over sodium sulfate and concentrated to dryness, yielding **13** (1.58 g, 88%).

**4.1.7. General procedure for the synthesis of compounds $9a_{3-5}$**

Compound **8a** (0.04 g, 0.1 mmol), 4-methylpiperazine or morpholine or 1-BOC-homopiperazine (0.15 mmol), acetic acid (0.06 mL, 1.02 mmol) and $NaB(OAc)_3H$ (0.055 g, 0.24 mmol) were, in that order, added to dry THF (4 mL). The mixture was irradiated with microwaves for 10 min at 130 °C. Additional amine (0.15 mol) and $NaB(OAc)_3H$ (0.055 g, 0.24 mmol) were added, and the mixture was irradiated at 130 °C for 5 min. This gave 100% conversion to product. The reaction mixture was filtered and concentrated. The residue was dissolved in methanol (1.5 mL) and concd HCl (0.5 mL) and irradiated using microwaves at 100 °C for 5 min. The mixture was filtered and purified by flash chromatography over silica gel to give the desired compounds **$9a_{3-5}$**.

**4.1.7.1. Ethyl 1-methyl-5-((4-methylpiperazin-1-yl)methyl)-2-((phenylsulfonyl)methyl)-1*H*-indole-3-carboxylate ($9a_3$).** Elution with benzene/MeOH (9:1) afforded **$9a_3$** (0.045 g, 94%) as a brownish-yellow powder; mp: 94–96 °C; $^1$H NMR ($CDCl_3$): $\delta$ 1.3 (t, $J$ = 7.2 Hz, 3H, –$OCH_2CH_3$); 2.57 (s, 3H, 4-$CH_3$ of piperazinyl); 2.75–3.05 (m, 8H, 8*H* of piperazinyl); 3.75 (s, 2H, –$CH_2$N<); 3.95 (s, 3H, –$NCH_3$); 4.05 (q, $J$ = 7.2 Hz, 2H, –$OCH_2CH_3$); 5.25 (s, 2H, –$CH_2$-sulfonyl–); 7.3–7.7 (m, 7H, –Φ*H* and –Ph*H*); 7.95 (s, 1H, –Φ*H*); MS: *m/z* 470.1 [$MH^+$].

**4.1.7.2. Ethyl 1-methyl-5-(morpholinomethyl)-2-((phenylsulfonyl)methyl)-1*H*-indole-3-carboxylate ($9a_4$).** Elution with benzene/MeOH (95:5) afforded **$9a_4$** (0.039 g, 85%) as an rose powder; mp: 109–111 °C; $^1$H NMR ($CDCl_3$): $\delta$ 1.3 (t, $J$ = 7.2 Hz, 3H, –$OCH_2CH_3$); 2.53 (s, 4H, 4*H* of morpholino); 3.65 (s, 2H, –$CH_2$N<); 3.75 (s, 4H, 4*H* of morpholino); 3.95 (s, 3H, –$NCH_3$); 4.05 (q, $J$ = 7.2 Hz, 2H, –$OCH_2CH_3$); 5.25 (s, 2H, –$CH_2$-sulfonyl–); 7.3–7.7 (m, 7H, –Φ*H* and –Ph*H*); 7.95 (s, 1H, –Φ*H*); MS: *m/z* 457.0 [$MH^+$].

**4.1.7.3. Ethyl 5-((1,4-diazepan-1yl)methyl)-1-methyl-2-((phenylsulfonyl)methyl)-1*H*-indole-3-carboxylate ($9a_5$).** Elution with benzene/MeOH/$NH_4OH$ (9:1:0.25) afforded **$9a_5$** (0.04 g, 83%) as a white powder; mp: 98–100 °C; $^1$H NMR ($CD_3OD$): $\delta$ 1.3 (t, $J$ = 7.2 Hz, 3H, –$OCH_2CH_3$); 2.0 (s, 1H, –N*H* of homopiperazine); 2.1 (m, 2H, 2*H* of homopiperazine); 2.95 (t, 2H, 2*H* of homopiperazine); 3.1 (t, 2H, 2*H* of homopiperazine); 3.35 (t, 4H, 4*H* of homopiperazine); 3.90 (s, 3H, –$NCH_3$); 4.0 (s, 2H, –$CH_2$N<); 4.15 (q, $J$ = 7.2 Hz, 2H, –$OCH_2CH_3$); 5.4 (s, 2H, –$CH_2$-sulfonyl–); 7.4–7.75 (m, 7H, –Φ*H* and –Ph*H*); 8.05 (s, 1H, –Φ*H*); MS: *m/z* 470.1 [$MH^+$].

