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Original article

5-Acetyl-2-arylbenzimidazoles as antiviral agents. Part 4

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ABSTRACT

Within a project aimed at discovering new Flaviviridae inhibitors, new variously substituted 2-phenylbenzimidazoles were synthesized and evaluated in cell-based assays for cytotoxicity and antiviral activity against viruses representatives of the three genera of the Flaviviridae family, i.e.: *Pestivirus* (BVDV), *Flavivirus* (YFV) and *Hepacivirus* (HCV). Title compounds were also tested against RNA viruses representative of other single-stranded, positive-sense (ssRNA$^+$) negative-sense (RNA$^-$), or double-stranded (dsRNA) genomes, as well as against representatives of two DNA virus families.

Nine compounds showed activity against BVDV (EC_{50} = 0.8–8.0 μM), compound **31** being the most potent (EC_{50} = 0.80 μM) and selective (SI = CC_{50}/EC_{50} = >100). When tested in an HCV replicon assay, compound **31** resulted again the most potent, displaying an EC_{50} value of 1.11 μM and an SI of 100. Besides inhibiting BVDV, two compounds (**35** and **38**) showed a moderate activity also against YFV (EC_{50} = 13 μM). Interestingly, **35** was moderately active also against RSV (EC_{50} = 25 μM).

1. Introduction

Our interest on the chemistry and biological properties of benzimidazoles has led us to synthesize numerous derivatives endowed with analgesic–antiinflammatory [1,2] and choleretic [3,4] activities, as well as with a chloropromazine-like activity in the conditioned avoidance response [5,6] and, more recently, with antiproliferative and antiviral activities [7–10]

In this context, we have described the series of 2-[4-substituted naphthyl]- (**1**) [7], 2-[4-substituted biphenyl]- (**2**) [8], 2-[4-substituted styryl]-benzimidazoles (**3**) [9] and 1-substituted-2-[(benzotriazol-1/2-yl)methyl benzimidazoles (**4**) and (**5**) [10], endowed with selective activity, at micromolar level, against Respiratory Syncytial virus (RSV) and some members of the Flaviviridae family (Fig. 1).

The latter includes single-stranded positive-sense RNA (ssRNA$^+$) viruses, which cause significant diseases in humans and animals. They are distributed into three genera: the *Hepacivirus* genus includes, as sole representative, the Hepatitis C virus (HCV) [agents such as GB virus-A and GB virus-A-like, GB virus-D and GBV-C or hepatitis G virus, although closely related to HCV, represent unassigned members of Flaviviridae]; the *Flavivirus* genus comprises Dengue Fever, Yellow Fever (YFV), West Nile, Japanese Encephalitis and Tick-Borne Encephalitis viruses; the *Pestivirus* genus comprises Bovine Viral Diarrhoea (BVDV), Border Disease and Classical Swine Fever viruses.

HCV is a major cause of human hepatitis [11]. The WHO estimates that over 170 million people worldwide are presently infected by this virus [12,13]. Most infections become persistent, and about 60% progress towards chronic liver disease. Chronic HCV infection can lead to development of cirrhosis, hepatocellular carcinoma and liver failure [14,15]. Pegylated interferon, in combination with ribavirin, is used in the clinic for HCV infections. Unfortunately, this therapy has limited efficacy and is often associated with severe, adverse events [16].

Flaviviruses are human pathogens prevalent throughout the world and cause a range of acute febrile illnesses, encephalitides and hemorrhagic diseases. Although an effective vaccine against YFV has been available since the late 1930s, its use is not systematic in many areas [17].

Pestivirus infections of domesticated livestock cause significant economic losses worldwide. They cause a range of clinical manifestations, including abortion, teratogenesis, respiratory problems, chronic wasting disease, immune system dysfunction and predisposition to secondary viral and bacterial infections. BVDV can also

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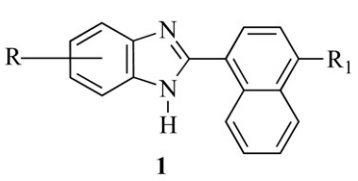

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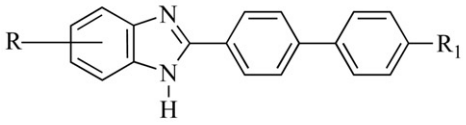

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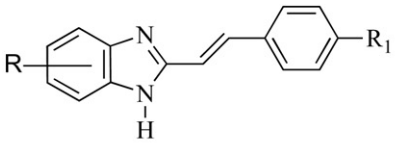


3

R=H; 5-Me; 5,6-diMe, 5-Cl; 5,6-diCl; 5-CF_3; 5-$COCH_3$;
R_1= H; NO_2; NH_2; $NHCOCH_3$;

YFV (EC_{50} μM)

1a, 5,6-diMe; R_1=H 6
1b, 5-Cl; R_1= NH_2 27
1c, 5,6-diCl; R_1=NH_2 16

2d, R=$COCH_3$; R_1=NO_2

YFV (EC_{50} =0.5μM); CVB-2 (EC_{50} =1 μM)

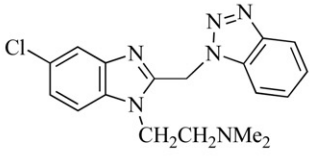
3e, R=5-$COCH_3$; R_1=NO_2

BVDV(EC50=1.7 μM)

3f; R=H; R_1=NH_2;
3g; R=Cl; R_1=NH_2

CVB-2, (EC_{50} values of 7 - 8 μM respectively)

3h, R=CF_3; R_1=$NHCOCH_3$
RSV (EC_{50}= 1 μM)



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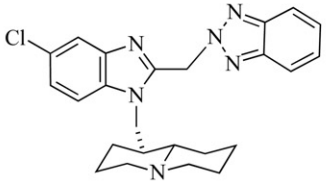


5

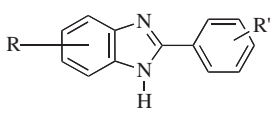
Anti RSV EC_{50}=0.03 μM

Fig. 1. Chemical structure and antiviral activity of the series of 2-arylbenzimidazoles previously described [7,8,9,10].

establish a persistent infection (PI) in animals, that succumb or remain viraemic throughout life and serve as continuous virus reservoirs. Furthermore, BVDV also shows the ability to cross the placenta of susceptible animals causing a variety of foetal infections [18,19].

With the exception of YFV, no vaccines exist against Flaviviridae pathogens, as well as no selective antiviral drugs are yet available in the clinic to prevent and/or treat their infections. The only exceptions are two protease inhibitors, recently approved by FDA for the treatment of HCV infections [20–23]. Hence the need to continue studies aimed at identifying new lead compounds targeted at virus-specifc steps of the Flaviviridae replication cycle.

As part of a pluriennal program in this area [7–10], very recently we described new 2-phenylbenzimidazoles [compounds **6** in Fig. 2], which have been evaluated in cell-based assays for cytotoxicity and activity against a panel of RNA and DNA viruses [24]. 39 compounds exhibited antiviral activity at concentrations comprised between 0.1 and 10 μM, and four of them were outstanding for their potency against VV (**6a**, R = 5,6-diCl; R_1 = 4-NO_2) or BVDV (**6b**, R = 5-NO_2; R_1 = H; **4c**, R = 5-NO_2; R_1 = $COCH_3$; **6d**, R = 5,6-diCl; R_1 = $COCH_3$), with EC_{50} = 0.1, 1.5, 0.8 and 1.0 μM,



6

R=H; 5-CF_3; 5-$COCH_3$; 5,6-diCl;
R'=2-NO_2; 2,4-diNO_2; 4-OH; 4-OCH_3; 3-OCH_3; 2,4-diOCH_3; 3,5-diOCH_3; 2,3,4-triOCH_3; 4-F;2,6-diF; 4-NO_2; 2-NO_2; 4-OCH_3;2-NH_2; 4-NH_2 ; 4-OCH_3

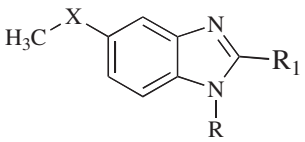
Fig. 2. Examples of 5R- and 2-aryl substituted benzimidazoles endowed with recorded antiflaviridae activities.

respectively. In enzyme assays, **6b** and **6d** inhibited at low μM concentrations the RNA-dependent RNA polymerase (RdRp) of BVDV and HCV, respectively. This suggested that 2-phenylbenzimidazoles could be attractive leads for further development of inhibitors of poxviruses, pestiviruses and even HCV. Therefore, we decided to carry on our program to evaluate whether this type of compounds, purposely modified, could be improved in both potency and spectrum of antiviral activity.

Focussing on the derivatization of the 5-acetyl group on the benzimidazole, by using typical reagents of carbonyl group as hydroxylamine, semicarbazide and thiosemicarbazide, we designed and synthesized derivatives **7**–**31** (Fig. 3) in order to evaluate the influence of this modification on the antiviral activity.

In support of our hypothesis was the example of the many benzimidazole derivatives reported in the literature, where the presence of a carboxylic or tetrazolyl group at position 5 of the benzimidazole ring [as in compounds **32**–**34** (Fig. 4)] allowed inhibition of the HCV RdRp at very low IC_{50} values [25–27].

In our opinion, the presence of an acetyl group in compounds **7**–**12**, owing to a comparable electronegativity with both carboxy and tetrazolyl groups mentioned above, and its transformation into the derivatives **13**–**31**, could represent a good strategy to improve the antiviral properties of this class of compounds in analogy with what observed in the known cases of enviroxime [28]a,b, 2-acetylpyridine thiosemicarbazones [29] and methisazone [30]. Thus, we decided to proceed in two stages. First, we afforded the simple modification of the acetyl group, as in compounds **7**–**31**. Once verified that this type of substitution could lead to selective BVDV inhibitors, we prepared benzimidazoles **35**–**61** (Fig. 5) in form of both simple acetyl derivatives and corresponding thiosemicarbazones and semicarbazones, characterized by an increased



7-31

Compd Ref	X	R	R_1
7[6]	C=O	H	Ph
8[6]	C=O	H	4'-Cl-Ph
9	C=O	H	4'- CF_3-Ph
10[6]	C=O	H	4'-OCH_3-Ph
11[10]	C=O	H	2',4'-diOCH_3-Ph
12[10]	C=O	H	2',3',4'-triOCH_3-Ph
13[6]	CH-OH	H	4'-OCH_3-Ph
14	CH-OH	H	2',4'-diOCH_3-Ph
15	CH-OH	H	2',3',4'-triOCH_3-Ph
16	C=N-OH	H	Ph
17	C=N-OH	H	4'-Cl-Ph
18	C=N-OH	H	4'- CF_3-Ph
19	C=N-OH	H	4'-OCH_3-Ph
20	C=N-OH	H	2',4'-diOCH_3-Ph
21	C=N-OH	H	2',3',4'-triOCH_3-Ph
22	C=N-NH-CO-NH_2	H	Ph
23	C=N-NH-CO-NH_2	H	4'-Cl-Ph
24	C=N-NH-CO-NH_2	H	4'- CF_3-Ph
25	C=N-NH-CO-NH_2	H	4'-OCH_3-Ph
26	C=N-NH-CO-NH_2	H	2',4'-diOCH_3-Ph
27	C=N-NH-CS-NH_2	H	Ph
28	C=N-NH-CS-NH_2	H	4'-Cl-Ph
29	C=N-NH-CS-NH_2	H	4'- CF_3-Ph
30	C=N-NH-CS-NH_2	H	4'-OCH_3-Ph
31	C=N-NH-CS-NH_2	H	2',4'-diOCH_3-Ph

Fig. 3. First series of the new designed compounds **7**–**31**.

lipophilicity due to the cyclohexyl substitution at position 1, as for the above mentioned compounds **32**–**34**.

In addition, we tested the effect of the isosteric replacement, at position 2, of unsubstituted and substituted phenyl rings with different aromatic rings, such as furan and pyridine, which are present in the potent HCV RdRp inhibitors **62**–**63** (Fig. 6) described by other authors [31,32].

In this paper we present the design and synthesis of the above described derivatives and their evaluation for cytotoxicity and antiviral activity in cell-based assays.

2. Results and discussion

5-acetyl-2-arylbenzimidazole derivatives were tested in cell-based assays for cytotoxicity and antiviral activity against representative members of a number of virus families (Tables 1and 2). In addition to the Flaviviridae BVDV and YFV, among ssRNA$^+$ viruses we tested a retrovirus (human immunodeficiency virus type-1, HIV-1), two picornaviruses, human enterovirus B (coxsackie virus type B5, CVB-5) and human enterovirus C (polio virus type-1, Sb-1). Among ssRNA$^-$ viruses we tested a paramyxovirus (human respiratory syncytial virus, RSV) and a rhabdovirus (vesicular stomatitis virus (VSV). Among double-stranded RNA (dsRNA) viruses, we tested reovirus type-1 (Reo-1). Two representatives of DNA viruses were also included: human herpesvirus 1 (herpes simplex type-1 HSV-1) and vaccinia virus (VV). Efavirenz, 2′-C-methyl-guanosine, 2′-C-ethynyl-cytidine, 6-azauridine, mycophenolic acid and acyclovir were used as reference inhibitors. Cytotoxicity was evaluated in parallel with the antiviral activity.

As far as the activity against Flaviviridae is concerned, nine compounds (**26**–**28**, **30**, **31**, **53**, **56**, **57** and **60**) exhibited selective activity against BVDV in the low micromolar range (EC_{50} = 0.8–8.0 μM). In particular, derivatives **28** and **31** showed the most potent anti-BVDV activity (EC_{50} = 0.83 ± 0.09 and 0.80 ± 0.06 μM, respectively; dose–response curve in Fig. 7A) and, like compounds **26**, **57** and **60**, were non cytotoxic at concentrations up to 100 μM.

Other compounds (**11**, **20**, **36**–**38**, **42** and **61**) exhibited activity against BVDV at higher μM concentrations (EC_{50} = 16–23 μM). Notewhorthy, compound **38** exhibited a selective, although not-very potent, activity also against YFV (CC_{50} > 100 μM; EC_{50} = 13.4 ± 1.1 μM), whereas compound **35** showed activity against YFV (CC_{50} > 100 μM; EC_{50} = 13.2 ± 1.5 μM), but not against BVDV.

When tested against representatives of other virus families (Tables 1and 2), 5-acetyl-2-arylbenzimidazoles resulted mainly inactive. Exceptions were: compound **20** and **35** which, besides BVDV, also inhibited CVB-5 (CC_{50} > 100 μM; EC_{50} = 43 μM) and RSV (CC_{50} > 100 μM; EC_{50} = 25 μM), respectively; and compound **43**, active only against CVB-5 (CC_{50} > 100 μM; EC_{50} = 47 μM).

SAR studies allow to conclude that derivatization of the 5-acetyl group led to a significant improvement in the anti-BVDV activity (compounds **7**–**11**); transformation into ketoximes allowed to achieve higher anti-BVDV potency (**20**); the conversion into semicarbazones also allowed to achieve an increase in potency (**26**); finally, the replacement of a semicarbazide with a thiosemicarbazide to obtain the thiosemicarbazones allowed to achieve fairly low EC_{50} values (**28** and **31**). On the contrary, no significant extension of the antiviral spectrum was obtained, the only exception being compounds **20**, **35** and **37**.

