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Short communication

Coumarins hinged directly on benzimidazoles and their ribofuranosides to inhibit hepatitis C virus

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ABSTRACT

A new compound library that contained 20 hinged benzimidazole–coumarin hybrids and their β-D-ribofuranosides was established. The anti-hepatitis C virus (HCV) activity of all novel coumarin derivatives, which were obtained by use of organic synthetic methods, was tested. Two of these hybrids exhibited appealing EC_{50} values of as low as 3.0 and 5.5 μM. The best selectivity index was 14. The incorporation of a D-ribofuranose into the hinged hybrids provided the corresponding nucleosides with the β configuration, one of which inhibited HCV replication with an EC_{50} value of 20 μM. Additionally, the structure–activity relationship is elucidated on the basis of the functional groups that were attached to the nuclei of benzimidazole, coumarin, and ribofuranose of the hybrids.

1. Introduction

The World Health Organization has estimated that over 200 million people (~3.5% of the world's population) are chronically infected with hepatitis C virus (HCV). These chronic carriers are at risk of developing cirrhosis or liver cancer or both [1,2]. The HCV infection is a major cause of chronic liver disease. More than 50% of individuals who are exposed to HCV develop chronic hepatitis and 20% of chronically infected individuals develop cirrhosis [3,4]. The classical treatment involves the use of interferon α-2 or its pegylated form, either alone or in combination with ribavirin [5]. A sustained response is observed in only around 40% of the patients and, dauntingly, this treatment is associated with serious side effects [6–8]. Recently, Boceprevir and Telaprevir have been approved by the U.S. Food and Drug Administration to treat genotype 1 chronic hepatitis C [9], while the combined therapy with peginterferon α-2 and ribavirin is still required [10,11]. Boceprevir is also associated with aneamia and has a side effect of producing a metallic taste in the mouth. Both symptoms affect almost half of all patients [9]. More than half of patients who are medicated with Telaprevir suffer from rashes [9]. In 3–6% of patients, the rash is sufficiently severe that treatment must be halted. Owing to the urgent need for new drugs [12,13], compounds with functional scaffolds and anti-HCV activity are being developed and synthesized.

The approach presented herein to hinging benzimidazoles and coumarins together exploits their individual biological activities. Derivatives of benzimidazole [14,15] and coumarin [16,17] have clinical potential in the treatment of breast cancer [18,19], leukemia [20,21], and tumor cells [22,23]. For example, Rajitha et al. [24]

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synthesized a series of derivatives of 3-(2-benzimidazolyl) coumarin with anticancer activity *in vitro*. Yakout [25] synthesized benzimidazole–coumarin hybrids that exhibit antitumor activities and molluscicidal properties against *Biomphalaria alexandrina* snails. Lee, Wang, et al. [26] prepared cyano-containing coumarin–benzimidazoles, which strongly inhibit the proliferation of various cancer cells (such as U87, B16, HeLa, DLD-1, and HIH 3T3). Furthermore, some derivatives of benzimidazole [27–31] have an anti-HCV effect, so do the derivatives of coumarin [32–35]. Moreover, the benzimidazole nuclei and substituted coumarin moieties can be linked together via a methylenethio spacer, and the resultant hybrids can inhibit HCV replication [36].

This investigation discloses new findings that various benzimidazoles can be directly hinged on coumarins with various substituents. The corresponding β-ribofuranosides of hinged hybrids were also prepared. Some of these new hinged compounds potently inhibited HCV and their structure–activity relationship (SAR) is presented.

2. Chemistry

2.1. Syntheses and spectral characteristics of hinged benzimidazole–coumarin hybrids

To examine the effects of different substituents, we synthesized a series of hinged benzimidazole–coumarin hybrids according to Scheme 1. The treatment of various phenylenediamines **1a**–**e** with 3-(ethoxycarbonyl)coumarins **2a**–**g** and 85% *o*-phosphoric acid in water gave the desired hybrids **3a**–**m** with yields from 65 to 87%. All of these products were purified by recrystallization to afford the hybrids with purity >95.9% for antiviral assays.

