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Original research paper

Synthesis of some benzimidazole derivatives endowed with 1,2,3-triazole as potential inhibitors of hepatitis C virus

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New derivatives of 2-thiobenzimidazole incorporating triazole moiety were synthesized, characterized and tested *in vitro* for antiviral activity against hepatitis C virus (HCV) and hepatitis B virus (HBV). Their cytotoxicity was determined by the reduction in the number of viable cell. All of the synthesized compounds are inactive against HBV and some showed activity against HCV. In particular, two compounds showed significant activity, 2-{4-[(1-benzoylbenzimidazol-2-ylthio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-(*p*-nitro-phenyl)-acetamide (**13**) and 2-(4-{[1-(*p*-chlorobenzoyl)-benzimidazol-2-ylthio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-(*p*-nitrophenyl)-acetamide (**17**). The results give an insight into the importance of the substituent at position 2 of benzimidazole for the inhibition of HCV.

Keywords: benzimidazole, triazole, anilide, antiviral, HCV, HBV

Hepatitis C virus (HCV), a member of the *Hepacvirus* genus of the family *Flavivridae*, is a major etiological agent of human liver disease. HCV has infected an estimated 170 million people worldwide. HCV infection is often asymptomatic; however, it frequently causes chronic hepatitis, which progresses to the end-stage liver diseases, such as liver cirrhosis and hepatocellular carcinoma. For a long time, the therapeutic combination of pegylated alpha interferon (peg-IFN) and the nucleoside analogue ribavirin were used for

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HCV treatment and were found to have a sustained viral response (SVR) rate of ~50 % in genotype 1 and 4 of HCV-infected patients (1). Recently, a new anti-HCV agent (Sofosbuvir^R) was added to the above mentioned combination and produced improvement in the SVR up to ~90 % (1). However, peg-IFN and ribavirin should be included for successful treatment, particularly in the treatment of genotype 4. Combined therapy of HCV infections with peg-IFN is associated with serious side effects such as depression, flu-like symptoms, fatigue and hemolytic anemia caused by ribavirin (2, 3).

Therefore, finding a single compound for the treatment of HCV that promises to cure the majority of patients without the complications of combined therapy and/or the need for painful injections would be a good achievement.

Owing to its isosterism with indole and purine nuclei present in many fundamental cellular components and bioactive compounds, the benzimidazole ring represents a kind of privileged substructure from which several important drugs used in different therapeutic areas have been obtained, especially antitumor and antiviral agents. Among the antiviral benzimidazoles, an important position is held by compounds acting against HIV-1, HCV and respiratory syncytial virus (RSV) (4, 5).

The benzimidazole scaffold is common in different anti-HCV agents with different modes of action. Derivatives of bis-benzimidazolemethane were discovered to be highly potent, reversible and selective serine protease inhibitors (6, 7). Furthermore, by replacing the 2-methylbenzimidazole moiety of this compound with 2-pyrimidomethylenethio moiety or its bioisosteric 2-pyrimidomethyleneamino group was shown to have a positive impact on the inhibitory activity against HCV (8). Moreover, several 2-substituted benzimidazoles showed good antiviral activity (9, 10).

The antiviral activity was found in all the subsets of benzimidazole derivatives, but it was not evenly distributed among them. The activity is a result of several interacting structural features, which may direct it *vs.* specific viruses, modulating also their potency (11).

On the other hand, the 1,2,3-triazole core has been applied in many synthetic approaches. Several compounds containing this heterocycle exhibited a broad range of biological activities including against HIV and HCV (12–15).

Considering the importance of 2-substituted benzimidazole and triazole nucleus, it was thought worthwhile to design and synthesize some new 2-substituted benzimidazole derivatives connected to differently substituted anilides with triazole moiety (Fig. 1) and to screen them for their potential antiviral activity. This approach may provide scaffolds in which pharmacophores can be arranged so as to yield potent and hopefully selective inhibitors.

EXPERIMENTAL

Chemistry

Reagents and solvents. – All reagents and solvents were obtained from commercial suppliers and were used without further purification. The starting materials, 2-(prop-2-ynylthio)-1*H*-benzimidazole (1, 16), 2-azido-*N*-phenylacetamide (**3a**), 2-azido-*N*-(*p*-chlorophenyl)acetamide (**3b**), 2-azido-*N*-(*p*-bromophenyl)acetamide (**3c**), 2-azido-*N*-(*p*-methylphenyl)

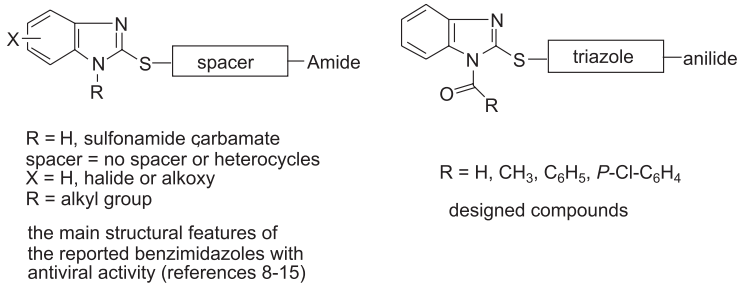


Fig. 1. Rationale for the design of target compounds **4–18.**

acetamide (**3d**) and 2-azido-*N*-(*p*-nitrophenyl)acetamide (**3e**) were prepared according to reported procedures (17).