Introduction of a cyclohexyl substituent at position 1 (derivatives **35**–**61**) failed to prove useful in improving the anti-BVDV potency. However, compounds **35**–**38**, **42**, **48**, **53**, **57**, **59**–**61** resulted active against this virus, the most potent being, again, the thiosemicarbazones (**53**, **57**, **60**, **61**). Moreover, susbstitution with cyclohexyl (derivatives **35**–**41**) allowed the appearance of antiviral activities against different viruses: YFV and RSV, in the case of compounds **35** and **37**, and YFV in the case of compound **38**.

Structure–activity relationship studies indicate that the acetyl group as such, or its corresponding hydroxyethyl derivatives, are unable to promote antiviral activity in the series of *N*-1-unsubstituted benzimidazoles. Only when the phenyl bears a 2,4-dimethoxy group at position 2 (as in the above cited compound **11**), the improvement in anti-BVDV activity resulted higher than that obtained previously [24]. As a cycloexyl substitution at position 1 of the benzimidazole takes place, some compounds (**35**–**38**, **42**, **48**, **53**, **57**, **59**–**61**) exhibit anti-BVDV activity, the most active being those bearing the most lipophilic susbstituents in the phenyl ring (2,4di-OCH_3 = Cl > OCH_3). Bioisosteric replacement of the phenyl with a furanyl or pyridinyl ring was partially successful in the case of **59** (EC_{50} = 26 μM) and fairly good in the case of compound **60** (EC_{50} = 8 μM).

Due to its interesting activity against BVDV, compound **31** was also tested against HCV in a subgenomic replication assay that allows viral replication in a human hepatoma cell line (GS4.1). In

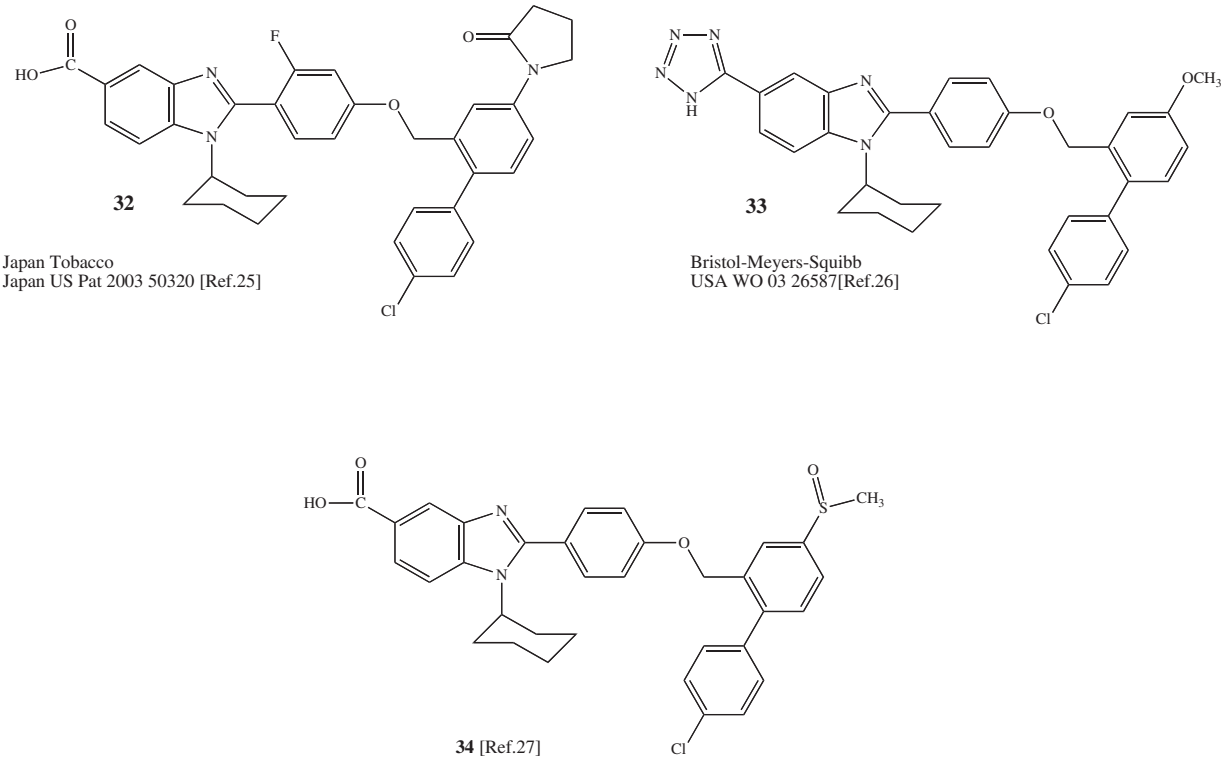


Fig. 4. Examples of benzimidazole compounds patented as HCV polymerase inhibitors.

this assay, **31** selectively inhibits the HCV replication with an EC_{50} of 1.11 ± 0.15 μM (Table 3, dose–response curve in Fig. 7B).

Studies on the inhibition of BVDV and HCV recombinant RNA polymerases, in vitro selection of BVDV resistant mutants, definition of the molecular basis of resistance, as well as in silico studies aimed at detailing drug-RdRps interactions and mode of action of the most potent 5-acetyl-2-arylbenzimidazoles will be the object of separate publications.

2.1. Chemistry

The preparation of compounds **7**–**31** and **35**–**61** was achieved according to the sequence of reactions of Scheme 1. From the commercially available bromoacetophenone (**a**) (Janssen), we prepared the necessary intermediates (**b**,**c**) following a known procedure for (**b**) [6], whereas (**c**) was obtained for the first time in DMSO at 70 °C for 3 h. The diamine (**d**) was previously described by W. Borsche and J. Barthenheier [30], while (**e**) is a new compound. Ring closure to benzimidazoles was accomplished in two different ways. As the N-unsubstitued diamine (**d**) was used, condensation with the bisulfite salts of the aldehydes **f**–**m** yielded the desired compounds **7**–**12**, **42**–**43**. In the case of the *N*-cyclohexyl substituted diamine (**e**), we had to carry on the reactions with the aldehydes **f**–**n** in DMF/water (30:1), at room temperature for 1 h, in the presence of oxone to obtain compounds **35**–**41**. In both cases yields were very satisfactory (ranging from 58 to 98%). The intermediates **7**, **8**, **10**-**12** have been already described by some of us (G.P., M.L) as referenced in Fig. 3, while compounds **9**, **42**, **43** are described here for the first time. Compounds **13**–**15** were obtained on reduction by means of $NaBH_4$ in aqueous ethanol of the parent **10**–**12**. The ketoximes **16**–**21** were obtained from the ketones **7**–**12** by reaction with hydroxylamine. The acetyl derivatives **7**–**12** and **35**–**43** were alternatively converted into the corresponding semicarbazones **22**–**26** and **44**–**52**, respectively, and thiosemicarbazones **27**–**31** and **53**–**61**, respectively, using in turn semicarbazide hydrochloride and thiosemicarbazide in refluxing ethanol and in the presence of sodium acetate or glacial acetic acid, respectively.

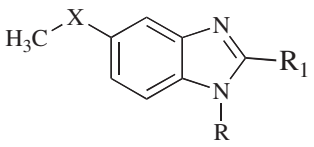
The elucidation of all novel compounds was supported by elemental analyses and ^{1}H NMR spectra, that are fully consistent with the described structures. In addition, for certain compounds (**14**, **17**, **22**, **24**, **25**, **29**, **39**, **48**, **50**, **53**, **55**, **61**) ^{13}C NMR spectra were recorded to further ascertain the localization of the carbon atoms external to the hetero-rings. Examination of these ^{13}C NMR spectra (vide infra) allowed us to confirm that a few variations are concerned with the presence of substituents in the phenyl moiety at position 2 of the benzimidazole ring, whereas the chemical shifts of C-8, C-9, and of C=S and C=O in side chains, were almost coincident (Table 4).

3. Experimental section

3.1. General remarks

Melting points were carried out with a Kofler hot stage melting point apparatus and are uncorrected. Infrared spectra were recorded as nujol mulls on NaCl plates with a Perkin–Elmer 781 IR spectrophotometer and are expressed in ν (cm^{-1}). UV spectra are qualitative and were recorded in nm for solutions in EtOH with a Perkin–Elmer Lamba 5 spectrophotometer. Nuclear magnetic resonance (^{1}H NMR) spectra were determined in $CDCl_3$, DMSO-d_6, $CDCl_3$/DMSO-d_6 (in the ratio 1:3) and were recorded in a Varian XL-200 (200 MHz). Chemical shifts (δ scale) are reported in parts per million (ppm), downfield from tetramethylsilane (TMS) as internal standard. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; br s, broad singlet; dd, doublet of doublet.

The assignment of exchangeable protons (OH and NH) was confirmed by the addition of D_2O. Mass spectra were performed on



35-61

Compd	X	R	R_1
35	C=O	Cyclohexyl	Ph
36	C=O	Cyclohexyl	4'-Cl-Ph
37	C=O	Cyclohexyl	4'- CF_3-Ph
38	C=O	Cyclohexyl	4'-OCH_3-Ph
39	C=O	Cyclohexyl	2',4'-diOCH_3-Ph
40	C=O	Cyclohexyl	3'-Furyl
41	C=O	Cyclohexyl	2'-Pyridinyl
42	C=O	H	3'-Furyl
43	C=O	H	Cyclohexyl
44	C=N-NH-CO-NH_2	Cyclohexyl	Ph
45	C=N-NH-CO-NH_2	Cyclohexyl	4'-Cl-Ph
46	C=N-NH-CO-NH_2	Cyclohexyl	4'- CF_3-Ph
47	C=N-NH-CO-NH_2	Cyclohexyl	4'-OCH_3-Ph
48	C=N-NH-CO-NH_2	Cyclohexyl	2',4'-diOCH_3-Ph
49	C=N-NH-CO-NH_2	Cyclohexyl	3'-Furyl
50	C=N-NH-CO-NH_2	Cyclohexyl	2'-Pyridinyl
51	C=N-NH-CO-NH_2	H	3'-Furyl
52	C=N-NH-CO-NH_2	H	Cyclohexyl
53	C=N-NH-CS-NH_2	Cyclohexyl	Ph
54	C=N-NH-CS-NH_2	Cyclohexyl	4'-Cl-Ph
55	C=N-NH-CS-NH_2	Cyclohexyl	4'- CF_3-Ph
56	C=N-NH-CS-NH_2	Cyclohexyl	4'-OCH_3-Ph
57	C=N-NH-CS-NH_2	Cyclohexyl	2',4'-diOCH_3-Ph
58	C=N-NH-CS-NH_2	Cyclohexyl	3'-Furyl
59	C=N-NH-CS-NH_2	Cyclohexyl	2'-Pyridinyl
60	C=N-NH-CS-NH_2	H	3'-Furyl
61	C=N-NH-CS-NH_2	H	Cyclohexyl

Fig. 5. Second series of the new designed compounds **35**–**61**.

combined HP 5790-HP 5970 GC/MS apparatus or with a combined Liquid Chromatograph-Agilent 1100 series Mass Selective Detector (MSD). Analytical thin-layer chromatography (TLC) was performed on Merck silica gel F-254. Pure compounds showed a single spot in TLC. For flash chromatography, Merck silica gel 60 was used with a particle size 0.040–0.063 mm (230–400 mesh ASTM). Elemental analyses were performed on a Perkin–Elmer 2400 instrument at Laboratorio di Microanalisi, Dipartimento di Chimica, Università di Sassari, Italy, and results were within ±0.4% of theoretical values.

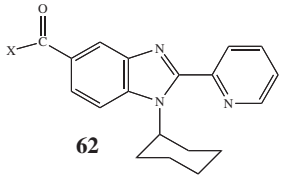
3.2. Chemistry

3.2.1. Intermediates

The diamine (**d**) was not commercially available and was purposely prepared as described by W. Borsche and J. Barthenheier [33]. The bisulphite compounds (**f**–**m**) were obtained in high yields from the commercially available corresponding aldehydes (Aldrich) with $Na_2S_2O_5$ in ethanol, according to the procedure used by Shriner and Land [34].

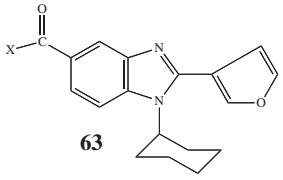
*3.2.1.1. 1-(4-(Cyclohexylamino)-3-nitrophenyl)ethanone (**b**).* To a solution of **a** (1 g, 41mmole) in DMSO (4 ml), cyclohexylamine (0.85 g, 87 mmol) was added dropwise. The brown-yellow mixture was then heated at 70 °C for 3 h under magnetic stirring. On cooling, it was diluted with water and the formed precipitate was collected and dried on air. Compound **b** (1.03 g, 96% yield) was yellow orange solid [R_f = 0,36 (EP-EA 9:1)]; m.p, 115–118 °C. Anal. calc. $C_{14}H_{18}N_2O_3$ %: C, 64.10; H, 6.92; N, 10.68; found: C, 63.98; H, 7.10; N, 10.72. ν_{max} (nujol) cm^{-1}: 3583, 3339, 1671, 1613; λ_{max} (EtOH) nm: 308, 269, 198. ^{1}H NMR ($CDCl_3$) δ: 8,79 (1H, d, *J* 1.6, H-3), 8.52 (1H, br s, *J* 8.0, NH), 8.04 (1H, dd, *J* 8.4 and 1.6, H-5), 6.92 (1H, d, *J* 8.4, H-6), 3.66–3.50 (1H, m, CH cyclohexyl), 2.56 (3H, s, CH_3CO), 2.18–1.30 (10H, m, cyclohexyl).

*3.2.1.2. 1-(3-Amino-4-(cyclohexylamino)phenyl)ethanone (**e**).* A solution of (**c**) (2 g, 762 mmol) in ethanol (120 ml) was added of 0.2 g of 10% palladised charcoal and hydrogenated under moderate pressure at 20 °C within 2 h. Then, the mixture was filtered off through filter paper and the solvent removed under vacuum. A glassy residue of (**e**) (1.77 g, 97% yield) was pure at TLC [R_f = 0.30, EP-EA 6:4]; m.p. 86–88 °C; Anal. calc. for $C_{14}H_{20}N_2O$: C, 72.38; H, 8.68; N, 12.06; found: C, 72.50; H, 8.51; N, 12.00. ν_{max} (nujol) cm^{-1}: 3583, 3370, 1642, 1587; λ_{max} (EtOH) nm: 349, 258, 216; ^{1}H NMR ($CDCl_3$) δ: 7.50 (1H, dd, *J* 8.6 and 2.0, H-5), 7.39 (1H, d, *J* 1.8, H-3), 6.58 (1H, d, *J* 8.4, H-6), 3.45–3.30 (1H, m, CH cyclohexyl), 2.49 (3H, s, CH_3CO), 2.15–1.30 (10H, m, cyclohexyl).