**4.1.8. General procedure for the synthesis of compounds $9b_{1-2}$**

Compound **8b** (0.04 g, 0.1 mmol), dimethylamine or pyrrolidine (0.12 mmol), acetic acid (0.095 mL, 1.02 mmol) and $NaB(OAc)_3H$ (0.074 g, 0.21 mmol) were, in that order, added to dry THF (4 mL). The mixture was irradiated with microwaves for 10 min at 130 °C. Additional amine (0.12 mmol) and $NaB(OAc)_3H$

(0.074 g, 0.21 mmol) were added, and the mixture was irradiated at 130 °C for 5 min. This gave 100% conversion to product. The reaction mixture was filtered and concentrated. The residue was dissolved in methanol (1.5 mL) and concd HCl (0.5 mL) and irradiated using microwaves at 100 °C for 5 min. The mixture was filtered and purified by flash chromatography over silica gel (benzene/methanol, 95:5) to give the desired compounds **9b$_{1-2}$**.

**4.1.8.1. Ethyl 5-((dimethylamino)methyl)-1,2-(dimethyl)-1*H*-indole-3-carboxylate (9b$_1$).** Bone powder (0.033 g, 75%); mp: 67–69 °C; $^1$H NMR ($CDCl_3$): $\delta$ 1.45 (t, $J$ = 7.2 Hz, 3H, –OCH$_2$C*H*$_3$); 2.65 (s, 6H, –N(C*H*$_3$)$_2$); 2.83 (s, 3H, –C*H*$_3$); 3.77 (s, 3H, –NC*H*$_3$); 4.30 (s, 2H, –C*H*$_2$– N<); 4.45 (q, $J$ = 7.2 Hz, 2H, –OC*H*$_2$CH$_3$); 7.45 (d, 1H, –Φ*H*); 7.60 (dd, $J$ = 8.55, 1.75 Hz, 1H, –Φ*H*); 8.15 (s, 1H, –Φ*H*); MS: *m/z* 275.0 [MH$^+$].

**4.1.8.2. Ethyl 5-((dimethylamino)methyl)-1,2-(dimethyl)-1*H*-indole-3-carboxylate (9b$_2$).** Nut-brown powder (0.04 g, 83%); mp: 125–127 °C; $^1$H NMR ($CDCl_3$): $\delta$ 1.45 (t, $J$ = 7.2 Hz, 3H, –OCH$_2$C*H*$_3$); 1.59 (m, 4H, –pyrrolidinyl *H*); 2.25 (m, 4H, –pyrrolidinyl *H*); 2.83 (s, 3H, –C*H*$_3$); 3.77 (s, 3H, –NC*H*$_3$); 4.30 (s, 2H, –C*H*$_2$– N<); 4.45 (q, $J$ = 7.2 Hz, 2H, –OC*H*$_2$CH$_3$); 7.45 (d, 1H, –Φ*H*); 7.78 (dd, $J$ = 8.55, 1.75 Hz, 1H, –Φ*H*); 8.15 (s, 1H, –Φ*H*); MS: *m/z* 301.0 [MH$^+$].

### 4.1.9. Synthesis of ethyl 5-(hydroxymethyl)-1-methyl-2-((phenyl sulfonyl)methyl)-1*H*-indole-3-carboxylate (10a)

To an ice-cooled solution of the corresponding aldehyde **8a** (0.12 g, 0.3 mmol) in THF (1.5 mL) was added $NaBH_4$ (0.029 g, 0.75 mmol) in methanol (3.8 mL). Then, the resulting mixture was stirred at rt for 1 h. After complete conversion, the solvent was distilled off under reduced pressure. Then, water was added, and the resulting mixture was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over $Na_2SO_4$ and evaporated under reduced pressure. The desired product was purified by chromatography on silica gel (hexane/EtOAc 6:4) to give **10a** (0.106 g, 91%) as white solid; mp: 172–174 °C; $^1$H NMR ($CDCl_3$): $\delta$ 1.3 (t, $J$ = 7.2 Hz, 3H, –OCH$_2$C*H*$_3$); 2.0 (s, 1H, –CH$_2$O*H*); 3.97 (s, 3H, –NC*H*$_3$); 4.05 (q, $J$ = 7.2 Hz, 2H, –OC*H*$_2$CH$_3$); 4.82 (s, 2H, –C*H*$_2$-sulfonyl–); 5.28 (s, 2H, –C*H*$_2$OH); 7.4–7.75 (m, 7H, –Φ*H* and –Ph*H*); 8.05 (s, 1H, –Φ*H*); MS: *m/z* 388.1 [MH$^+$].