Procedures. – Melting points are uncorrected and were determined on an electrothermal melting point apparatus (Stuart Scientific, model SMP3, UK). Precoated silica gel plates (Kieselgel 0.25 mm, 60G F254, Merck, Germany) were used for TLC monitoring of the reactions. The developing solvent system CH_2Cl_2/CH_3OH (9.5:0.5, *V*/*V*) was used and the spots were detected at 254 nm using an ultraviolet lamp (model CM-10, Spectroline, USA).

IR spectra (KBr discs) were recorded on a Shimadzu IR-470 spectrometer (Shimadzu, Japan). ^1H (500 MHz) and ^{13}C NMR (125 MHz) spectra were obtained with JEOL JNM-ECA 500 spectrometer (JEOL, Japan) with tetramethylsilane (TMS) as an internal standard. Chemical shifts were expressed in δ-value (ppm) relative to TMS. DMSO-d_6 was used as a solvent. Addition of deuterium oxide was used for the detection of exchangeable protons. Mass spectra were recorded with a JEOL JMS600 mass spectrometer.

*2-Acyl-1-propargyl-thiobenzimidazoles (**2a–c**). General procedure*. – To a suspension of 2-(prop-2-ynyl-thio)-1*H*-benzimidazole (**1**) (188 mg, 1 mmol) (16) and potassium carbonate anhydrous (145 mg, 1.05 mmol, 1.05 equivalent) in dry acetone (10 mL), the appropriate acyl halide (1.05 equivalent) was added. The reaction mixture was stirred for 12–18 h at ambient temperature. Acetone was evaporated, the residue was treated with water and then extracted with CH_2Cl_2 (3 x 10 mL). The organic extract was washed with water, dried over anhydrous Na_2SO_4 and then concentrated. The obtained crude products were recrystallized from DMF/H_2O (7:3).

*2-{4-[(1-Acylbenzimidazol-2-yl-thio)methyl]-1*H*-1,2,3-triazol-1-yl}-N-(unsubstituted/substituted phenylacetamides (**4–18**). General procedure*. – To a solution of propargyl derivatives **1** or **2a–c** (1.3 mmol) in a mixture of THF/H_2O (12 mL, 2:1), the reported azide derivatives **3a–e** (17) (1.6 mmol), copper sulphate (0.13 mmol) and sodium ascorbate (0.26 mmol) were added. The resulting mixture was stirred at room temperature for 20 h, and the residue was extracted with CH_2Cl_2 (3 x 15 mL). The organic phase was washed with water, dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by column chromatography using CH_2Cl_2/methanol (9:1) as an eluent to give pure products (**4–18).**

Physical and spectral data of the newly synthesized compounds (**2a–c** and **4–18)** are collected in Tables I and II.

Table I. Physical and ESI-HRMS data of the newly synthesized compounds

Compound		M. p. (°C)	Yield (%)	Mol. formula	ESI-HRMS (calcd./found)
2a	1-Acetyl-2-propyn-2-ylthiobenzimidazole	163–165	86	$C_{12}H_{11}N_2OS$	231.0592 231.0586
2b	1-Benzoyl-2-propyn-2-ylthiobenzimidazole	131–132	81	$C_{17}H_{13}N_2OS$	293.0749 293.0753
2c	1-*p*-Chlorobenzoyl-2-propyn-2-ylthiobenzimidazole	129–130	72	$C_{17}H_{12}ClN_2OS$	327.0359 327.0365
4	2-{4-[(1-Acetylbenzimidazol-2-ylthio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-phenylacetamide	196–198	89	$C_{20}H_{19}N_6O_2S$	407.1297 407.1290
5	2-{4-[(1-Acetylbenzimidazol-2-ylthio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-(*p*-chlorophenyl)-acetamide	201–203	93	$C_{20}H_{18}ClN_6O_2S$	441.0900 441.0896
6	2-{4-[(1-Acetylbenzimidazol-2-ylthio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-(*p*-bromophenyl)-acetamide	202–204	83	$C_{20}H_{18}BrN_6O_2S$	485.0395 485.0391
7	2-{4-[(1-Acetylbenzimidazol-2-ylthio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-(*p*-methylphenyl)-acetamide	207–209	88	$C_{21}H_{21}N_6O_2S$	421.1447 421.1451
8	2-{4-[(1-Acetylbenzimidazol-2-ylthio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-(*p*-nitrophenyl)-acetamide	195–197	87	$C_{20}H_{18}N_7O_4S$	452.1141 452.1141
9	2-{4-[(1-Benzoylbenzimidazol-2-ylthio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-phenylacetamide	198–199	91	$C_{25}H_{21}N_6O_2S$	469.1447 469.1450
10	2-{4-[(1-Benzoylbenzimidazol-2-ylthio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-(*p*-chlorophenyl)acetamide	199–201	90	$C_{25}H_{20}ClN_6O_2S$	503.1057 503.1060
11	2-{4-[(1-Benzoylbenzimidazol-2-ylthio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-(*p*-bromophenyl)-acetamide	206–208	89	$C_{25}H_{20}BrN_6O_2S$	547.0552 547.0544
12	2-{4-[(1-Benzoylbenzimidazol-2-ylthio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-(*p*-methylphenyl)-acetamide	197–199	89	$C_{26}H_{23}N_6O_2S$	483.1603 483.1604
13	2-{4-[(1-Benzoylbenzimidazol-2-ylthio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-(*p*-nitrophenyl)-acetamide	201–203	91	$C_{25}H_{20}N_7O_4S$	514.1298 514.1292
14	2-{4-[(1-(*p*-Chlorobenzoyl)-benzimidazol-2-ylthio) methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-phenyl-acetamide	196–198	92	$C_{25}H_{20}ClN_6O_2S$	503.1057 503.1058
15	2-(4-{[1-(*p*-Chlorobenzoyl)-benzimidazol-2-ylthio) methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-(*p*-chlorophenyl) acetamide	206–208	87	$C_{25}H_{19}Cl_2N_6O_2S$	537.0667 537.0669