62

X= OH HCV NS5B polymerase IC_{50}= 4.3 μM
[Ref.31]

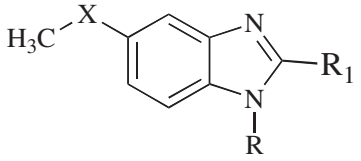


63

X= OH HCV NS5B polymerase IC_{50}= 1.6 μM
[Ref.32]

Fig. 6. Further examples of benzimidazole compounds described as HCV polymerase inhibitors.

Table 1
Cytotoxicity and antiviral activity of 2-phenylbenzimidazole derivatives (**7**–**31**) against representatives of ssRNA$^+$ (HIV-1, BVDV, YFV, CBV-5, Sb-1), ssRNA$^-$ (RSV, VSV), dsRNA (Reo-1) and dsDNA (VV, HSV-1) viruses.



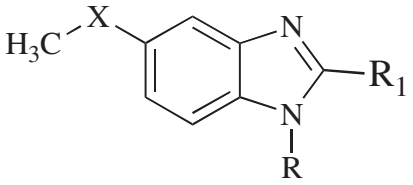
Compds.	*X*	*R*	*R1*	MT-4	HIV-1	MDBK	BVDV	BHK	YFV	Reo-1	VERO 76	CVB-5	Sb-1	RSV	VSV	VV	HSV-1
				CC_{50}[a]	EC_{50}[b]	CC_{50}[c]	EC_{50}[d]	CC_{50}[e]	EC_{50}[f]	EC_{50}[g]	CC_{50}[h]	EC_{50}[i]	EC_{50}[j]	EC_{50}[k]	EC_{50}[l]	EC_{50}[m]	EC_{50}[n]
7	C=O	H	Ph	ND	ND	>100	100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
8	C=O	H	Ph(4′-Cl)	ND	ND	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
9	C=O	H	Ph(4′- CF_3)	ND	ND	>100	15	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
10	C=O	H	Ph(4′-OCH_3)	24	>24	58	>58	40	>40	ND	>100	>100	>100	ND	ND	>100	ND
11	C=O	H	Ph(2′,4′-OCH_3)	50	>50	73	**21**	50	>50	ND	>100	>100	>100	ND	ND	>100	ND
12	C=O	H	Ph(2′,3′,4′-OCH_3)	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
13	CH–OH	H	Ph(4′-OCH_3)	≥100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
14	CH–OH	H	Ph(2′,4′-OCH_3)	≥100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
15	CH–OH	H	Ph(2′,3′,4′-OCH_3)	79	>79	60	>60	60	>60	>100	≥100	>100	>100	>100	ND	>100	>100
16	CN–OH	H	Ph	ND	ND	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
17	CN–OH	H	Ph(4′-Cl)	20	>20	37	18	33	>33	ND	ND	ND	ND	ND	ND	ND	ND
18	CN–OH	H	Ph(4′- CF_3)	ND	ND	55	>55	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
19	CN–OH	H	Ph(4′-OCH_3)	ND	ND	44	>44	44	>44	>100	>100	>100	>100	ND	ND	>100	ND
20	CN–OH	H	Ph(2′,4′-OCH_3)	≥100	>100	>100	17	>100	>100	>100	>100	**43**	>100	>100	>100	>100	>100
21	CN–OH	H	Ph(2′,3′,4′-OCH_3)	51	>51	>100	>100	>100	>100	>100	>100	>100	>100	ND	ND	>100	>100
22	C=N–NH–CO–NH_2	H	Ph	ND	ND	>100	50	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
23	C=N–NH–CO–NH_2	H	Ph(4′-Cl)	ND	ND	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
24	C=N–NH–CO–NH_2	H	Ph(4′- CF_3)	ND	ND	>100	45	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
25	C=N–NH–CO–NH_2	H	Ph(4′-OCH_3)	ND	ND	25	11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
26	C=N–NH–CO–NH_2	H	Ph(2′,4′-OCH_3)	21	≥21	>100	**2.55**	≥100	>100	>100	80	>80	>80	ND	>80	80	ND
27	C=N–NH–CS–NH_2	H	Ph	≥100	>100	32	**3.80**	32	>32	>32	>100	>100	>100	>100	>100	ND	ND
28	C=N–NH–CS–NH_2	H	Ph(4′-Cl)	2	>2	>100	**0.85**	>100	>100	>100	>100	>100	>100	ND	>100	>100	ND
29	C=N–NH–CS–NH_2	H	Ph(4′- CF_3)	2	>2	5	>1.8	5	>5	ND	22	>22	>22	ND	ND	ND	ND
30	C=N–NH–CS–NH_2	H	Ph(4′-OCH_3)	5	>5	13	**1.85**	13	>13	>13	>100	>100	>100	ND	>100	>100	ND
31	C=N–NH–CS–NH_2	H	Ph(2′,4′-OCH_3)	72	>72	>100	**0.80**	28	>28	>28	>100	>100	>100	>100	>100	ND	>100
Reference compounds																	
Efavirenz				40	0.002												
2′-C-methyl-guanosine						>10	1.1	>10	1.9								
2′-C-methyl-cytidine								>100		16							
2′-C-ethynyl-cytidine											>100	27	23				
6-aza-uridine											≥12.5			1.2			
Mycophenolic acid											≥12.5					1.5	
Acycloguanosine											>100						3

Data represent mean values for three independent determinations. Variation among duplicate samples was less than 15%.
ND = Not determined.
[a] Compound concentration (μM) required to reduce the proliferation of mock-infected MT-4 cells by 50%, as determined by the MTT method.
[b] Compound concentration (μM) required to achieve 50% protection of MT-4 cells from HIV-1 induced cytopathogenicity, as determined by the MTT method.
[c] Compound concentration (μM) required to reduce the viability of mock-infected MDBK cells by 50%, as determined by the MTT method.
[d] Compound concentration (μM) required to achieve 50% protection of MDBK cells from BVDV-induced cytopathogenicity, as determined by the MTT method.
[e] Compound concentration (μM) required to reduce the viability of mock-infected BHK cells by 50%, as determined by the MTT method.
[f] Compound concentration (μM) required to achieve 50% protection of BHK cells from YFV-induced cytopathogenicity, as determined by the MTT method.
[g] Compound concentration (μM) required to achieve 50% protection of BHK cells from Reo-1-induced cytopathogenicity, as determined by the MTT method.
[h] Compound concentration (μM) required to reduce the viability of mock-infected VERO-76 cells by 50% as determined by the MTT method.
[i] Compound concentration (μM) required to reduce the plaque number of CVB-5 by 50% in VERO-76 monolayers.
[j] Compound concentration (μM) required to reduce the plaque number of Sb-1 by 50% in VERO-76 monolayers.
[k] Compound concentration (μM) required to reduce the plaque number of RSV by 50% in VERO-76 monolayers.
[l] Compound concentration (μM) required to reduce the plaque number of VSV by 50% in VERO-76 monolayers.
[m] Compound concentration (μM) required to reduce the plaque number of VV by 50% in VERO-76 monolayers.
[n] Compound concentration (μM) required to reduce the plaque number of HSV-1 by 50% in VERO-76 monolayers.

*3.3. General procedure for the preparation of the 2-[R-substitutedphenyl]-5-acetyl-benzimidazoles (**7**–**12**) and 2-[cycloakyl or heteroaryl]-5-acetyl-benzimidazoles (**42**–**43**)*

(A)-A mixture of 5-acetyl-1,2-diaminobenzene (**d**) (10 mmol) and of either sodium hydroxy(*R*-substituted-phenyl-1-yl)methanesulfonate (**f**–**k**) or sodium hydroxy(cyclohexyl or furan-3-yl) methanesulfonate (**l**–**m**) (11 mmol) was refluxed for 4 h in 80 mL of ethanol. After cooling, an excess of the sodium salt was filtered off through filter paper and the mother liquors were evaporated to dryness under reduced pressure. The solid residues, coloured from yellow to dark brown, were resuspended in dry ether and then purified, if necessary, by recrystallization from EtOH/H_2O, or by silica gel column chromatography, using a mixture of the solvent indicated under the single compounds described below. Compounds **7**, **8**, **10**, **11**, **12** have been described previously, as referenced in Fig. 3.

Table 2
Cytotoxicity and antiviral activity of 2-phenylbenzimidazole derivatives (**35**–**61**) against representatives of ssRNA$^+$ (HIV-1, BVDV, YFV, CBV-5, Sb-1), ssRNA$^-$ (RSV, VSV), dsRNA (Reo-1) and dsDNA (VV, HSV-1) viruses.



Compds.	*X*	*R*	*R*1	MT-4	HIV-1	MDBK	BVDV	BHK	YFV	Reo-1	VERO 76	CVB-5	Sb-1	RSV	VSV	VV	HSV-1
				CC_{50}[a]	EC_{50}[b]	CC_{50}[c]	EC_{50}[d]	CC_{50}[e]	EC_{50}[f]	EC_{50}[g]	CC_{50}[h]	EC_{50}[i]	EC_{50}[j]	EC_{50}[k]	EC_{50}[l]	EC_{50}[m]	EC_{50}[n]
35	C=O	c-hexyl	Ph	37	>37	>100	60	100	**13.2**	>100	80	>80	>80	**25**	>80	>80	>80
36	C=O	c-hexyl	Ph(4′-Cl)	58	>58	>100	**23**	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
37	C=O	c-hexyl	Ph(4′-CF_3)	41	>41	52	**23**	33	>33	>33	35	>35	>35	20	>35	>35	>35
38	C=O	c-hexyl	Ph(4′-OCH_3)	27	>27	>100	**21**	>100	**13.4**	>100	30	>30	>30	>30	>30	>30	>30
39	C=O	c-hexyl	Ph(2′,4′-OCH_3)	21	>21	21	>21	8.5	>8.5	>85	70	>70	>70	>70	>70	>70	>70
40	C=O	c-hexyl	3′-Furyl	63	>63	>100	>100	77	>77	ND	80	>80	>80	ND	ND	>80	>80
41	C=O	c-hexyl	2′-Pyridinyl	70	>70	>100	>100	≥100	>100	ND	>90	>90	>90	ND	>90	>90	>90
42	C=O	H	3′-Furyl	>100	>100	≥100	**22**	98	>98	ND	>100	>100	>100	ND	>100	>100	>100
43	C=O	H	c-hexyl	>100	>100	>100	>100	>100	>100	>100	>100	**47**	>100	ND	>100	>100	>100
44	C=N–NH–CO–NH_2	c-hexyl	Ph	ND	ND	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
45	C=N–NH–CO–NH_2	c-hexyl	Ph(4′-Cl)	ND	ND	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
46	C=N–NH–CO–NH_2	c-hexyl	Ph(4′-CF_3)	ND	ND	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	ND
47	C=N–NH–CO–NH_2	c-hexyl	Ph(4′-OCH_3)	ND	ND	54	>54	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
48	C=N–NH–CO–NH_2	c-hexyl	Ph(2′,4′-OCH_3)	23	>23	36	22	19	>19	ND	20	>20	>20	ND	ND	>20	>20
49	C=N–NH–CO–NH_2	c-hexyl	3′-Furyl	ND	ND	>100	100	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
50	C=N–NH–CO–NH_2	c-hexyl	2′-Pyridinyl	ND	ND	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
51	C=N–NH–CO–NH_2	H	3′-Furyl	ND	ND	>100	60	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
52	C=N–NH–CO–NH_2	H	c-hexyl	ND	ND	>100	44	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
53	C=N–NH–CS–NH_2	c-hexyl	Ph	20	10	24	**4.5**	38	**9**	>38	20	>20	>20	>20	>20	>20	>20
54	C=N–NH–CS–NH_2	c-hexyl	Ph(4′-Cl)	17	≥17	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
55	C=N–NH–CS–NH_2	c-hexyl	Ph(4′-CF_3)	30	≥30	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
56	C=N–NH–CS–NH_2	c-hexyl	Ph(4′-OCH_3)	39	>39	32	**7**	ND	ND	ND	40	>40	>40	>40	>40	>40	>40
57	C=N–NH–CS–NH_2	c-hexyl	Ph(2′,4′-OCH_3)	17	≥17	>100	**4**	17	>17	>17	20	>20	>20	>20	>20	>20	>20
58	C=N–NH–CS–NH_2	c-hexyl	3′-Furyl	21	>21	>100	>100	>100	>100	ND	25	>25	>25	ND	>25	>25	>25
59	C=N–NH–CS–NH_2	c-hexyl	2′-Pyridinyl	23	>23	41	**26**	24	>24	ND	20	>20	>20	ND	>20	>20	>20
60	C=N–NH–CS–NH_2	H	3′-Furyl	86	>86	>100	**8**	>100	>100	>100	100	>100	100	>100	>100	>100	>100
61	C=N–NH–CS–NH_2	H	c-hexyl	48	>48	>100	**16**	24	>24	ND	>100	>100	>100	>100	>100	>100	ND
Reference compounds																	
Efavirenz				40	0.002												
2′-C-methyl-guanosine						>10	1.1	>10	1.9								
2′-C-methyl-cytidine								>100		16							
2′-C-ethynyl-cytidine											>100	27	23				
6-aza-uridine											≥12.5			1.2			
Mycophenolic acid											≥12.5					1.5	
Acycloguanosine											>100						3

Data represent mean values for three independent determinations. Variation among duplicate samples was less than 15%.
ND = Not determined.
[a] Compound concentration (μM) required to reduce the proliferation of mock-infected MT-4 cells by 50%, as determined by the MTT method.
[b] Compound concentration (μM) required to achieve 50% protection of MT-4 cells from HIV-1 induced cytopathogenicity, as determined by the MTT method.
[c] Compound concentration (μM) required to reduce the viability of mock-infected MDBK cells by 50%, as determined by the MTT method.
[d] Compound concentration (μM) required to achieve 50% protection of MDBK cells from BVDV-induced cytopathogenicity, as determined by the MTT method.
[e] Compound concentration (μM) required to reduce the viability of mock-infected BHK cells by 50%, as determined by the MTT method.
[f] Compound concentration (μM) required to achieve 50% protection of BHK cells from YFV-induced cytopathogenicity, as determined by the MTT method.
[g] Compound concentration (μM) required to achieve 50% protection of BHK cells from Reo-1-induced cytopathogenicity, as determined by the MTT method.
[h] Compound concentration (μM) required to reduce the viability of mock-infected VERO-76 cells by 50% as determined by the MTT method.
[i] Compound concentration (μM) required to reduce the plaque number of CVB-5 by 50% in VERO-76 monolayers.
[j] Compound concentration (μM) required to reduce the plaque number of Sb-1 by 50% in VERO-76 monolayers.
[k] Compound concentration (μM) required to reduce the plaque number of RSV by 50% in VERO-76 monolayers.
[l] Compound concentration (μM) required to reduce the plaque number of VSV by 50% in VERO-76 monolayers.
[m] Compound concentration (μM) required to reduce the plaque number of VV by 50% in VERO-76 monolayers.
[n] Compound concentration (μM) required to reduce the plaque number of HSV-1 by 50% in VERO-76 monolayers.