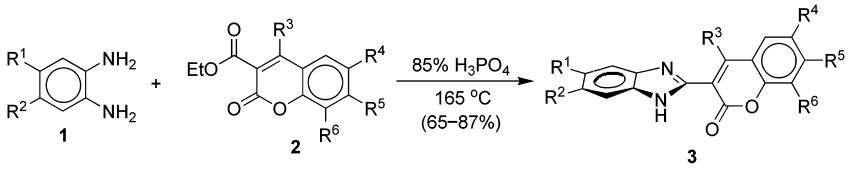
The structures of hinged hybrids **3a**–**m** were confirmed by their spectroscopic characteristics. For instance, the desired hybrid **3d** was prepared by the condensation of diamine **1b** with coumarin **2a**. The exact mass of **3d** was measured as 290.1056, which is very close to its theoretical value of 290.1055. The characteristic N=C–NH carbon resonance at 153.13 ppm was observed in its ^{13}C NMR spectrum; this carbon in the corresponding starting material **2a** (i.e., C(=O)OEt) resonated at 162.97 ppm. In the ^{1}H NMR spectrum of **1b**, the two aromatic =C–H protons exhibited a singlet at 6.49 ppm. After diamine **1b** was coupled with **2a**, these two protons in the product **3d** resonated at 7.52 ppm. Additionally, the β proton of the lactenone moiety in the starting material **2a** exhibited a singlet at 8.62 ppm. The corresponding proton in the product **3d** appeared as a singlet at 9.64 ppm.

By the same procedure, the coupling reactions **4** + **2a** → **5**, **1a** + **6** → **7**, and **8** + **2g** → **9** were successfully completed as shown in Scheme 2. The hinged hybrids **5** and **7** contained a naphthalene unit but the hinged hybrid **9** contained an imidazopyridine nucleus.

2.2. Synthesis of 3-(benzimidazol-2′-yl)coumarin ribofuranosides and their identification as β anomers

To attach a ribofuranose moiety to hinged benzimidazole–coumarin hybrids, compounds **3i**,**n** were firstly silylated with *N*,*O*-bistrimethylsilylacetamide (BSA, Scheme 3) [37,38]. The intermediates were then coupled with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose in the presence of Me_3SiOTf at 80 °C to yield the corresponding nucleosides **10i**,**n**, respectively. Under alkaline conditions, all acetyl-protecting groups therein were removed by use of saturated methanolic ammonia to give the triols **11i** and **11n**. Purification by HPLC generated these two hybrids in 85% and 88% yields, respectively, with purities of >96.6%.

The C1-protons of *N*-ribofuranosides **11i** and **11n** resonated at 5.47 and 5.78 ppm, respectively, yielding a doublet with $J = 7.2$ Hz. The β epimers contain C1-protons with a more upfield chemical shift in the ^{1}H NMR spectra than the corresponding nucleosides with an α configuration – ~6.6 ppm for the α epimers and ~5.9 ppm for the β epimers [39,40]. Furthermore, the ^{2}J value of these protons in the β epimers is larger than that of those in the α epimers, being ~4.7 Hz for the α epimers and ~6.2 Hz for the β epimers. On the basis of these information, the β configuration was attributed to the products **11i**,**n**.