16	2-(4-{[1-(*p*-Chlorobenzoyl)-benzimidazol-2-ylthio] methyl}-1*H*-1,2,3-triazol-1-yl)-*N*-(*p*-bromophenyl) acetamide	211–212	91	$C_{25}H_{19}BrClN_6O_2S$	581.0162 581.0157
17	2-(4-{[1-(*p*-Chlorobenzoyl)-benzimidazol-2-ylthio) methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-(*p*-nitrophenyl) acetamide	191–193	84	$C_{25}H_{19}ClN_7O_4S$	548.0908 548.0903
18	2-{4-[(1*H*-Benzimidazol-2-ylthio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-(*p*-methylphenyl)-acetamide	195–197	88	$C_{19}H_{19}N_6O_2S$	379.1341 379.1330

Antiviral activity

HepG2.2.15.7 cells and HepG2.2.15 clone, producing a high amount of HBV, Luc-neo#2 and subgenomic HCV replicon cells with luciferase reporter used in testing anti-HBV and anti-HCV activity, respectively, were established and provided by Drs. Takaji Wakita and Koichi Watashi (Department of Virology II, National Institute of Infectious Diseases, Tokyo, Japan). All compounds, **4**–**18**, were screened for their anti-HBV and anti-HCV activity using the reported method (18, 19). Reference inhibitors were used: lamivudine (3TC) for HBV and telaprevir (TLV) for HCV (1). Cytotoxicity was evaluated by the reduction in the number of viable cells using the tetrazolium dye method (18, 19). Tetracolor One® (Seikagaku Corporation, Japan) is a water-soluble tetrazolium dye that is used to determine the cell viability.

Each compound was dissolved in DMSO to produce 20 mmol L^{-1} stock solution. Each point in the dose-response curve represents the average of 3 readings.

Anti-HBV activity. – Determination of anti-HBV activity of benzimidazole derivatives was based on the inhibition of virus-induced cytopathicity. HepG2.2.15.7 cells (1×10^4 cells per well) were inoculated into a microtiter plate. After incubation for 24 h, the cells were cultured in the presence of various concentrations of the tested compounds. After incubation for 3 and 6 days, the culture medium was replaced with fresh one containing an appropriate concentration of the compound. The cells were further incubated for 3 days. At the end of incubation, the culture supernatants were collected and examined for their HBV DNA levels by real-time PCR. The cells were examined for their viability by the tetrazolium dye method (18, 19).

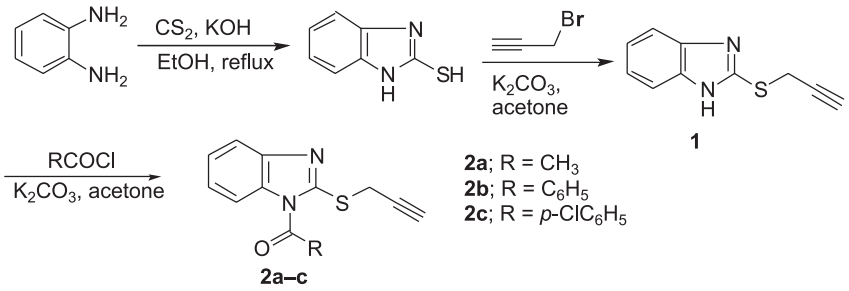
Anti-HCV activity. – The LucNeo#2 cells (5×10^3 cells per well) were cultured in a 96-well plate in the absence of G418, an aminoglycoside commonly used to generate stable replicons for RNA viruses, such as hepatitis C virus, West Nile virus, and bovine viral diarrhoea virus (BVDV), and in the presence of various concentrations of the compounds. After incubation for 3 days, the culture medium was removed, and the cells were washed once with phosphate buffer saline (PBS). Lysis buffer was added to each well, and the lysate was transferred to the corresponding well of a non-transparent 96-well plate. The luciferase activity was measured by addition of the luciferase reagent in a lucerfrease assay kit using a luminometer with automatic injectors (18, 19).