*3.3.1. 2-(4-Trifluoromethylphenyl)-5-acetyl-1H-benzimidazole (**9**)*

Yield: 98%. M.p. 230–232 °C (EtOH/H_2O). Anal. calc. for: $C_{16}H_{11}F_3N_2O$: C, 63.16; H, 3.64; F, 18.73; N, 9.21; O, 5.26. Found %: C, 63.00; H, 3.80; N, 9.10. ν_{max} (Nujol) cm^{-1}: 3290, 1666; UV (EtOH) nm: 319, 261, 220; ^1H NMR ($CDCl_3$-DMSO-d_6) δ: 8.21 (2H, d, *J* 8.0, H-3′,5′), 8.27 (1H, s, H-4), 7.91 (1H, dd, *J* 8.4 and 1.6, H-6), 7.87 (2H, d, *J* 8.6, H-2′,6′), 7.67 (1H, d, *J* 8.4, H-7), 2.66 (3H, s, CH_3CO).

*3.3.2. 2-(3-Furanyl)-5-acetyl-1H-benzimidazole (**42**)*

Yield: 68%. R_f = 0.18 (EP-EA 4:6); m.p. 215–218 °C. Anal. calc. for :$C_{13}H_{10}N_2O_2$: C, 69.02; H, 4.46; N, 12.38; found: C, 68.82; H, 4.58; N,

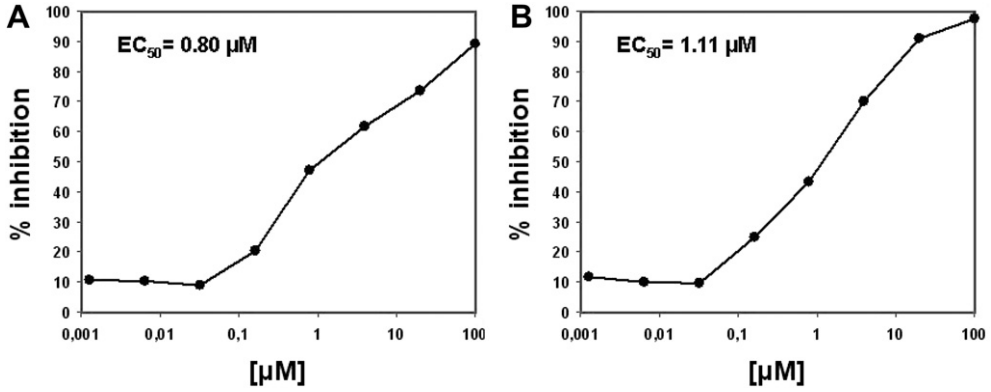


Fig. 7. Compound **31**. Dose–response curves from cell-based assays against BVDV (**A**) and HCV (**B**).

12.30; ν_{max} (nujol) cm^{-1}: 3260, 1659; λ_{max} (EtOH) nm: 308, 247, 228; ^{1}H NMR ($CDCl_3$-DMSO-d_6) δ: 8.36 (1H, dd, *J* 1.8 and 0.8, H-2′), 8.19 (1H, s, H-4), 7.85 (1H, dd, *J* 8.6 and 1.6, H-6), 7.68 (1H,dd, *J* 1.8 and 1.6, H-4′), 7.59 (1H, d, *J* 8.6, H-7), 7.11 (1H, dd, *J* 1.8 and 0.8, H-5′), 2.65 (3H, s, CH_3CO).

*3.3.3. 2-(Cyclohexyl)-5-acetyl-1H-benzimidazole (**43**)*

Yield: 58% after chromatography [R_f = 0.23 (EP-EA 4:6)]; m.p. 45–48 °C. Anal. calc. for: $C_{15}H_{18}N_2O$: C, 74.35; H, 7.49; N, 11.56; Found: C, 74.28; H, 7.70; N, 11.47. ν_{max} (nujol) cm^{-1}: 1660; λ_{max} (EtOH) nm: 268, 214; ^{1}H NMR ($CDCl_3$) δ: 8.22 (1H, s, H-4), 7.84 (1H, dd, *J* 8.6 and 1.6, H-6), 7.58 (1H, d, *J* 8.6, H-7), 3.04–2.24 (1H, m, CH cyclohexyl), 2.66 (3H, s, CH_3CO), 2.24–1.18 (10H, m, cyclohexyl).

*3.4. General procedure for the preparation of the 2-[aryl,cycloalkyl or heteroaryl]-5-acetyl-benzimidazoles (**35**–**41**)*

(B) To a solution of (**e**) (258 mmol) in DMF-water in the ratio 30:1 (4 mL), a solution of the appropriate aldheyde (284 mmol) and of oxone ($2KHSO_5$–$KHSO_4$–K_2SO_4) (167 mmol) were added. The mixture was kept under stirring at rt and after 1 h was poured dropwise into a stirred solution constituted by 1 M K_2CO_3 (1.80 mL) and water (40 ml). The solid precipitates (**35**–**41**) were collected, thoroughly washed with water and left to dry on air. In the case of compounds **40**–**41**, the aqueous phase was decanted and the organic residue was taken up with chloroform. Then, the chloroform solution was dried over anhydrous sodium sulphate and evaporated. Purification of all compounds was performed by silica gel flash column chromatography, eluting with a mixture of petrol ether (PE) ethylacetate (EA) in the ratio indicated under the R_f of each compound, as described below.

Table 3
Comparative activity of compound **31** against BVDV and HCV in cell-based assays.

Compd	Cell-based[a]			
	BVDV		HCV-1b	
	[b]**CC50**	[c]**EC50**	[d]**CC50**	[e]**EC50**
31	>100	0.80 ± 0.06	11.3 ± 1.5	1.11 ± 0.15

[a] Data represent mean values for three independent determinations.
[b] Compound concentration (μM) required to reduce the viability of mock-infected MDBK cells by 50%, as determined by the MTT method.
[c] Compound concentration (μM) required to achieve 50% protection of MDBK cells from BVDV-induced cytopathogenicity, as determined by the MTT method.
[d] Compound concentration (μM) required to reduce the viability of GS4.1 cells by 50%, as described in the Experimental section.
[e] Compound concentration (μM) required to achieve 50% protection of GS4.1 cells from cytopathogenicity, as described in the Experimental section.

*3.4.1. 1-Cyclohexyl-2-phenyl-5-acetyl-1 H-benzimidazole (**35**)*

Yield: 69%. R_f = 0.31 (PE-EA 6:4); m.p. 153–155 °C; Anal. calc. for $C_{21}H_{22}N_2O$: C, 79.21; H, 6.96; N, 8.80; C, 78.96; H, 7.03; N, 8.70. ν_{max} (nujol) cm^{-1}: 1672. λ_{max} (EtOH) nm: 242, 199. ^{1}H NMR ($CDCl_3$) δ: 8.42 (1H, s, H-4), 7.98 (1H,dd, *J* 8.0 and 1.6, H-6), 7.70 (1H, d, *J* 8.0, H-7), 7.65–7.55 (3H, m, H-3′,4′,5′), 7.54 (2H, m, H-2′,6′), 4.44–4.24 (1H, m, CH cyclohexyl), 2.64 (3H, s, CH_3CO), 2.42–1.26 (10H, m, cyclohexyl).

*3.4.2. 1-Cyclohexyl-2-(4-chlorophenyl)-5-acetyl-1H-benzimidazole (**36**)*

Yield: 76%. R_f = 0.38 (PE-EA 7:3); m.p. 182–184 °C; Anal. calc. for $C_{21}H_{21}ClN_2O$: C, 71.48; H, 6.00; Cl, 10.05; N, 7.94; found: C, 71.25; H, 6.40; Cl, 9.90; N, 7.85. ν_{max} (nujol) cm^{-1}: 1674. λ_{max} (EtOH) nm: 251, 199. ^{1}H NMR ($CDCl_3$) δ: 8.41 (1H, s, H-4), 7.97 (1H,dd, *J* 8.0 and 1.6, H-6), 7.69 (1H, d, *J* 8.6, H-7), 7.59 (2H, d, *J* 8.6, H-3′,5′), 7.53 (2H, d, *J* 8.6, H-2′-6′), 4.42–4.24 (1H, m, CH cyclohexyl), 2.69 (3H, s, CH_3CO), 2.40–1.20 (10H, m, cyclohexyl).

*3.4.3. 1-Cyclohexyl-2-(4-trifluoromethylphenyl)-5-acetyl-1H-benzimidazole (**37**)*

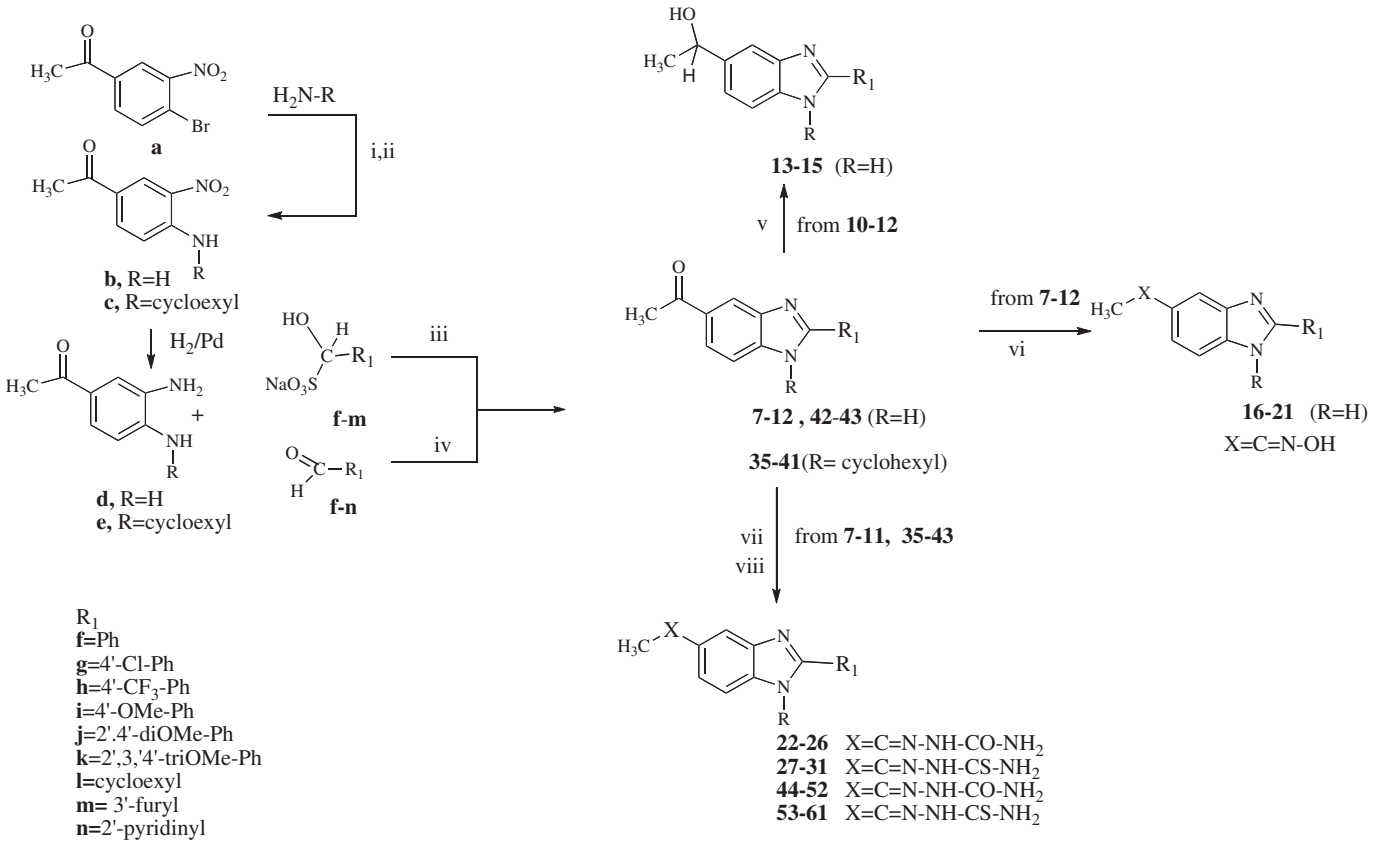
Yield: 78%. R_f = 0.44 (PE-EA 7:3); m.p. 158–160 °C; Anal. calc.% for $C_{22}H_{21}F_3N_2O$: C, 68.38; H, 5.48; F, 14.75; N, 7.25; found C, 68.03; H, 5.80; N, 7.11. ν_{max} (nujol) cm^{-1}: 1678. λ_{max} (EtOH) nm: 255, 228. ^{1}H NMR ($CDCl_3$) δ: 8.44 (1H, s, H-4), 7.99 (1H,dd, *J* 8.6 and 1.4, H-6), 7.85 (2H, d, *J* 8.4, H-3′,5′), 7.84 (2H, d, *J* 8.4, H-2′-6′), 7.72 (1H, d, *J* 8.4, H-7), 4.38–4.26 (1H, m, CH cyclohexyl) 2.69 (3H, s, CH_3CO), 2.45–1.25 (10H, m, cyclohexyl).

*3.4.4. 1-Cyclohexyl -2-(4-methoxyphenyl)-5-acetyl-1H-benzimidazole (**38**)*

Yield: 82%. R_f = 0.31 (PE-EA 1:1); m.p. 138–140 °C; Anal. calc. for $C_{22}H_{24}N_2O_2$: C, 75.83; H, 6.94; N, 8.04; found: C, 76.01; H, 6.68; N, 7.98. ν_{max} (nujol) cm^{-1}: 3583, 1664. λ_{max} (EtOH) nm: 257, 201. ^{1}H NMR ($CDCl_3$) δ: 8.40 (1H, s, H-4), 7.95 (1H,dd, *J* 8.8 and 1.6, H-6), 7.68 (1H, d, *J* 8.8, H-7), 7.59 (2H, d, *J* 8.0, H-3′,5′), 7.06 (2H, d, *J* 8.0, H-2′-6′), 4.48–4.28 (1H, m, CH cyclohexyl), 3.90 (3H, s, OCH_3), 2.68 (3H, s, CH_3CO), 2.40–1.26 (10H, m, cyclohexyl). LC/MS: 349 (M + 1).

*3.4.5. 1-Cyclohexyl-2-(2,4-dimethoxyphenyl)-5-acetyl-1H-benzimidazole (**39**)*

Yield: 90%.R_f = 0.30 (PE-EA 1:1); m.p. 76–78 °C; Anal. calc.% for $C_{23}H_{26}N_2O_3$: C, 72.99; H, 6.92; N, 7.40; found: C, 73.10; H, 7.04; N, 7.20. ν_{max} (nujol) cm^{-1}: 1672. λ_{max} (EtOH) nm: 285, 239, 203. ^{1}H NMR ($CDCl_3$) δ: 8.39 (1H, s, H-4), 7.94 (1H,dd, *J* 8.0 and 1.6, H-6), 7.66 (1H, d, *J* 8.4, H-7), 7.43 (1H, d, *J* 8.4, H-6′), 6.64 (1H, dd, *J* 8.4 and 1.2, H-5′), 6.58 (1H, d, *J* 2.2, H-3′), 4.06–3.90 (1H, m, CH cyclohexyl), 3.90 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 2.68 (3H, s, CH_3CO), 2.30–1.20



Scheme 1. i, In ethanol in sealed tube at 100 °C; ii, in DMSO at 70 °C for 3 h; iii, in refluxing ethanol for 4–5 h; iv, in DMF at rt for 1 h in the presence of oxone; v, NaBH4 in ethanol/water; vi, NH2OH in ethanol; vii, semicarbazide hydrochloride in ethanol and in the presence of sodium acetate; viii, thiosemicarbazide in ethanol, water and acetic acid.