### 4.1.10. Synthesis of ethyl 5-((1*H*-imidazol-1-yl)methyl)-1-methyl-2-((phenyl sulfonyl)methyl)-1*H*-indole-3-carboxylate (9a$_6$)

To a solution of compound **10a** (0.06 g, 0.15 mmol) in acetonitrile (1.5 mL) was added CDI (0.127 g, 0.75 mmol) and imidazole (0.021 g, 0.3 mmol). The resulting solution was heated to reflux for 16 h. After cooling to ambient temperature, the reaction mixture was diluted with water and extracted with ethyl acetate. The combined organic phases were washed with brine, dried over $Na_2SO_4$ and evaporated under reduced pressure. The desired product was purified by chromatography on silica gel (dichloromethane/methanol, 98:2) to obtain **9a$_6$** (0.044 g, 67%) as a pearl-white powder; mp: 95–97 °C; $^1$H NMR ($CDCl_3$): $\delta$ 1.3 (t, $J$ = 7.2 Hz, 3H, –OCH$_2$C*H*$_3$); 3.97 (s, 3H, –NC*H*$_3$); 4.05 (q, $J$ = 7.2 Hz, 2H, –OC*H*$_2$CH$_3$); 5.28 (s, 4H, –C*H*$_2$-sulfonyl– and –C*H*$_2$N<); 6.95 (s, 1H, 2-1*H* of imidazolyl) 7.05–7.2 (m, 2H, 4,5-2*H* of imidazolyl); 7.4–7.75 (m, 7H, –Φ*H* and –Ph*H*); 7.9 (s, 1H, –Φ*H*); MS: *m/z* 438.1 [MH$^+$].

## 4.2. Biological assay

### 4.2.1. Cell lines and culture conditions

The human hepatoma-derived cell line Huh-7.5 was grown in high-glucose Dulbecco's modified Eagles' medium (DMEM; Life Technologies) supplemented with 2 mM L-glutamine, 100 U/mL of penicillin, 100 μg/mL of streptomycin, and 10% fetal bovine serum.[13] Cells were sub-cultivated twice per week, with a 1:4 split ratio.

### 4.2.2. In vitro anti-HCV assay

The antiviral activities of compounds **9a$_{1-5}$**, **9b$_{1-2}$** against HCV were measured in Huh-7.5 hepatoma cells using replication and entry assays as previously described by Pacini et al.[14] For the replication assay, an RNA transcript of a replicon containing a firefly luciferase reporter was transfected in Huh-7.5 cells; these were subsequently plated on 96-well plates in the presence of test compounds and Arbidol. For the infection assay, viral particles containing a subgenomic HCV RNA carrying a firefly luciferase reporter gene were used to infect naive Huh-7.5 cells; these were plated in a 96-well format in the presence of test compounds and Arbidol. After three days of incubation luciferase activity was measured with a Bright-Glo luciferase assay system (Promega) following the manufacturer's instructions. The $IC_{50}$ and selectivity indices of the evaluated compounds and Arbidol were calculated.

### 4.2.3. Cytotoxicity assay

Cytotoxicity induced by the test compounds in cultures of cells was also determined.

The toxicity of compounds **9a$_{1-5}$** and **9b$_{1-2}$** in Huh-7.5 cells was evaluated by CellTiter-Blue Viability Assay (Promega) that is used to estimate the number of viable cells present in multiwell plates; the reagent is a buffered solution containing highly purified resazuin. Resazuin is dark blue in color and has little intrinsic fluorescence. Metabolically active cells can reduce resazuin to resorufin, which is pink and highly fluorescent, with an excitation maximum of 579 nm and an emission maximum of 584 nm. Nonviable cells rapidly lose metabolic capacity, do not reduce the indicator dye, and thus do not generate a fluorescent signal.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.06.058.

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