Cytotoxicity assay. – LucNeo#2 cells (5 x 10^3 cells per well) were cultured in a 96-well plate in the absence of G418 and in the presence of various concentrations of the compounds. After incubation for 3 days, the culture supernatants (100 μL) were removed and the tetra-Color ONE (10 μL) was added to each well. The cells were further incubated at 37 °C for 1 h the absorbance of each well was measured at 450 nm with a microplate reader (18, 19).

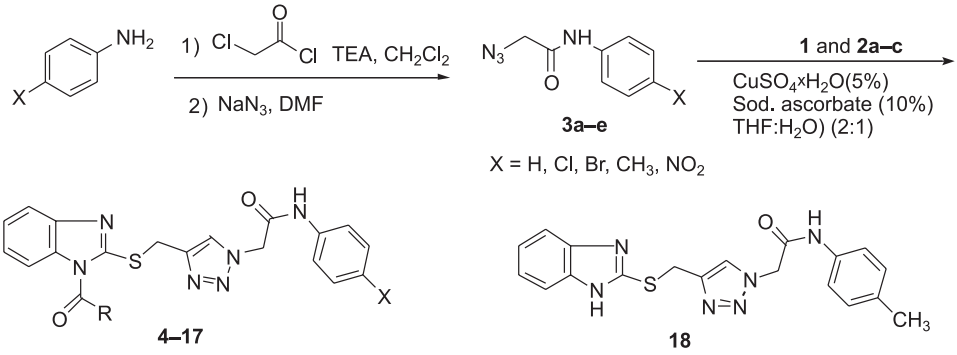
RESULTS AND DISCUSSION

Chemistry

In this report, hybrid molecules were prepared as illustrated in Schemes 1 and 2. Acylation of 2-(prop-2-ynyl-thio)-1*H*-benzimidazole (**1)** (16) with different acyl halides in the presence of anhydrous potassium carbonate in acetone afforded 1-acyl-2-propargyl-thio derivatives (**2a–c**). Structures of these compounds were established by IR, NMR and HRMS. In IR spectra, disappearance of the absorption band at 3410–3420 cm^{-1}, along with the appearance of a strong absorption band corresponding to the carbonyl group (1680–1700 cm^{-1}), confirmed *N*-acylation. In ^1H NMR spectra, 1-acetyl-2-propyn-2-yl-thiobenzimidazole (**2a**) was characterized by the appearance of a singlet at 2.86 ppm, while integration



Scheme 1.



Scheme 2.