(10H, m, cyclohexyl). ^{13}C NMR (DMSO-d_6) δ :197.54 (C=O), 162.31 (C-2), 158.32 (C-4′), 153.30 (C-2′), 143.13 (C-3a)), 136.67 (C-7a), 132.82 (C-6), 130.74 (C-5), 121.72 (C-4), 120.60 (C-7), 112.44 (C-1′), 111.62 (C-6′), 105.63 (C-5′), 98.34 (C-3′), 57.01 (C-1″-cyclohexane), 55.51 (OCH_3), 55.39 (OCH_3), 30.53 (C-2″,6″), 26.78 (CH_3), 25.59 (C-3″,5″), 24.54 (C-4″).

*3.4.6. 1-Cyclohexyl -2-[furan-3-yl]-5-acetyl-1H-benzimidazole (**40**)*

Yield: 62%. R_f = 0.38 (PE-EA 1:1); m.p. 117–118 °C; Anal. calc.% for $C_{19}H_{20}N_2O_2$: C, 74.00; H, 6.54; N, 9.08; found: C, 73.69; H, 6.82; N, 8.81. ν_{max} (nujol) cm^{-1}: 1672. λ_{max} (EtOH) nm: 245. ^{1}H NMR ($CDCl_3$) δ: 8.38 (1H, dd, *J* 8.0 and 0.8, H-2′), 7.96 (1H, dd, *J* 8.0 and 1.8, H-6), 7.90 (1H, s, H-4), 7.65 (1H, d, *J* 8.0, H-7), 7.60 (1H, dd, *J* 1.8 and 1.6, H-4′), 6.80 (1H, dd, *J* 1.4 and 0.6, H-5′), 4.60–4.40 (1H, m, CH cyclohexyl), 2.67 (3H, s, CH_3CO), 2.39–1.37 (10H, m, cyclohexyl).

*3.4.7. 1-Cyclohexyl -2-[pyridin-2-yl]-5-acetyl-1H-benzimidazole (**41**)*

Yield: 66%. R_f = 0.28 (PE-EA 1:1); m.p. 125–128 °C; Anal. calc.% for $C_{20}H_{21}N_3O$: C, 75.21; H, 6.63; N, 13.16; found: C, 74.89; H, 7.02; N, 13.00. ν_{max} (nujol) cm^{-1}: 1673. λ_{max} (EtOH) nm: 295, 237. ^{1}H NMR ($CDCl_3$) δ: 8.73 (1H, d, *J* 4.0, H-3′), 8.44 (1H, d, *J* 1.4, H-4), 8.24 (1H, d, *J* 8.0, H-6′), 7.94 (1H, dd, *J* 8.6 and 1.6, H-6), 7.90–7.84 (1H, m, H-5′), 7,75 (1H, d, *J* 8.8, H-7), 7.44–7.36 (1H, m, H-4′), 5.30–5.20 (1H, m, CH cyclohexyl), 2.69 (3H, s, CH_3CO), 2.42–1.20 (10H, m, cyclohexyl).

*3.5. General procedure for the reduction of the 2-[R-substitutedphenyl]-5-acetyl-benzimidazoles (**10**–**12**) into the derivatives (**13**–**15**)*

A suspension of **10**–**12** (34 mmol) and sodium borohydride (68 mmol) in ethanol (20 mL) was refluxed under stirring for 2 h. Then, the solvent was evaporated under vacuum. The gummy residue was shaken with water and eventually taken up with dry ether to give a solid that was recrystallized from ethanol or ethanol–water.

*3.5.1. 1-(2-(4-Methoxyphenyl)-1H-benzimidazol-5-yl)ethanol (**13**)*

Yield: 94%; m.p.222–224 °C as described [6].

*3.5.2. 1-(2-(2,4-Dimethoxyphenyl)-1H-benzimidazol-5-yl)ethanol (**14**)*

Yield: 80%; m.p. 217–218 °C; Anal. calc for $C_{17}H_{18}N_2O_3 + 0.25H_2O$: C, 67.42; H, 6.07; N, 9.25; found: C, 67.38; H, 6.11; N, 9.45. ^{13}C NMR (DMSO-d_6) δ: 161.87 (C-4′), 158.00(C-2′), 149.16 (C-2), 142.70 (C-3a), 141.50 (C-7a), 134.55 (C-5), 130.80 (C-6′), 117.35 (C-6), 112.91 (C-4), 111.19 (C-7), 108.24 (C-1′), 106.19 (C-5′), 98.55 (C-3′), 68.61 (C-8), 55.80 (2′-OCH_3), 55.48 (4′-OCH_3), 26.50 (CH_3).

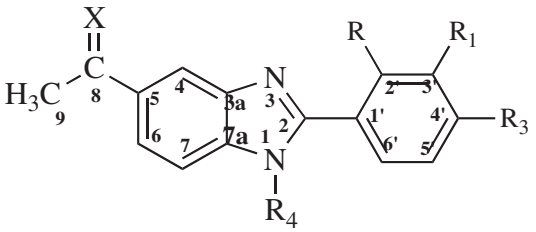
*3.5.3. 1-(2-(2,3,4-Trimethoxyphenyl)-1H-benzimidazol-5-yl) ethanol (**15**)*

Yield: 78%; m.p. 140–142 °C; Anal. calc for $C_{18}H_{20}N_2O_4 + 0.5H_2O$: C, 64.00; H, 6.27; N, 8.30; found: C, 63.83; H, 6.08; N, 8.35.

*3.6. General procedure for the preparation of the ketoximes **16**–**21***

A mixture of the ketone (**7**–**12**) (1.68 mmol) in ethanol (10 mL) and hydroxylamine hydrochloride (1.78 mmol) in water (1 mL) was heated under reflux for 2 h in the presence of a sodium hydroxide aqueous solution (3.56 mmol in 2 ml of water). On cooling, the mixture was diluted with water to cause

Table 4
^{13}C NMR spectra of compounds 14,17,22,24,25,29,39,48,50,53,55,61, representative of the list of Figs. 3 and 5.



Compd.	C-2	C-3a	C-4	C-5	C-6	C-7	C-7a	C-8 $X = O$ $X = NOH$ $X = N{-}NH$	C-9	C-1′*	C-2′*	C-3′*	C-4′*	C-5′*	C-6′*	X; N═NH−CO−(S)NH_2	Other chemical shifts
14	149.16	142.70	112.91	134.55	117.35	111.19	141.50	68.61	26.50	108.24	158.00	98.55	161.87	106.19	130.80		55.80 (2′-OCH_3), 55.48 (4′-OCH_3),
17	154.91	129.12	128.19	120.37	128.87	128.19	128.87	153.37	11.99	131.64	129.12	129.12	134.67	129.12	129.12		
22	152.11	129.05	130.04	126.50	120.87	112.92	130.04	145.10	13.85	132.88	126.50	129.05	129.05	129.05	126.50	157.60 C═O	
24	150.50	133.36	127.10	130.05	129.42	112.93	133.87	144.96	13.83	133.36	127.10	126.01	133.87	126.01	127.10	157.66 C═O	121.35 (CF_3)
25	152.13	136.20	120.58	132.62	122.34	114.45	139.50	145.02	13.82	112.90	128.11	114.35	160.76	114.35	128.11	157.50 C═O	55.38 (OCH_3),
29	151.71	137.78	112.97	128.18	114.27	113.60	138.14	148.50	14.50	133.04	126.93	129.25	122.29	129.25	126.93	178.81 C═S	131.00 (CF_3)
39	162.31	143.13	121.72	130.74	132.82	120.60	136.67	197.54	26.78	112.44	153.30	98.34	158.32	105.63.	111.62		57.01 (C-1″) ,55.51 (OCH_3), 55.39 (OCH_3), 30.53 (C-2″,6″), 25.59 (C-3″,5″), 24.54 (C-4″).
48	151.77	133.60	120.13	132.78	131.92	117.15	143.60	145.08	13.80	105.51	157.51	111.98	158.27	98.30	112.13	162.07 C═O	56.74 (C-1″), 55.45 (2′-OCH_3), 55.32(4′-OCH_3), 30.66 (C-2″,6″), 25.64 (C-3″,5″), 24.68 (C-4″),
50	144.80	142.95	121.22	134.76	113.10	117.68	137.55	148.96	13.76	N-1	150.85	125.53	132.74	124.40	150.32	157.45 C═O	56.45 (C-1″), 30.73 (C-2″,6″), 25.78 (C-3″,5″), 24.72 (C-4″),
53	153.99	143.31	118.21	129.78	121.05	112.55	134.45	148.76	14.42	130.60	128.76	129.30	131.69	129.30	128.76	178.69 C═S	56.61 (C-1″), 30.68 (C-2″,6″), 25.53 (C-3″,5″), 24.50 (C-4″)
55	160.30	148.83	112.90	120.82	117.96	112.45	143.25	153.99	14.42	134.46	130.77	114.20	131.56	114.20	130.77	178.67 C═S	122.64 (CF_3) 55.33 (C-1″), 30.66 (C-2″,6″), 25.55 (C-3″,5″), 24.53 (C-4″)
61	149.20	138.74	113.53	131.21	120.42	112.98	138.74	160.19	14.50	37.69	31.18	25.53	21.13	25.53	31.18	178.65 C═S	

*Superscript is referred to both aromatic and cyclohexane ring at position 2 of benzimidazole; Superscript is referred to cyclohexane ring at position 1 of benzimidazole.

precipitation of the oximes as crude solids, which were recrystallized from ethanol.

*3.6.1. 1-(2-Phenyl-1H-benzimidazol-5-yl)ethanone oxime (**16**)*

Yield: 75%; m.p. 236-231 °C; Anal. calc. for $C_{15}H_{13}N_3O + 0.5H_2O$: C, 69.21; H, 5.42; N, 16.14; found: C, 69.10; H, 5.47; N, 15.37.

*3.6.2. 1-(2-(4-Chlorophenyl)-1H-benzimidazol-5-yl)ethanone oxime (**17**)*

Yield: 65%; m.p. 254–257 °C; Anal. calc. for $C_{15}H_{12}ClN_3O + 1C_2H_6O$: C, 61.54; H, 5.47; N, 12.66; found: C, 61.43; H, 5.37; N, 12.81. ^{13}C NMR (DMSO-d_6) δ: 153.37 (C=NOH), 151.01 (C-2), 134.67 (C-4′), 131.64 (C-1′), 129.12 (C- 3′,5′, 2′,6′, 3a), 128.87 (C-6,7a) 128.19 (C-4,7), 120.37 (C-5), 11.99 (CH_3).

*3.6.3. 1-(2-(4-Trifluoromethylphenyl)-1H-benzimidazol-5-yl) ethanone oxime(**18**)*

Yield: 76%; m.p. 277–282 °C; Anal. calc. for $C_{16}H_{12}F_3N_3O$: C, 60.19; H, 3.79; N, 13.16; found: C, 60.08; H, 4.19; N, 12.97.

*3.6.4. 1-(2-(4-Methoxyphenyl)-1H-benzimidazol-5-yl)ethanone oxime(**19**)*

Yield: 86%; m.p. 275–280 °C; Anal. calc. for $C_{16}H_{15}N_3O_2$: C, 68.31; H, 5.37; N, 14.94; found: C, 68.28; H, 5.47; N, 14.87.

*3.6.5. 1-(2-(2,4-Dimethoxyphenyl)-1H-benzimidazol-5-yl) ethanone oxime (**20**)*

Yield:80%; m.p. 245–250 °C. Anal. calc. For $C_{17}H_{17}N_3O_3$: C, 65.58; H, 5.50; N, 13.50; found: C, 65.45; H, 5.70; N, 13.27.

*3.6.6. 1-(2-(2,3,4-Trimethoxyphenyl)-1H-benzimidazol-5-yl) ethanone oxime(**21**)*

Yield: 69%; m.p. 256–260 °C; Anal. calc. for $C_{18}H_{19}N_3O_4$: C, 63.33; H, 5.61; N, 12.31; found: C, 62.89; H, 5.71; N, 12.17.

*3.7. General procedure for the preparation of the semicarbazones (**22**–**26**) and (**44**–**52**)*

To a solution of the ketone (**7**–**12**) (**35**–**43**) (1 mmol) in ethanol (3 mL), an excess of semicarbazide hydrochloride (3.4 mmol) dissolved in an aqueous solution of sodium acetate (10 mmol in 10 mL of water) was added. The mixture was refluxed for 3 h (**24**–**26**), 4 h (**22**), 12 h (**23**) and 5 h (**35**–**43**). On cooling, the precipitates were collected and left to dry on air. The crude products were then purified on flash chromatography on silica gel column eluting with a mixture of chloroform/methanol, as indicated below to give rise to the semicarbazones (**22**–**26**) and (**44**–**52**).

*3.7.1. 2-(1-(2-Phenyl-1H-benzimidazol-5-yl)ethylidene) hydrazinecarboxamide (**22**)*

Yield: 41%; R_f = 0.36 ($CHCl_3$–MeOH 9:1); m.p. 153–155 °C; Anal. calc for $C_{16}H_{15}N_5O$: C, 65.52; H, 5.15; N, 23.88; found: C, 65.83; H, 5.08; N, 24.00 ν_{max} (nujol) cm^{-1}: 3471, 1666; λ_{max} (EtOH) nm: 323, 258, 206; ^{1}H NMR ($CDCl_3$-DMSO-d_6) δ: 12.80 (1H, br s, NH benzimidazole), 9.17 (1H, s, NH) 8.18 (1H, dd, *J* 8.0 and 1.6, H-6), 7.98 (1H, s, H-4), 7.73 (1H, d, *J* 8.0, H-7), 7.60–7.40 (5H, m, H-2′,3′,4′,5′,6′), 6.29 (1H, br s, NH), 2.32 (3H, s, CH_3C=N–).^{13}C NMR (DMSO-d_6) δ :157.60 (C=O, hydrazide), 152.11 (C-2), 145.10 (C=NH–NH), 132.88 (C-1′), 130.04 (C-), 129.05 (C-), 126.50 (C-5, 2′,6′), 120.87 (C-6), 112.92 (C-7), 13.86 (CH_3).

*3.7.2. 2-(1-(2-(4-Chlorophenyl)-1H-benzimidazol-5-yl)ethylidene) hydrazinecarboxamide (**23**)*

Yield: 29%; R_f = 0.31 ($CHCl_3$–MeOH 9:1); m.p. 162–164 °C; Anal. calc for $C_{16}H_{14}ClN_5O$: C, 58.63; H, 4.31; N, 21.37; found: C, 58.39; H, 4.80; N, 20.94; ν_{max} (nujol) cm^{-1}: 3469, 1731, 1584; λ_{max} (EtOH) nm: 329, 263, 204; ^{1}H NMR ($CDCl_3$-DMSO-d_6) δ: 12.76 (1H, br s, NH benzimidazole), 9.06 (1H, br s, NH), 8.18 (2H, d, *J* 8.2, H-3′,5′), 7.84 (1H, dd, *J* 8.2 and 1.2, H-6), 7.73 (1H, s, H-4), 7.68 (1H, d, *J* 8.6, H-7), 7.49 (2H, d, *J* 8.2, H-2′,6′), 6.20 (2H, br s, NH_2), 2.32 (3H, s, CH_3C=N–).