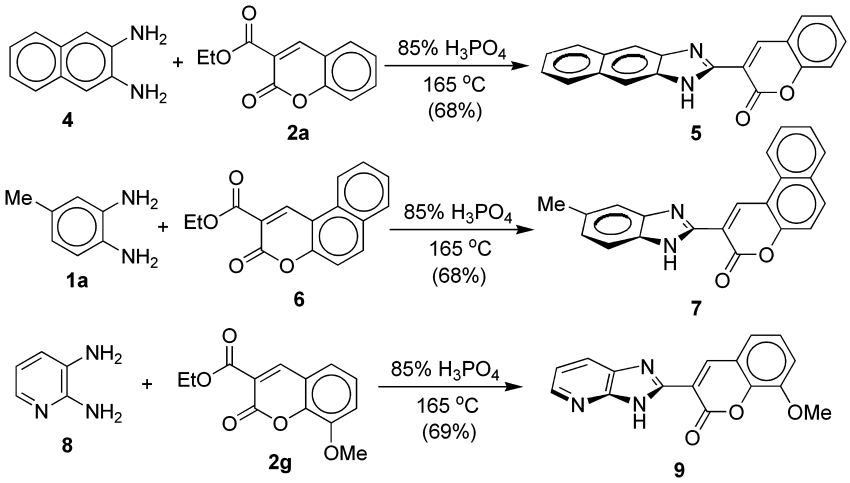


1	R^1:	R^2:
a.	Me	H
b.	Me	Me
c.	F	H
d.	H	H
e.	CO_2H	H

2	R^3:	R^4:	R^5:	R^6:
a.	H	H	H	H
b.	Me	H	H	H
c.	H	Br	H	H
d.	Me	H	OAc	H
e.	H	NO_2	H	H
f.	H	H	I	H
g.	H	H	H	OMe

3	R^1:	R^2:	R^3:	R^4:	R^5:	R^6:
a.	Me	H	H	H	H	H
b.	Me	H	Me	H	H	H
c.	Me	H	H	H	H	OMe
d.	Me	Me	H	H	H	H
e.	Me	Me	Me	H	H	H
f.	Me	Me	H	Br	H	H
g.	F	H	H	H	H	H
h.	F	H	Me	H	H	H
i.	H	H	Me	H	H	H
j.	H	H	Me	H	OAc	H
k.	CO_2H	H	H	H	H	H
l.	CO_2H	H	H	NO_2	H	H
m.	CO_2H	H	H	H	I	H

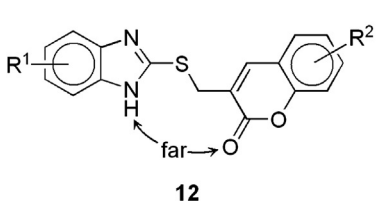
Scheme 1. Synthesis of hinged benzimidazole–coumarin hybrids **3a**–**m**.

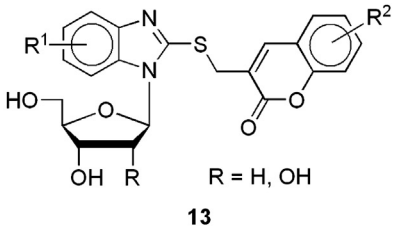


Scheme 2. Synthesis of heterocycle–coumarin derivatives **5**, **7**, and **9**.

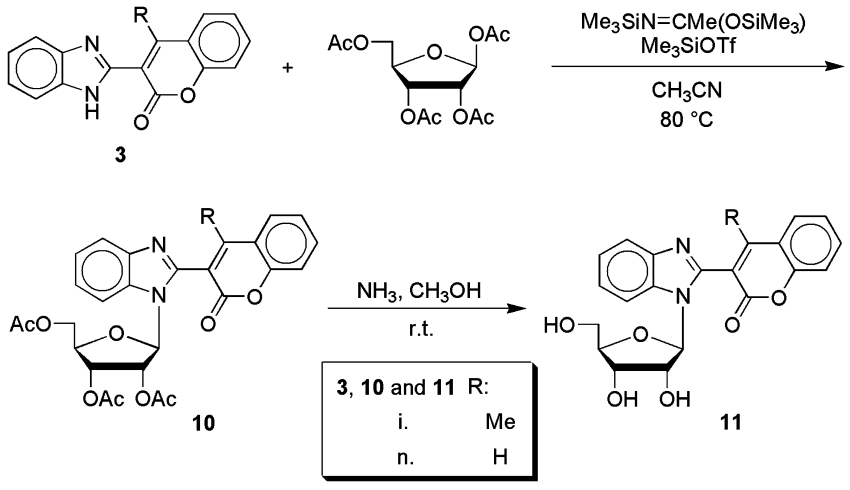
2.3. Intramolecular hydrogen bond in hinged benzimidazole–coumarin hybrids

In other works [36], the present authors utilized the $-SCH_2-$ linker to connect the benzimidazole and coumarin nuclei in the formation of various hybrid compounds **12** and **13**. The probability of the formation of an "intramolecular" hydrogen bond in hybrids **12** is low because the benzimidazole N–H proton is distant from the coumarin carbonyl group.