Table II. Spectral data of the newly synthesized compounds

Compd.	IR (ν, cm^{-1})	^{1}H NMR (500 MHz, DMSO-d_6) (δ, ppm)	^{13}C NMR (125 MHz, DMSO-d_6) (δ, ppm)
2a	3307 (spC-H), 3030, 1716 (C=O), 1473, 1456, 1371, 1315 (C=C)	7.83 (d, *J* = 7.9 Hz, 1H), 7.68 (d, *J* = 7.9 Hz, 1H), 7.40–7.35 (m, 2 H), 4.10 (s, 2H), 3.22 (s, 1H), 2.86 (s, 3H)	170.8, 154.2, 144.4, 133.8, 125.3, 124.4, 119.3, 115.0, 80.9, 74.7, 26.9, 21.0
2b	3307 (spC-H), 3010, 1697 (C=O), 1600, 1473, 1450, 1348, 1313 (C=C), 1193, 700, 648	7.83–7.65 (m, 6H), 7.31 (d, *J* = 7.2 Hz, 1H), 7.12 (t, *J* = 7.2 Hz, 1H), 6.60 (d, *J* = 8.2 Hz, 1H), 4.18 (s, 2H), 3.7 (s, 1H)	168.6, 153.9, 144.2, 134.8, 134.6, 133.6, 130.0, 129.9, 125.3, 124.1, 119.4, 114.4, 80.6, 75.0, 21.5
2c	3307 (spC-H), 3010, 1697 (C=O), 1600, 1473, 1450, 1348, 1313 (C=C), 1193, 700, 648	7.86 (d, *J* = 8.6 Hz, 2H), 7.75–7.69 (m, 3H), 7.33–7.31 (m, 1H), 7.18–7.16 (m, 1H), 6.71 (d, *J* = 8.0 Hz, 1H), 4.18 (s, 2H), 3.26 (s, 1H)	167.6, 153.8, 144.2, 139.7, 134.7, 132.3, 132.0, 130.2, 125.4, 124.3, 119.4, 114.5, 80.5, 75.1, 21.5
4	3460 (NH), 1706, 1608 (C=O), 1576, 1544, 184, 1475 (C=C), 715, 742	10.49 (s, 1H), 8.18 (s, 1H), 7.85 (d, *J* = 8.2 Hz, 1H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.60 (d, *J* = 8.2 Hz, 2 H), 7.39–7.35 (m, 4H), 7.12 (t, *J* = 8.2 Hz, 1H), 5.34 (s, 2H), 4.62 (s, 2H), 2.86 (s, 3H)	170.7, 165.2, 155.1, 144.5, 143.4, 139.4, 133.9, 129.9, 126.5, 125.2, 124.7, 124.3, 120.2, 119.4, 115.1, 53.2, 27.5, 27.0
5	IR3430 (NH), 1710, 1690 (C=O), 1607, 1581, 1548, 1456 (C=C), 840	10.63 (s, 1H), 8.18 (s, 1H), 7.85 (d, *J* = 8.0 Hz, 1H), 7.70 (d, *J* = 6.9 Hz, 1H), 7.62 (d, *J* = 6.9 Hz, 2 H), 7.43–7.35 (4H, m), 5.35 (s, 2H), 4.69 (s, 2H), 2.86 (s, 3H)	170.8, 165.4, 155.1, 144.6, 143.5, 133.9, 130.2, 129.9, 128.4, 126.5, 125.3, 124.4, 121.8, 119.5, 115.2, 53.2, 27.6, 27.1
6	3445 (NH), 1704, 1686 (C=O), 1604, 1582, 1554, 1485 (C=C), 712, 743	10.63 (s, 1H), 8.18 (s, 1H), 7.85–7.84 (m, 3H), 7.71 (s, 1H), 7.58–7.54 (m, 2H), 7.40–7.34 (m, 2H), 5.35 (s, 2H), 4.62 (s, 2H), 2.86 (s, 3H)	170.5, 165.3, 154.9, 144.5, 143.3, 138.6, 133.8, 132.6, 126.3, 125.2, 124.2, 122.1, 119.3, 116.3, 115.0, 53.1, 27.4, 26.9
7	3435 (NH), 1696, 1687 (C=O), 1608, 1554, 1492, 1455 (C=C), 830	10.4 (s, 1H), 8.17 (s, 1H), 7.85 (d, *J* = 7.6 Hz, 1H), 7.72 (d, *J* = 6.9 Hz, 2H), 7.47 (d, *J* = 8.2 Hz, 1H), 7.39–7.35 (m, 2H), 7.16 (d, *J* = 8.2 Hz, 2H), 5.32 (s, 2H), 4.62 (s, 2H), 2.86 (s, 3H), 2.92 (s, 3H)	170.7, 164.9, 155.1, 144.5, 143.3, 136.8, 133.9, 133.7. 130.2, 126.4, 125.2, 124.3, 120.2, 119.4, 115.1, 53.3, 27.5, 27.0, 21.4
8	3435 (NH), 1710, 1687 (C=O), 1607, 1556, 1518, 1456 (C=C), 837	11.10 (s, 1H), 8.27 (d, *J* = 9.2 Hz, 2H), 8.20 (s, 1H), 7.86–7.84 (m, 3H), 7.71 (d, *J* = 7.6 Hz, 1H), 7.40–7.35 (m, 2H), 5.43 (s, 2H), 4.62 (s, 2H), 2.77 (s, 3H)	170.7, 166.3, 163.3, 155.0, 145.4, 144.5, 143.5, 133.9, 126.5, 126.1, 125.2, 124.3, 120.0, 119.4, 115.1, 53.3, 27.5, 27.0
9	3405 (NH), 1702, 1671 (C=O), 1615, 1589, 1472, 1453 (C=C), 712, 743	10.49 (s, 1H), 8.20 (s, 1H), 7.82–7.80 (m, 1H), 7.73–7.72 (m, 2H), 7.65 (t, *J* = 7.9 Hz, 1H), 7.60–7.59 (m, 3H), 7.37–7.32 (m, 3H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.32 (t, *J* = 8.0 Hz, 1H), 7.14–7.10 (m, 2H), 5.34 (s, 2H), 4.69 (s, 2H)	168.7, 165.1, 154.7, 144.3, 143.2, 139.3, 134.9, 134.6, 133.7, 130.1, 130.0, 128.4, 126.5, 125.2, 124.7, 124.1, 120.2, 119.4, 114.4, 53.2, 27.9