*3.7.3. 2-(1-(2-(4-Trifluoromethylyphenyl)-1H-benzimidazol-5-yl) ethylidene) hydrazine carboxamide (**24**)*

Yield: 64%; R_f = 0.28 ($CHCl_3$–MeOH 9,5:0,5); m.p. 238–240 °C; Anal. calc for $C_{17}H_{14}$ F_3N_5O: C, 56.51; H, 3.91; N, 19.38; found: C, 56.18; H, 4.12; N, 19.20; ν_{max} (nujol) cm^{-1}: 3417, 1655, 1566; λ_{max} (EtOH) nm: 327, 264, 201; ^{1}H NMR ($CDCl_3$-DMSO-d_6) δ: 13.05 (1H, br s, NH benzimidazole), 9.25 (1H, br s, NH), 8.39 (2H, d, *J* 8.4, H-3′,5′), 8.07 (1H, d, *J* 1.4, H-4), 7.81 (2H,d, *J* 8.4, H-2′,6′), 7.74–7.45 (2H, m, H-6 and H-7), 6.35 (2H, br s, NH_2), 2.32 (3H, s, CH_3C=N–). ^{13}C NMR (DMSO-d_6) δ: 157.66 (C=O), 150,50 (C-2), 144.96 (C=N–NH–), 133.87 (C-7a, 4′), 133.36 (C-3a, 1′), 130.05 (C-5), 129.42 (C-6), 127.10 (C- 4, 2′,6′), 126.01 (C- 3′,5′), 121.35 (CF_3), 112.93 (C-7), 13.83 (CH_3).

*3.7.4. 2-(1-(2-(4-Methoxyphenyl)-1H-benzimidazol-5 yl) ethylidene)hydrazinecarboxamide (**25**)*

Yield: 23%; R_f = 0.25 ($CHCl_3$–MeOH 9,5:0,5); m.p. 127–129 °C; Anal. calc for $C_{17}H_{17}N_5O_2$: C, 63.15; H, 5.30; N, 21.66; found: C, 63.00; H, 5.70; N, 21.48; ν_{max} (nujol) cm^{-1}: 1677, 1572; λ_{max} (EtOH) nm: 327, 320, 266, 209; ^{1}H NMR ($CDCl_3$,DMSO-d_6) δ: 12.60 (1H, br s, NH benzimidazole), 9.11 (1H, br s, NH), 8.12(2H, d, *J* 8.0, H-3′,5′), 7.97 (1H, s, H-4), 7.69 (1H, d, *J* 8.4, H-7), 7.60–7.45 (1H, dd, *J* 9.2 and 1.6, H-6), 7.03 (2H, d, *J* 8.0, H-2′,6′), 6.23 (2H, br s, NH_2), 2.31 (3H, s, CH_3C=N–). ^{13}C NMR (DMSO-d_6) δ: 160.76 (C-4′), 157,50 (C=O), 152.13 (C-2), 145.01 (C=N–NH–), 139.50 (C-7a), 136.20 (C-3a), 132.62 (C-5), 128.11 (C-2′,6′), 122.34 (C-6), 120.58 (C-4), 114.45 (C-3′,5′,7), 112.90 (C-1′), 55.38 (OCH_3), 13.82 (CH_3).

*3.7.5. 2-(1-(2-(2,4-Dimethoxyphenyl)-1H-benzimidazol-5-yl) ethylidene)hydrazinecarboxamide (**26**)*

Yield: 58%; R_f = 0.22 ($CHCl_3$–MeOH 9,5:0,5); m.p.186–188 °C; Anal. calc. for $C_{18}H_{19}N_5O_3$: C, 61.18; H, 5.42; N, 19.82; found: C, 60.94; H, 5.80; N, 19.51; ν_{max} (nujol) cm^{-1}: 3194, 1683, 1609; λ_{max} (EtOH) nm: 330, 262, 204; ^{1}H NMR ($CDCl_3$-DMSO-d_6) δ: 9.36 (1H, br s, NH), 8.25 (1H, d, *J* 9.2, H-6′), 8.13 (1H, s, H-4), 7.88 (1H, dd, *J* 8.6 and 1.4, H-6), 7.65 (1H, d, *J* 8.6, H-7), 6.77 (2H, s, H-5′), 6.40 (2H, br s, NH_2), 4.09 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 2.30 (3H, s, CH_3C=N–).

*3.7.6. 2-(1-(1-Cyclohexyl-2-phenyl-1H-benzimidazol-5-yl) ethylidene)hydrazinecarboxamide (**44**)*

Yield: 94%. R_f = 0.18 ($CHCl_3$–MeOH 9:1); m.p. 228–230 °C; Anal. calc. for $C_{22}H_{25}N_5O$: C, 70.38; H, 6.71; N, 18.65; found: C, 70.10; H, 6.84; N, 18.56; ν_{max} (nujol) cm^{-1}: 3457, 1672, 1583; λ_{max} (EtOH) nm: 255, 203; ^{1}H NMR ($CDCl_3$) δ: 8.40 (1H, dd, *J* 8.0 and 1.6, H-6), 8.10 (1H, s, H-4), 7.75 (1H, d, *J* 8.0, H-7), 7.72–7.60 (3H, m, H-3′,4′,5′), 7.60–7.50 (2H, m, H-2′,6′), 4.50–4.23 (1H, m, CH cyclohexyl), 2.31 (3H, s, CH_3C=N–), 2.08–1.18 (10H, m, cyclohexyl).

*3.7.7. 2-(1-(2-(4-Chlorophenyl)-1-cyclohexyl-1H-benzimidazol-5-yl)ethylidene)hydrazinecarboxamide(**45**)*

Yield: 51%. R_f = 0.35 ($CHCl_3$–MeOH 9.5:0.5); m.p. 212–214 °C; Anal. calc. for $C_{22}H_{24}ClN_5O$: C, 64.46; H, 5.90; Cl, 8.65; N, 17.09; found: C, 64.16; H, 6.30; Cl, 8.15; N, 17.40. ν_{max} (nujol) cm^{-1}: 3473, 1681, 1585; λ_{max} (EtOH) nm: 244; ^{1}H NMR (DMSO-d_6) δ: 9.26 (1H, br s, NH)), 8.62 (2H, d, *J* 8.4, H-3′,5′), 8.01 (1H, s, H-4), 7.81 (1H, dd, *J* 8.0 and 68.13; H, 6.71; N, 17.27 1,6, H-6), 7.72 (1H, d, *J* 8.6, H-7), 7.56 (2H, d, *J* 8.4, H-2′-6′), 6.34 (2H, br s, NH_2), 4.39–4.18 (1H, m, CH cyclohexyl), 2.29 (3H, s, CH_3C=N–), 2.04–1.10 (10H, m, cyclohexyl).

*3.7.8. 2-(1-(1-Cyclohexyl-2-(4-(trifluoromethyl)phenyl)-1H-benzimidazol-5-yl)ethylidene)hydrazine carboxamide (**46**)*

Yield: 50%. R_f = 0.36 ($CHCl_3$–MeOH 9.5:0.5); m.p. 215–218 °C; Anal. calc. for $C_{23}H_{24}F_3N_5O$: C, 62.29; H, 5.45; N, 15.79; found: C, 62.40; H, 5.15; N, 15.95. ν_{max} (nujol) cm^{-1}: 3643, 1681, 1570; λ_{max} (EtOH) nm: 266, 227, 203; ^1H NMR ($CDCl_3$-DMSO-d_6) δ: 8.59 (1H, br s, NH), 8.07 (1H, s, H-4), 7.94–7.65 (6H, m, H-6,7,2′,3′,5′,6′), 5.80 (2H, br s, NH_2), 4.44–4.20 (1H, m, CH cyclohexyl), 2.33 (3H,s, CH_3C=N–), 2.12–1.18 (10H, m, cyclohexyl).

*3.7.9. 2-(1-(1-Cyclohexyl-2-(4-methoxyphenyl)-1H-benzimidazol-5-yl)ethylidene)hydrazine carboxamide (**47**)*

Yield: 83%. R_f = 0.35 ($CHCl_3$–MeOH 9.5:0.5); m.p. 213–215 °C; Anal. calc. for $C_{23}H_{27}N_5O_2$: C, 68.13; H, 6.71; N, 17.27; found: C, 68.31; H, 6.98; N, 17.20. ν_{max} (nujol) cm^{-1}: 3468, 1679, 1583; λ_{max} (EtOH) nm: 258, 202; ^1H NMR ($CDCl_3$-DMSO-d_6) δ: 9.23 (1H, br s, NH), 7.98 (1H, s, H-4), 7.78 (1H, dd, *J* 8.0 and 1.6, H-6), 7.67 (1H, d, *J* 8.0, H-7), 7.58 (2H, d, *J* 8.2, H-3′,5′), 7.09 (2H, d, *J* 8.2, H-2′-6′), 6.34 (2H, br s, NH_2), 4.45–4.20 (1H, m, CH cyclohexyl), 3.89 (3H, s, OCH_3), 2.30 (3H, s, CH_3C=N–), 2.02–1.20 (10H, m, cyclohexyl).

*3.7.10. 2-(1-(1-Cyclohexyl-2-(2,4-dimethoxyphenyl)-1H-benzimidazol-5-yl)ethylidene)hydrazine carboxamide (**48**)*

Yield: 79%.R_f = 0.26 ($CHCl_3$–MeOH 9,5:0,5); m.p. 224–226 °C; Anal. calc. for $C_{24}H_{29}N_5O_3$: C, 66.19; H, 6.71; N, 16.08; found: C, 65.91; H, 6.77; N, 16.02. ν_{max} (nujol) cm^{-1}: 3483, 1683, 1591; λ_{max} (EtOH) nm: 284, 253, 203; ^1H NMR ($CDCl_3$) δ: 8.05 (1H, s, H-4), 7.70 (1H,dd, *J* 8.0 and 1.6, H-6), 7.60 (1H, d, *J* 8.0, H-7), 7.42 (1H, dd, *J* 8.4 and 2.4, H-4′), 6.61 (1H, dd, *J* 8.2 and 2.0, H-5′), 6.57 (1H, d, *J* 2.0, H-3′), 3.90 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 2.30 (3H, s, CH_3C=N–), 2.22–1.20 (10H, m, cyclohexyl). ^{13}C NMR (DMSO-d_6) δ :162.07 (C=O), 158.27 (C-4′), 157.51 (C-2′), 151.77 (C-2), 145.04 (C=N–NH), 143.61 (C-7a), 133.60 (C-3a), 132.78 (C-5), 131.92 (C-6), 120.13 (C-4), 117.15 (C-7), 112.13 (C-6′), 111.98 (C-3′), 105.51 (C-1′), 98.30 (C-5′), 56.74 (C-1″ cyclohexane), 55.45 (2′-OCH_3), 55.32(4′-OCH_3), 30.66 (C-2″,6″), 25.64 (C-3″,5″), 24.68 (C-4″), 13.80 (CH_3).

*3.7.11. 2-(1-(1-Cyclohexyl-2-(furan-3-yl)-1H-benzimidazol-5-yl) ethylidene)hydrazinecarboamide (**49**)*

Yield: 83%. R_f = 0.54 ($CHCl_3$–MeOH 9:1); m.p. 270–272 °C; Anal. calc. for $C_{20}H_{23}N_5O_2$: C, 65.73; H, 6.34; N, 19.16; found: C, 65.80; H, 6.21; N, 18.94. ν_{max} (nujol) cm^{-1}: 3469, 1680, 1584; λ_{max} (EtOH) nm: 295, 252, 206; ^1H NMR ($CDCl_3$) δ: 8.30 (1H, br s, NH), 8.08 (1H, dd, *J* 1.8 and 0.8, H-2′), 7.89 (1H, s, H-4), 7.69 (1H, dd, *J* 8.4 and 1.6, H-6), 7.63 (1H, dd, *J* 1.8 and 1.6, H-4′), 7.60 (1H, d, *J* 8.6, H-7 partially obscured), 6.80 (1H, dd, *J* 1.8 and 0.8, H-5′), 6.19 (1H, br s, NH), 5.14 (2H, br s, NH_2), 4.61–4.37 (1H, m, CH cyclohexyl), 2.31 (3H,s, CH_3C=N–), 2.18–1.20 (10H, m, cyclohexyl).

3.7.12. 2-(1-(1-Cyclohexyl-2-(pyridin-2-yl)-1H-benzimidazol-5-yl) ethylidene)hydrazinecarboxamide (50)

Yield: 83%. R_f = 0.42 ($CHCl_3$–MeOH 9,5:0,5); m.p. 185–188 °C; Anal. calc. for $C_{21}H_{24}N_6O$: C, 67.00; H, 6.43; N, 22.32.Found: C, 67.15; H, 6.73; N, 22.11. ν_{max} (nujol) cm^{-1}: 3848, 1681, 1580; λ_{max} (EtOH) nm: 271, 232, 201; ^1H NMR ($CDCl_3$) δ: 8.72 (1H, dd, *J* 4.0 and 1.8, H-3′), 8,22 (1H, dd, *J* 8.0 and 1.2, H-6), 8.12 (1H, d, *J* 8.0, H-7), 7.93–7.80 (1H, m, H-5′), 7.72 (1H, s, H-4), 7.42–7.35 (1H, m, H-4′-6′), 5.60–5.40 (1H, m, CH cyclohexyl), 2.32 (3H, s, CH_3C=N–), 2.18–1.20 (10H, m, cyclohexyl). ^{13}C NMR (DMSO-d_6) δ: 157.46 (C=O), 150.85 (C-2′ pyridinyl), 150.32 (C-6′), 148.96 (C=N–NH), 144.80 (C-2), 142.95 (C-3a), 137.55 (C-7a), 134.76 (C-5), 132.74 (C-4′), 125.35 (C-3′), 124.40 (C-5′), 121.22 (C-4), 117.68 (C-7), 113.10 (C-6), 56.45 (C-1″ cyclohexane), 30.73 (C-2″,6″), 25.78 (C-3″,5″), 24.72 (C-4″), 13.76 (CH_3).