10	3425 (NH), 1710, 1668 (C=O), 1626, 1593, 1529, 1474 (C=C), 837	10.64 (s, 1H), 8.19 (s, 1H), 7.82–7.80 (m, 4H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.66–7.62 (m, 4H), 7.42 (d, *J* = 7.9 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.32 (t, *J* = 8.0 Hz, 1H), 7.13 (t, *J* = 8.0 Hz, 1H), 5.35 (s, 2H), 4.69 (s, 2H)	168.8, 165.4, 154.8, 144.4, 143.3, 138.4, 135.0, 134.7, 133.8, 130.2, 130.1, 129.9, 128.4, 126.6, 125.3, 124.2, 121.8, 119.5, 114.4, 53.3, 28.0
11	3435 (NH), 1710, 1669 (C=O), 1591, 1518, 1495, 1466 (C=C), 842	10.63 (s, 1H), 8.19 (s, 1H), 7.82–7.80 (m, 3H), 7.72 (d, *J* = 8.2 Hz, 1H), 7.66–7.64 (m, 2H), 7.58–7.54 (m, 4H), 7.33–7.11 (m, 2H), 6.62 (d, *J* = 8.2 Hz, 1H), 5.35 (s, 2H), 4.69 (s, 2H)	168.7, 165.4, 154.7, 144.3, 143.2, 138.7, 134.9, 134.6, 133.7, 132.7, 130.1, 130.0, 126.5, 125.2, 124.1, 122.1, 119.4, 116.4, 114.4, 53.2, 27.9
12	3415 (NH), 1704, 1670 (C=O), 1593, 1551, 1561, 1451 (C=C), 831	10.41 (s, 1H), 8.18 (s, 1H), 7.81–7.80 (m, 2H), 7.72 (d, *J* = 7.9 Hz, 1H), 7.65 (t, *J* = 7.9 Hz, 3H), 7.48 (d, *J* = 7.9 Hz, 1H), 7.32 (t, *J* = 7.6 Hz, 1H), 7.17–7.12 (m, 3H), 6.62 (d, *J* = 8.0 Hz, 1H), 5.32 (s, 2H), 4.69 (s, 2H), 2.29 (s, 3H)	168.7, 164.9, 154.7, 144.3, 143.1, 136.8, 134.9, 134.6, 133.7, 133.6, 130.2, 130.1, 130.0, 126.5, 125.2, 124.1, 120.2, 119.4, 114.4, 53.2, 27.9, 21.4
13	3420 (NH), 1714, 1670 (C=O), 1616, 1593, 1561, 1451 (C=C), 834	11.10 (s, 1H), 8.27 (d, *J* = 9.0 Hz, 2H), 8.21 (s, 1H), 7.86–7.70 (m, 4H), 7.72 (d, *J* = 7.6 Hz, 1H), 7.65 (t, *J* = 8.0 Hz, 2H), 7.32 (t, *J* = 7.6 Hz, 1H), 7.12 (t, *J* = 7.6 Hz, 2H), 6.62 (d, *J* = 8.0 Hz, 1H), 5.44 (s, 2H), 4.70 (s, 2H)	168.7, 166.3, 154.7, 145.4, 144.3, 143.5, 143.3, 134.9, 134.7, 133.7, 130.1, 130.0, 126.5, 126.0, 125.2, 124.1, 120.0, 119.4, 114.4, 53.3, 27.9
14	3425 (NH), 1704, 1680 (C=O), 1626, 1593, 1558, 1475 (C=C), 830	10.49 (s, 1H), 8.19 (s, 1H), 7.85 (d, *J* = 8.2 Hz, 2H), 7.73 (d, *J* = 8.2 Hz, 3H), 7.60 (d, *J* = 8.0 Hz, 2H), 7.38–7.32 (m, 3H), 7.12 (t, *J* = 8.2 Hz, 1H), 6.73 (d, *J* = 8.2 Hz, 1H), 5.34 (s, 2H), 4.69 (s, 2H)	167.7, 165.1, 154.6, 144.3, 143.1, 139.5, 139.3, 134.8, 132.4, 132.2, 130.2, 129.9, 126.5, 125.3, 124.7, 124.2, 120.1, 119.4, 114.5, 53.2, 27.9
15	3420 (NH), 1710, 1682 (C=O), 1622, 1592, 1552, 1452 (C=C), 842	10.59 (s, 1H), 8.14 (s, 1H), 7.80 (d, *J* = 8.6 Hz, 2H), 7.67 (d, *J* = 8.6 Hz, 2H), 7.57 (d, *J* = 8.5 Hz, 3H), 7.37 (t, *J* = 8.5 Hz, 2H), 7.28 (t, *J* = 7.5 Hz, 1H), 7.12 (t, *J* = 7.5 Hz, 1H), 6.67 (t, *J* = 8.2 Hz, 1H), 5.30 (s, 2H), 4.64 (s, 2H)	167.8, 165.3, 154.6, 144.3, 143.2, 139.5, 138.3, 134.8, 132.4, 132.2, 130.2, 129.8, 129.3, 126.5, 125.3, 124.2, 121.7, 119.4, 114.5, 53.2, 27.9
16	3430 (NH), 1704, 1674 (C=O), 1613, 1593, 1559, 1486 (C=C), 837	10.59 (s, 1H), 8.16 (s, 1H), 7.82 (d, *J* = 8.2 Hz, 2H), 7.70 (d, *J* = 8.2 Hz, 2H), 7.56–7.50 (m, 2H), 7.58–7.54 (m, 3H), 7.30 (t, *J* = 8.2 Hz, 1H), 7.14 (t, *J* = 8.2 Hz, 1H), 6.70 (d, *J* = 8.2 Hz, 1H), 5.32 (s, 2H), 4.66 (s, 2H)	167.2, 164.9, 154.1, 143.8, 142.7, 139.0, 138.2, 134.3, 132.2, 131.9, 131.7, 129.7, 126.0, 124.8, 123.7, 121.6, 118.9, 115.9, 114.0, 52.7, 27.5
17	3445 (NH), 1712, 1675 (C=O), 1603, 1551, 1519, 1453 (C=C), 837	11.10 (s, 1H), 8.27 (d, *J* = 9.3 Hz, 2H), 8.21 (s, 1H), 7.86–7.84 (m, 4H), 7.74–7.72 (m, 3H), 7.33 (t, *J* = 7.2 Hz, 1H), 7.17 (t, *J* = 7.2 Hz, 1H), 6.72 (d, *J* = 8.2 Hz, 1H), 5.44 (s, 2H), 4.70 (s, 2H)	167.7, 166.3, 154.6, 145.4, 144.3, 143.5, 143.2, 139.5, 134.8, 132.4, 132.1, 130.2, 126.5, 126.1, 125.3, 124.2, 120.0, 119.4, 114.5, 53.3, 27.9
18	3415 (NH), 1673 (C=O), 1608, 1554, 1492, 1455 (C=C), 830	10.36 (s, 1H), 8.10 (s, 1H), 7.52–7.43 (m, 5H), 7.38–7.10 (m, 4 H), 5.27 (s, 2H), 4.66 (s, 2H), 2.23 (s, 3H)	163.8, 149.7, 143.6,143.2, 135.8, 132.7, 131.2, 129.2, 125.3,121.7,121.2, 119.2, 117.5, 110.4, 52.2, 25.8, 20.4