*3.7.13. 2-(1-(1-Cyclohexyl-2-(furan-3-yl)-1H-benzimidazol-5-yl) ethylidene)hydrazinecarbothioamide (**51**)*

Yield: 38%. R_f = 0,23 (EP-EA 4:6); m.p. 205–206 °C; Anal. calc. for $C_{20}H_{23}N_5OS$: C, 62.97; H, 6.08; N, 18.36; found: C, 62.48; H, 6.15; N, 18.14. ν_{max} (nujol) cm^{-1}: 3120, 1619; λ_{max} (EtOH) nm: 303, 232; ^1H NMR ($CDCl_3$) δ: 8.77 (1H, br s, NH), 8.09 (1H, dd, *J* 0,8 and 1.8, H-2′), 7.89 (1H, s, H-4), 7.70 (1H, dd, *J* 8,6 and 1.6, H-6), 7.62 (1H, d, *J* 8.6, H-7), 7.60 (1H, dd, *J* 1.8 and 1.6, H-4′), 7.40 (1H, br s, NH), 6.80 (1H, dd, *J* 1,8 and 0,8, H-5′), 6.36 (2H, br s, NH_2), 4.30–4.20 (1H, m, CH cyclohexyl), 2.39 (3H, s, CH_3C=N–), 2.37–1.20 (10H, m, cyclohexyl).

*3.7.14. 2-(1-(1-Cyclohexyl-2-(pyridin-2-yl)-1H-benzimidazol-5-yl) ethylidene)hydrazinecarbothioamide (**52**)*

Yield: 83%. R_f = 0,27 ($CHCl_3$–MeOH 9.8:0,2); m.p. 194–196 °C; Anal. calc. for $C_{21}H_{24}N_6S$: C, 64.26; H, 6.16; N, 21.41; found: C, 64.06; H, 6.24; N, 21.18. ν_{max} (nujol) cm^{-1}: 3140, 1611; λ_{max} (EtOH) nm: 307; ^1H NMR ($CDCl_3$) δ: 8.80 (1H, br s, NH), 8.72 (1H, dd, *J* 4,8 and 1.8, H-3′), 8.22 (1H, dd, *J* 8.0 and 1.4, H-6), 8.14 (1H, s, H-4), 7.93–7.80 (1H, m, H-5′), 7.80–7.68 (1H, m, H-7), 7.50–7.35 (2H, m, H-4′,6′), 6.42 (1H, br s, NH), 5.60–5.40 (1H, m, CH cyclohexyl), 2.38 (3H, s, CH_3C=N–), 2.37–1.20 (10H, m, cyclohexyl).

*3.8. General procedure for the preparation of the thiosemicarbazones (**27**–**31**) and (**53**–**61**)*

To a stated amount of ketone (**7**–**12**) (**35**–**43**) (42 mmole) in ethanol (10 mL), a slight excess of thiosemicarbazide (44 mmole) dissolved in a mixture of water (12 mL) and glacial acetic acid (0.8 mL) was added. The mixture was the refluxed for 1 h (**27**–**30**), 6 h (**31**, **40**, **59**), 9 h (**58**), 12 h (**60**, **61**) under stirring. On cooling, the precipitate formed was collected and thoroughly washed with water. The crude products, as amorphous powder coloured from cream-yellow to brown, were recrystallized from ethanol–water.

*3.8.1. 2-(1-(2-Phenyl-1H-benzimidazol-5-yl)ethylidene) hydrazinecarbothioamide (**27**)*

Yield: 77%; R_f = 0.35 ($CHCl_3$–MeOH 9.5:0.5); m.p. 180–185 °C; Anal. calc. for $C_{16}H_{15}N_5S + H_2O$: C 58.64; H, 5.19; N, 21.38; found: C, 58.39; H, 4.91; N, 20.92. ν_{max} (nujol) cm^{-1}: 3418, 1588; λ_{max} (EtOH) nm: 331, 258, 204; ^1H NMR ($CDCl_3$-DMSO-d_6) δ: 10.10 (1H, br s, NH), 8.20 (1H, dd, *J* 8.0 and 1.6, H-6), 8.06 (1H, s, H-4), 7.90–7.82 (3H, m, H-3′-4′-5′), 7.72 (2H, br s, NH_2), 7.64 (1H, d, *J* 8.0, H-7), 7.68–7.44 (2H, m, H-2′-6′), 2.43 (3H, s, CH_3CO).

*3.8.2. 2-(1-(2-(4-Chlorophenyl)-1H-benzimidazol-5-yl)ethylidene) hydrazinecarbothioamide (**28**)*

Yield: 63%; R_f = 0.35 ($CHCl_3$–MeOH 9.5:0.5); m.p. 200–205 °C; Anal. calc. for $C_{16}H_{14}ClN_5S$; C, 55.89; H, 4.10; N, 20.37; found: C, 55.63; H, 4.60; N, 19.82. ν_{max} (nujol) cm^{-1}: 3421, 1589; λ_{max} (EtOH) nm: 332, 261, 202; ^1H NMR ($CDCl_3$-DMSO-d_6) δ: 10.16 (1H, br s, NH), 8.20 (1H, d, *J* 8.6, H-7), 8.12 (1H, s, H-4), 7.96 (1H, dd, *J* 8.6 and 1,4, H-6), 7.80 (2H, br s, NH_2), 7.64 (2H, d, *J* 9. H-3′,5′), 7.60 (2H, d, *J* 8.8, H-2′,6′), 2.43 (3H, s, CH_3CO).

*3.8.3. 2-(1-(2-(4-Trifluoromethylphenyl)-1H-benzimidazol-5-yl) ethylidene)hydrazinecarbothioamide (**29**)*

Yield: 64%; R_f = 0.33 ($CHCl_3$–MeOH 9.5:0.5); m.p.190–193 °C; Anal. calc. for $C_{17}H_{14}F_3N_5S$: C, 54.10; H, 3.74; N, 18.56; found; C, 54.19; H, 3.85; N, 18.70; ν_{max} (nujol) cm^{-1}: 3387, 1619; λ_{max} (EtOH) nm: 327, 262, 204; ^1H NMR ($CDCl_3$) δ: 10.07 (1H, br s, NH), 8.40 (1H,dd, *J* 8.0 and 1.2, H-6), 8.30 (1H, s, H-4), 8.00 (1H, d, *J* 8.0, H-7), 7.85 (2H, d, *J* 8.6, H-3′,5′), 7.74 (2H, br s, NH_2), 7.61 (2H, d, *J* 8.6, H-2′-6′), 2.42 (3H, s, CH_3C=N–). ^{13}C NMR (DMSO-d_6) δ: 178.81 (C=S), 151.71 (C-2), 148.50 (C=N–NH), 138.14 (C-7a), 137.78 (C-3a), 133.04

(C-1′), 131.00 (CF_3), 129.25 (C-3′,5′), 128.18 (C-5), 126.93 (C-2′,6′), 122.29 (C-4′), 114.27 (C-6), 113.60 (C-7), 112.97 (C-4), 14.50 (CH_3).

3.8.4. 2-(1-(2-(4-Methoxyphenyl)-1H-benzimidazol-5-yl) ethylidene)hydrazinecarbothioamide (**30**)

Yield: 55%; R_f = 0.35 ($CHCl_3$–MeOH 9.5:0.5); m.p.230–235 °C; Anal. calc. for $C_{17}H_{17}N_5OS$: C, 60.16; H, 5.05; N, 20.63; found: C, 59.95; H, 4.82; N, 21.10; ν_{max} (nujol) cm^{-1}: 3434, 1608; λ_{max} (EtOH) nm: 333, 265, 202; ^{1}H NMR (DMSO-d_6) δ: 10.31 (1H, br s, NH), 8.29 (1H, s, H-4), 8.18 (1H, dd, *J* 8.0 and 1.6, H-6), 8.10 (1H, d, *J* 8.0, H-7), 7.99 (2H, br s, NH_2), 7.80 (2H, br s, NH_2), 7.68 (2H, d, *J* 8.0, H-3′,5′), 7.25 (2H, d, *J* 8.8, H-2′,6′), 3.89 (3H, s, OCH_3), 2.40 (3H,s, CH_3C=N–).

3.8.5. 2-(1-(2-(2,4-Dimethoxyphenyl)-1H-benzimidazol-5-yl) ethylidene)hydrazinecarbothioamide (**31**)

Yield: 53%; R_f = 0.35 (PE-EA 2:8); m.p. 175–178 °C; Anal. calc. for $C_{18}H_{19}N_5O_2S$: C, 58.52; H, 5.18; N, 18.96; found: C, 58.60; H, 5.32; N, 18.64; ν_{max} (nujol) cm^{-1}: 3628, 1611; λ_{max} (EtOH) nm: 338, 262, 205; ^{1}H NMR (DMSO-d_6) δ: 10.19 (1H, br s, NH), 8.24 (1H, d, *J* 9.0, H-6′), 8.05 (1H, s, H-4), 7.89 (1H, dd, *J* 8.6 and 1.4, H-6), 7.56 (1H, d, *J* 8.0, H-7), 6.76 (1H, s, H-3′), 6.72 (1H, dd, *J* 9.0 and 1.6, H-5′), 4.02 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 2.37 (3H, s, CH_3C=N–).

3.8.6. 1-Cyclohexyl-2-(1-(2-phenyl-1H-benzimidazol-5-yl) ethylidene)hydrazinecarbothioamide (**53**)

Yield: 81%. R_f = 0.18 (EP-EA 1:1); m.p. 217–219 °C; Anal. calc. for $C_{22}H_{25}N_5S$: C, 67.49; H, 6.44; N, 17.89; found: C, 67.24; H, 6.59; N, 17.56. ν_{max} (nujol) cm^{-1}: 3583, 1603; λ_{max} (EtOH) nm: 319, 251, 201; ^{1}H NMR ($CDCl_3$) δ: 8.75 (1H, br s, NH), 8.13 (1H, s, H-4), 7.73 (1H, dd, *J* 8.0 and 1.6, H-6), 7.65 (1H, d, *J* 8.0, H-7), 7.60–7.50 (5H, m, H-2′,3′,4′,5′,6′), 7.41 (1H, br s, NH), 6.34 (2H, br s, NH_2), 4.45–4.32 (1H, m, CH cyclohexyl), 2.38 (3H, s, CH_3C=N–), 2.35–1.34 (10H, m, cyclohexyl). ^{13}C NMR (DMSO-d_6) δ :178.69 (C=S), 153.99 (C-2), 148.76 (C=N–NH), 143.31 (C-3a), 134.45 (C-7a), 131.69 (C-4′), 130.60 (C-1′), 129.78 (C-5), 129.30 (C-3′,5′), 128.76 (C-2′,6′), 121.05 (C-6), 118.21 (C-4), 112.55 (C-7), 56.61 (C-1″ cyclohexane), 30.68 (C-2″,6″), 25.53 (C-3″,5″), 24.50 (C-4″), 14.42 (CH_3).

3.8.7. 1-Cyclohexyl-2-(1-(2-(4-chlorophenyl)-1H-benzimidazol-5-yl)ethylidene)hydrazinecarbothio amide (**54**)

Yield: 83%. R_f = 0.29 (EP-EA 6:4); p.f. 228–230 °C; Anal. calc. for $C_{22}H_{24}ClN_5S$: C, 62.03; H, 5.68; Cl, 8.32; N, 16.44; found: C, 61.85; H, 5.90; Cl, 8.20; N, 16.19. ν_{max} (nujol) cm^{-1}: 3583, 1602; λ_{max} (EtOH) nm: 319, 280, 254, 202; ^{1}H NMR ($CDCl_3$) δ: 8.73 (1H, br s, NH), 8.11 (1H, s, H-4), 7.74 (1H, dd, *J* 8.0 and 1.6, H-6), 7.65 (1H, d, *J* 8.6, H-7), 7.60 (2H, d, *J* 9.0, H-3′,5′), 7.52 (2H, d, *J* 8.6, H-2′-6′), 7.40 (1H, br s, NH), 6.34 (2H, br s, NH_2), 4.42–4.24 (1H, m, CH cyclohexyl), 2.38 (3H, s, CH_3C=N–), 2.38–1.25 (10H, m, cyclohexyl).

3.8.8. 1-Cyclohexyl-2-(1-(2-(4-trifluoromethylphenyl)-1H-benzimidazol-5-yl)ethylidene)hydrazine carbothioamide (**55**)

Yield: 86%. R_f = 0.29 (EP-EA 6:4); m.p. 228–230 °C; Anal. calc. for $C_{23}H_{24}F_3N_5S$: C, 60.11; H, 5.26; N, 15.24; found: C, 60.34; H, 5.77; N, 15.41. ν_{max} (nujol) cm^{-1}: 3392, 1612; λ_{max} (EtOH) nm: 327, 313, 202; ^{1}H NMR ($CDCl_3$) δ: 8.80 (1H, br s, NH), 8.14 (1H, s, H-4), 7.85 (1H, dd, *J* 9.2 and 1.6, H-6), 7.80 (1H, d, *J* 9, H-7), 7.75 (2H, d, *J* 8.8, H-3′,5′), 7,66 (2H, d, *J* 8.8, H-2′-6′), 7.41 (1H, br s, 1H, NH_2), 6.46 (1H, br s, 1H, NH_2), 4.42–4.24 (1H, m, CH cyclohexyl), 2.38 (3H,s, CH_3C=N–), 2.34–1.25 (10H, m, cyclohexyl). ^{13}C NMR (DMSO-d_6) δ :178.67 (C=S), 160.30 (C-2), 153.99 (C=N–NH), 148.83 (C-3a), 143.25 (C-7a), 134.46 (C-1′), 131.56 (C-4′), 130.77 (C-2′,6′), 122.64 (CF_3), 120.82 (C-5), 117.96 (C-6), 114.20 (C-3′,5′), 114.90 (C-4), 112.45 (C-7), 55.33 (C-1″), 30.66 (C-2″,6″), 25.55 (C-3″,5″), 24.53 (C-4″), 14.42 (CH_3).

3.8.9. 1-Cyclohexyl-2-(1-(2-(4-methoxyphenyl)-1H-benzimidazol-5-yl)ethylidene)hydrazine carbothioamide (**56**)

Yield: 86%. R_f = 0.39 (PE-EA 3:7); m.p. 250–252 °C; Anal. calc. for $C_{23}H_{27}N_5OS$: C, 65.53; H, 6.46; N, 16.61; found: C, 65.67; H, 6.36; N, 16.80; ν_{max} (nujol) cm^{-1}: 3413, 1610; λ_{max} (EtOH) nm: 319, 259, 203; ^{1}H NMR ($CDCl_3$) δ: 8.76 (1H, br s, NH), 8.11 (1H, s, H-4), 7.74 (1H, dd, *J* 8.0 and 1.6, H-6), 7.65 (1H, d, *J* 8.6, H-7), 7.60 (2H, d, *J* 8.8, H-3′,5′), 7.41 (1H, br s, NH), 7.05 (2H, d, *J* 8.8, H-2′-6′), 6.34 (2H, br s, NH_2), 4.42–4.24 (1H, m, CH cyclohexyl), 3.90 (3H, s, OCH_3), 2.38 (3H, s, CH_3C=N–), 2.38–1.25 (10H, m, cyclohexyl). LC/MS: 422 (M + 1).