of signals at δ 8–7 ppm confirmed the structures of 1-benzoyl-2-propyn-2-yl-thiobenzimidazole (**2b**) and 1-*p*-chlorobenzoyl-2-propyn-2-yl-thiobenzimidazole (**2c**). In ^{13}C NMR, the presence of a signal in the region δ 170–168 ppm confirmed acylation. Further, reacting compounds **2a–c** with azido compounds **3a–e** (17) using the Cu alkyne-azide cycloaddition (CuAAC) (20–23) provided the target compounds **4–18** in excellent yields (85–90 %). In particular, in each cycloadduct, the proton associated with the 1,2,3-triazole moiety was identified as a singlet with δ between 8.18 and 8.20 ppm. The structures of all the new compounds were confirmed also by mass spectral data (ESI-HRMS) where the found and calculated mass units are identical up to 4 decimal digits. All physical and spectral data of the new compounds are given in Tables I and II.

Antiviral activity

Target compounds were designed based on the available data (Fig. 1) (8–10). Benzimidazoles substituted at position-2 with amide, urea and/or heterocycle showed good anti-HCV and/or anti-HBV activity. Accordingly, and in search of a potent antiviral agent, 2-mercaptobenzimidazoles were linked to anilides with a triazole ring. It was proposed that the triazole ring along with sulphur atom would improve the activity of lead molecules. All synthesized compounds considered in this work were evaluated for antiviral activity against HBV and HCV.

Cytotoxicity was evaluated in parallel with the antiviral activity. The strategy followed in the antiviral screening study is based on using two concentrations (1 and 10 μmol L^{-1}) of each of the test compounds. Test compounds showed no significant activity against HBV and weak activity toward HCV (Figs. 2a and b). Among the tested compounds, 2-{4-[(1-benzoylbenzimidazol-2-ylthio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-(*p*-nitrophenyl)-acetamide (**13**) and 2-(4-{[1-(*p*-chlorobenzoyl)benzimidazol-2-ylthio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-(*p*-nitrophenyl)acetamide (**17**) showed significant activity and were subjected to further investigation at various concentrations in comparison with the reference drug telaprevir (TLV). Judging by the results, fifty percent effective concentrations (EC_{50}) of HCV inhibition for compounds **13** and **17** were 7.8 and 7.6 μmol L^{-1}, respectively, and the 50 % cytotoxic concentrations (CC_{50}) were 16.9 and 21.1 μmol L^{-1}, thus resulting in selectivity indices of 2–3 (Fig. 3). For telaprevir see also ref. 24. Consulting the inhibitory values for the compounds as anti-HCV agents, we found that the anilide part having an electron withdrawing group, notably nitro group, showed comparatively better activity than other substituents. The results could be explained according to the Topliss scheme (25, 26), which starts with the unsubstituted phenyl. In this work, 2-{4-[(1-acetylbenzimidazol-2-yl-thio)methyl]-1H-1,2,3-triazol-1-yl}-*N*-(*p*-chlorophenyl)-acetamide (**5**) with *p*-chlorophenyl showed higher affinity than 2-{4-[(1-acetylbenzimidazol-2-yl-thio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-phenylacetamide (**4**) with unsubstituted phenyl. For the same reason, 2-{4-[(1-benzoylbenzimidazol-2-yl-thio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-(*p*-chlorophenyl)acetamide (**10**) and 2-(4-{[1-(*p*-chlorobenzoyl)benzimidazol-2-yl-thio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-(*p*-chlorophenyl)acetamide (**15**) showed higher activity than 2-{4-[(1-benzoylbenzimidazol-2-yl-thio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-phenylacetamide (**9**) and 2-{4-[(1-(*p*-chlorobenzoyl) benzimidazol-2-yl-thio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-phenyl-acetamide (**14**), which most probably can be attributed to a positive π-effect, a positive α-effect or to a combination of both. Since the potency of 2-{4-[(1-benzoylbenzimidazol-2-yl-thio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-(*p*-methylphenyl)-acetamide (**12**) with *p*-methylphenyl was lower than that of

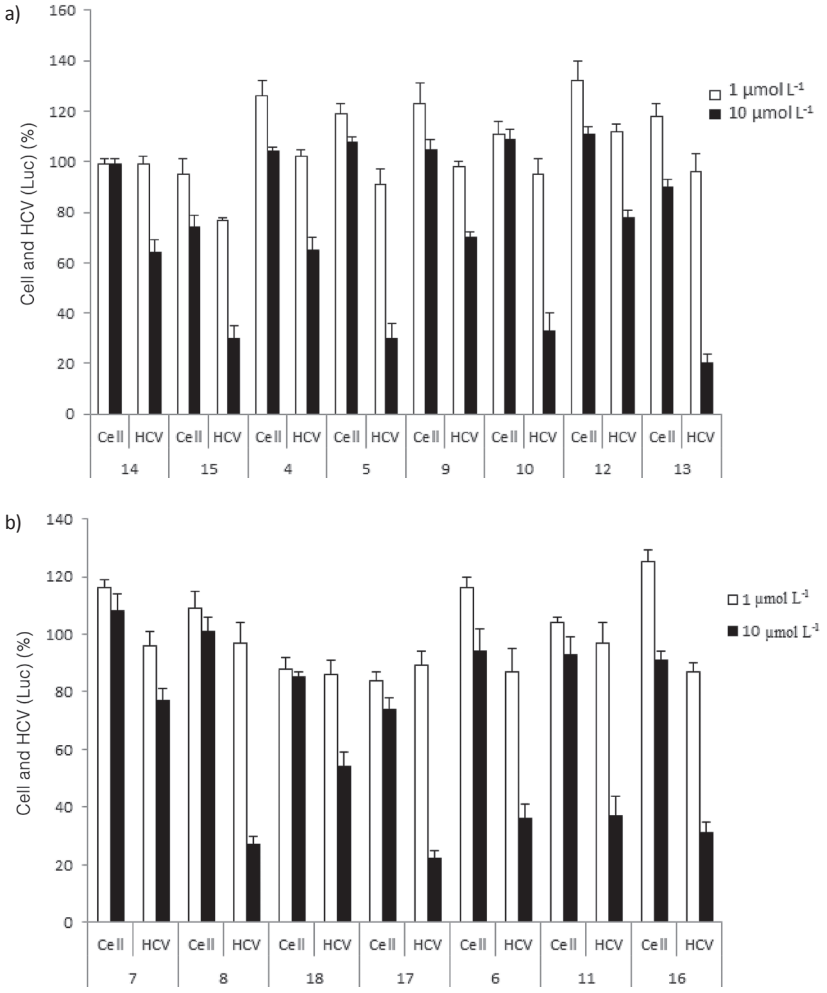


Fig. 2. Anti-HCV and cytotoxicity of compounds: a) **4, 5, 9, 10, 12–15**, and b) compounds **6–8**, **11, 16–18** (SD, $n = 3$).

2-{4-[(1-(*p*-chlorobenzoyl)benzimidazol-2-yl-thio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-phenyl-acetamide (**14**), it may be concluded that the positive π-effect is dominant. Accordingly, *p*-nitrophenyl derivatives were synthesized [2-{4-[(1-acetylbenzimidazol-2-yl-thio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-(*p*-nitrophenyl)-acetamide (**8**), 2-{4-[(1-benzoylbenzimidazol-2-yl-thio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-(*p*-nitrophenyl)-acetamide (**13**) and 2-(4-{[1-(*p*-chloro-benzoyl)benzimidazol-2-yl-thio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-(*p*-nitrophenyl)acetamide (**17**). As expected, the latter compounds (**13** and **17**) showed the highest inhibitory activity among all the synthesized compounds (Fig. 3).

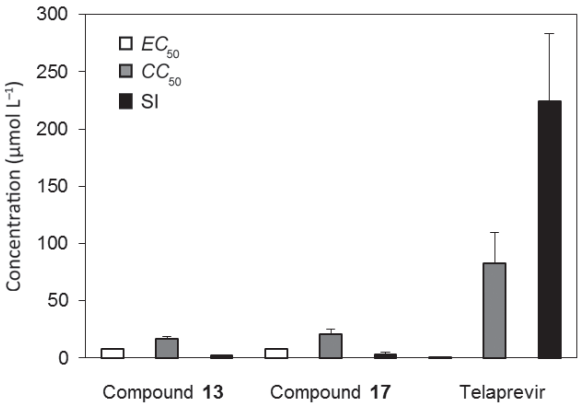


Fig. 3. Fifty percent effective concentration of HCV inhibition (*EC*50), 50 % cytotoxic concentration (*CC*50) and *CC*50 to *EC*50 ratio (selectivity index, SI) for compounds **13** and **17** and reference drug telaprevir (SD, $n = 3$).

CONCLUSIONS

In summary, we have described the synthesis of a series of hybrid molecules, which combined two heterocycles (benzimidazole and 1,2,3-triazole) in excellent yields using simple, efficient, and fast routes by taking advantage of the click chemistry strategy. These benzimidazole/triazoles were tested against HBV and HCV. Among compounds reported in this study, compounds 2-{4-[(1-benzoylbenzimidazol-2-yl-thio)methyl]-1*H*-1,2,3-triazol--1-yl}-*N*-(*p*-nitrophenyl)acetamide (**13**) and 2-(4-{[1-(*p*-chlorobenzoyl)benzimidazol-2-yl-thio)methyl]-1*H*-1,2,3-triazol-1-yl}-*N*-(*p*-nitrophenyl)acetamide (**17**) showed significant activity against HCV.

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