3.8.10. 1-Cyclohexyl-2-(1-(2-(2,4-dimethoxyphenyl)-1H-benzimidazol-5-yl)ethylidene)hydrazine carbothioamide (**57**)

Yield: 86%. R_f = 0.25 (EP-EA 3:7); m.p. 134–136 °C; Anal. calc. for $C_{24}H_{29}N_5O_2S$: C, 63.83; H, 6.47; N, 15.51; found: C, 63.54; H, 6.77; N, 15.42. ν_{max} (nujol) cm^{-1}: 3583, 1614; λ_{max} (EtOH) nm: 319, 253, 203; ^{1}H NMR ($CDCl_3$) δ: 8.76 (1H, br s, NH), 8.11 (1H, s, H-4), 7.70 (1H, dd, *J* 8.2 and 1.4, H-6), 7.62 (1H, d, *J* 8.2, H-7), 6.62 (1H, dd, *J* 8.6 and 2.4, H-5′), 6.57 (1H, d, *J* 2,0, H-3′), 6.36 (2H, br s, NH_2), 4.10–3.90 (1H, m, CH cyclohexyl), 3.90 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 2.37 (3H, s, CH3C=N–), 2.34–1.20 (10H, m, cyclohexyl).

3.8.11. 2-(1-(1-Cyclohexyl-2-(furan-3-yl)-1H-benzimidazol-5-yl) ethylidene)hydrazinecarbothioamide (**58**)

Yield: 38%. R_f = 0.23 (EP-EA 4:6); m.p. 205–206 °C; Anal. calc. for $C_{20}H_{23}N_5OS$: C, 62.97; H, 6.08; N, 18.36; found: C, 62.48; H, 6.15; N, 18.14. ν_{max} (nujol) cm^{-1}: 3120, 1619; λ_{max} (EtOH) nm: 303, 232; ^{1}H NMR ($CDCl_3$) δ: 8.77 (1H, br s, NH), 8.09 (1H, dd, *J* 0.8 and 1.8, H-2′), 7.89 (1H, s, H-4), 7.70 (1H, dd, *J* 8.6 and 1.6, H-6), 7.62 (1H, d, *J* 8.6, H-7), 7.60 (1H, dd, *J* 1.8 and 1.6, H-4′), 7.40 (1H, br s, NH), 6.80 (1H, dd, *J* 1.8 and 0.8, H-5′), 6.36 (2H, br s, NH_2), 4.30–4.20 (1H, m, CH cyclohexyl), 2.39 (3H, s, CH_3C=N–), 2.37–1.20 (10H, m, cyclohexyl).

3.8.12. 2-(1-(1-Cyclohexyl-2-(pyridin-2-yl)-1H-benzimidazol-5-yl) ethylidene)hydrazinecarbothioamide (**59**)

Yield: 83%. R_f = 0.27 ($CHCl_3$–MeOH 9.8:0.2); m.p. 194–196 °C; Anal. calc. for $C_{21}H_{24}N_6S$: C, 64.26; H, 6.16; N, 21.41; found: C, 64.06; H, 6.24; N, 21.18. ν_{max} (nujol) cm^{-1}: 3140, 1611; λ_{max} (EtOH) nm: 307; ^{1}H NMR ($CDCl_3$) δ: 8.80 (1H, br s, NH), 8.72 (1H, dd, *J* 4.8 and 1.8, H-3′), 8.22 (1H, dd, *J* 8.0 and 1.4, H-6), 8.14 (1H, s, H-4), 7.93–7.80 (1H, m, H-5′), 7.80–7.68 (1H, m, H-7), 7.50–7.35 (2H, m, H-4′,6′), 6.42 (1H, br s, NH), 5.60–5.40 (1H, m, CH cyclohexyl), 2.38 (3H, s, CH_3C=N–), 2.37–1.20 (10H, m, cyclohexyl).

3.8.13. 2-(1-(2-(Furan-3-yl)-1H-benzimidazol-5-yl)ethylidene) hydrazinecarbothioamide (**60**)

Yield: 80%; R_f = 0.23 (PE-EA 3:7); m.p. 159–162 °C; Anal. calc. for $C_{14}H_{13}N_5OS$: C, 56.17; H, 4.38; N, 23.40; found: C, 56.41; H, 4.19; N, 23.10. ν_{max} (nujol) cm^{-1}: 3433, 1613. λ_{max} (EtOH) nm: 327, 319, 250, 205. ^{1}H NMR ($CDCl_3$-DMSO-d_6) δ: 9.94 (1H, s, NH), 8.31 (1H, dd, *J* 1.8 and 0.8, H-2′), 8.0 (2H, br s, NH_2), 7.95 (1H, s, H-4), 7,76 (1H, dd, *J* 8.2 and 1.6, H-6), 7.61 (1H, dd, *J* 1.8 and 0.8, H-4′), 7.52 (1H, d, *J* 8.2, H-7), 7.01 (1H, dd, *J* 1.8 and 0.8, H-5′), 2.42 (3H, s, CH_3C=N–).

3.8.14. 2-(1-(2-Cyclohexyl-1H-benzimidazol-5-yl)ethylidene) hydrazinecarbothioamide (**61**)

Yield: 75%. R_f = 0.27 (EP-EA 3:7); m.p. 145–148 °C. Anal. calc. for $C_{16}H_{21}N_5S$: C, 60.92; H, 6.71; N, 22.20; found: C, 61.06; H, 6.51; N, 22.02. ν_{max} (nujol) cm^{-1}: 3370, 1589. λ_{max} (EtOH) nm: 317, 225, 204; ^{1}H NMR ($CDCl_3$-DMSO-d_6) δ: 10.0 (1H, br s, NH), 8.14 (1H, s, H-4), 8.06 (1H, br s, NH), 7.73 (1H, dd, *J* 8.6 and 1.6, H-6), 7.65 (2H, br s, NH_2), 7.44 (1H, d, *J* 8.6, H-7), 2.88 (1H, m, CH cyclohexyl), 2.39 (3H, s, CH_3C=N–), 2.18–1.25 (10H, m, cyclohexyl). ^{13}C NMR (DMSO-d_6)

δ :178.65 (C=S), 160.19 (C=N), 149.20 (C-2), 138.74 (C-3^a,7^a), 131.21 (C-5), 120 (C-6), 113.53 (C-4), 112.98 (C-7), 37.69 (C-1″), 31.18 (C-2″,6″), 21.13 (C-4″), 14.50 (CH_3).

4. Biology

4.1. Test compounds

Compounds were dissolved in DMSO at 100 mM, and then diluted in culture medium.

4.2. Cells and viruses

Cell lines were purchased from American Type Culture Collection (ATCC). The absence of mycoplasma contamination was checked periodically by the Hoechst staining method. Cell lines supporting the multiplication of RNA and DNA viruses were the following: $CD4^+$ human T-cells containing an integrated HTLV-1 genome (MT-4); Madin Darby Bovine Kidney (MDBK) [ATCC CCL 22 (NBL-1) *Bos taurus*]; Baby Hamster Kidney (BHK-21) [ATCC CCL 10 (C-13) *Mesocricetus auratus*] and Monkey kidney (Vero 76) [ATCC CRL 1587 *Cercopithecus aethiops*]. Viruses were purchased from American Type Culture Collection (ATCC), with the exception of Yellow Fever Virus (YFV), and Human Immunodeficiency Virus type-1 (HIV-1). Viruses representative of positive-sense, single-stranded RNAs ($ssRNA^+$) were: (i) Retroviridae: the III_B laboratory strain of HIV-1, obtained from the supernatant of the persistently infected H9/III_B cells (NIH 1983); (ii) Flaviviridae: yellow fever virus (YFV) [strain 17-D vaccine (Stamaril Pasteur J07B01)] and bovine viral diarrhoea virus (BVDV) [strain NADL (ATCC VR-534)]; (iii) Picornaviridae: human enterovirus B [coxsackie type B5 (CVB-5), strain Ohio-1 (ATCC VR-29)], and human enterovirus C [poliovirus type-1 (Sb-1), Sabin strain Chat (ATCC VR-1562)]. Viruses representative of negative-sense, single-stranded RNAs (ssRNA-) were: (iv) Paramyxoviridae: human respiratory syncytial virus (RSV) [strain A2 (ATCC VR-1540)]; (v) Rhabdoviridae: vesicular stomatitis virus (VSV) [lab strain Indiana (ATCC VR 1540)]. The virus representative of double-stranded RNAs (dsRNA) Reoviridae was reovirus type-1 (Reo-1) [simian virus 12, strain 3651 (ATCC VR-214)]. DNA virus representatives were: (vi) Poxviridae: vaccinia virus (VV) [vaccine strain Elstree–Lister (ATCC VR-1549)]; (vii) Herpesviridae: human herpes 1 (HSV-1) [strain KOS (ATCC VR-1493)].

4.3. Cytotoxicity assays

Cytotoxicity assays were run in parallel with antiviral assays. Exponentially growing MT-4 cells were seeded at an initial density of 1×10 [5] cells/ml in 96-well plates in RPMI-1640 medium, supplemented with 10% foetal bovine serum (FBS), 100 units/mL penicillin G and 100 μg/mL streptomycin. Cell cultures were then incubated at 37 °C in a humidified, 5% CO_2 atmosphere, in the absence or presence of serial dilutions of test compounds. Cell viability was determined after 96 h at 37 °C by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method [35]. MDBK and BHK cells were seeded in 24-well plates at an initial density of 6×10 [5] and 1×10 [6] cells/mL, respectively, in Minimum Essential Medium with Earle's salts (MEM-E), L-glutamine, 1 mM sodium pyruvate and 25 mg/L kanamycin, supplemented with 10% horse serum (MDBK) or 10% foetal bovine serum (FBS) (BHK). Cell cultures were then incubated at 37 °C in a humidified, 5% CO_2 atmosphere in the absence or presence of serial dilutions of test compounds. Cell viability was determined after 48–96 h at 37 °C by the MTT method. Vero-76 cells were seeded in 24-well plates at an initial density of 4×10 [5] cells/mL, in Dulbecco's Modified Eagle Medium (D-MEM) with L-glutamine and 25 mg/L kanamycin, supplemented with 10% FBS. Cell cultures were then incubated at 37 °C in a humidified, 5% CO_2 atmosphere in the absence or presence of serial dilutions of test compounds. Cell viability was determined after 48–96 h at 37 °C by the crystal violet staining method.

4.4. Antiviral assays

Compound's activity against HIV-1 was based on inhibition of virus-induced cytopathogenicity in MT-4 cell acutely infected with a multiplicity of infection (m.o.i.) of 0.01. Briefly, 50 μL of RPMI containing 1×10 [4] MT-4 cells were added to each well of flat-bottom microtitre trays, containing 50 μL of RPMI without or with serial dilutions of test compounds. Then, 20 μL of a HIV-1 suspension containing 100 $CCID_{50}$ were added. After a 4-day incubation at 37 °C, cell viability was determined by the MTT method. Compound's activity against YFV and Reo-1 was based on inhibition of virus-induced cytopathogenicity in BHK-21 cells acutely infected with a m.o.i. of 0.01. Compound's activity against BVDV was based on inhibition of virus-induced cytopathogenicity in MDBK cells acutely infected with a m.o.i. of 0.01. Briefly, BHK and MDBK cells were seeded in 96-well plates at a density of 5×10 [4] and 3×10 [4] cells/well, respectively, and were allowed to form confluent monolayers by incubating overnight in growth medium at 37 °C in a humidified CO_2 (5%) atmosphere. Cell monolayers were then infected with 50 μL of a proper virus dilution in maintenance medium [MEM-Earl with L-glutamine, 1 mM sodium pyruvate and 0.025 g/L kanamycin, supplemented with 0.5% inactivated FBS] to give an m.o.i of 0.01. After 1 h, 50 μL of maintenance medium, without or with serial dilutions of test compounds, were added. After a 3-/4-day incubation at 37 °C, cell viability was determined by the MTT method.

Compound's activity against CVB-5, Sb-1, VV, HSV-1 and RSV was determined by plaque reduction assays in infected cell monolayers. To this end, Vero 76-cells were seeded in 24-well plates at a density of 2×10 [5] cells/well and were allowed to form confluent monolayers by incubating overnight in growth medium [Dulbecco's Modified Eagle Medium (D-MEM) with L-glutamine and 4500 mg/L D-glucose and 0.025 g/L kanamycin, supplemented with 10% FBS] at 37 °C in a humidified CO_2 (5%) atmosphere. Then, monolayers were infected for 2 h with 250 μL of proper virus dilutions to give 50–100 PFU/well. Following removal of unadsorbed virus, 500 μL of maintenance medium [D-MEM with L-glutamine and 4500 mg/L D-glucose, supplemented with 1% inactivated FBS] containing 0.75% methyl–cellulose, without or with serial dilutions of test compounds, were added. Cultures were incubated at 37 °C for 2 (Sb-1 and VSV), 3 (CVB-5, VV and HSV-1) or 5 days (RSV) and then fixed with PBS containing 50% ethanol and 0.8% crystal violet, washed and air-dried. Plaques were then counted.

4.5. Linear regression analysis

The extent of cell growth/viability and viral multiplication, at each drug concentration tested, were expressed as percentage of untreated controls. Concentrations resulting in 50% inhibition (CC_{50} or EC_{50}) were determined by linear regression analysis.

4.6. HCV replicon assays

A human hepatoma cell line (Huh-7) bearing the HCV genotype 1b replicon (GS4.1 cells), kindly provided by C. Seeger (Fox Chase University, Philadelphia, PA, USA) through Idenix Pharmaceuticals, was grown in D-MEM supplemented with 10% FBS, 2 mM L-glutamine, 110 mg/L sodium pyruvate, 0.1 mM non-essential amino

acids, 100 U/mL penicillin, 100 μg/mL streptomycin and 0.5 mg/mL G418 (Invitrogen). For dose–response testing, GS4.1 cells were seeded in 96-well plates at a density of 7.5 × 10 [3] cells/well in 50 μL medium containing two-fold serial dilutions of test compounds (highest concentration, 75 μM). Huh-7 cells lacking the HCV replicon served as negative controls. Plates were then incubated for 72 h at 37 °C in a humidified, 5% CO_2 incubator. Inhibition of HCV replication was measured by quantification of the viral NS4A protein using an enzyme-linked immunosorbent assay (ELISA) as follows: plates were fixed for 1 min with 1:1 acetone–methanol, washed twice with PBS containing 0.1% Tween 20, left for 1 h at room temperature with TNE buffer containing 10% FBS, and then incubated for 2 h at 37 °C with the anti-NS4A mouse monoclonal antibody A-236 (ViroGen, Watertown, MA, USA) diluted in the same buffer. After three washes with PBS containing 0.1% Tween 20, plates were incubated for 1 h at 37 °C with anti-mouse immunoglobulin G-peroxidase conjugate in TNE buffer containing 10% FBS. After further washing as above, the reaction was developed with *o*-phenylenediamine (Zymed, San Francisco, CA, USA). The reaction was stopped after 30 min with 2 N H_2SO_4 and absorbance was determined at 492 nm using a Sunrise Tecan (Durham, NC, USA) spectrophotometer. EC_{50} values were determined from % inhibition vs compound concentration data, using a sigmoidal non-linear regression analysis based on four parameters, with a Tecan Magellan software. For cytotoxicity evaluation, Huh-7 and GS4.1 cells were treated with compounds as described above, and cellular viability was monitored using the Cell Titer 96 Aqueous One Solution cell proliferation assay (Promega). CC_{50} values were determined from the % cytotoxicity vs compound concentration data with Tecan Magellan software, as described above.

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