The IR stretching vibrational frequency of the lactone carbonyl group of the hinged hybrids, of which **3d** is a representative example, was at 1712 cm^{-1} regardless of whether the hybrids were neat or dissolved in dichloromethane at concentrations of 0.10 M and 0.010 M (Fig. 1(a)). In contrast, the lactone carbonyl group wavenumber of the neat hybrid **11i** was at 1719 cm^{-1}, but it shifted to 1736 cm^{-1} when this hybrid was at a low concentration of 0.010 M in acetonitrile (see Fig. 1(b)). These results reveal the presence of an "*intra*molecular" hydrogen bond in the hybrid **3d**. Therefore, the lactone carbonyl stretching vibrational frequency was independent of its concentration in the solution. However, the N–H proton of benzimidazole was replaced by a ribofuranose moiety in the hybrid **11i**. Accordingly, the frequency that is referred to the "*inter*molecular" hydrogen bond frequency depended upon its concentration in solution.



Scheme 3. Synthesis of hinged benzimidazole–coumarin–ribofuranosides **10** and **11**.

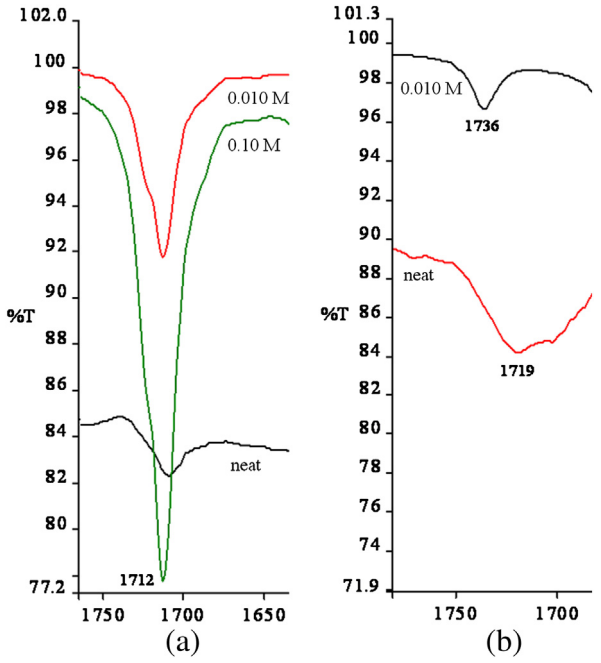


Fig. 1. (a) Vibrational frequency of the lactone carbonyl group in neat hybrid **3d** and in hybrid **3d** dissolved in dichloromethane at concentrations of 0.10 M and 0.010 M. (b) Vibrational frequency of lactone carbonyl group in neat hybrid **11n** and in hybrid **3d** dissolved in acetonitrile at a concentration of 0.010 M.

3. Pharmacology: anti-HCV activity

The antiviral activity of hybrid compounds **3a**–**m**, **5**, **7**, **9**, **10i**,**n**, and **11i**,**n** in the HCV genotype 1b subgenomic replicon system in Huh 5-2 cells was evaluated according to the procedures that have been described previously [41,42]. From the obtained dose–response curves, the concentration of a compound that inhibited virus replication by 50% (EC_{50}) and the concentration of a compound that reduced host cell metabolism by 50% (CC_{50}) were calculated. The results in Table 1 were used to determine the selectivity index ($SI = CC_{50}/EC_{50}$), a measure for the therapeutic window of the compound in the assay system. However, even though an EC_{50} may be obtained in the antiviral assay from the dose–response curve, the bioassayed compounds may not be regarded as hits. Selective inhibitors in the replicon assay were only applied to the hybrids when they were observed to inhibit significantly (>70%) virus replication at the concentrations that did not have an adverse effect on the metabolism of the host cell. The observed antiviral effect of other compounds was probably related to their pleiotropic or aspecific effects on the host cell.

Table 1
Anti-metabolic and antiviral effect of hinged hybrids on HCV 1b subgenomic replicon replication in Huh 5-2 cells.[a]

Compound	CC_{50}[b] (μM)	EC_{50}[c] (μM)	SI[d]
3a	2.3	2.3	1.0
3b	29	28	1.0
3c	12	12	1.0
3d	23	3.0	*7.9*
3e	77	40	2.0
3f	172	40	4.3
3g	13	84	0.15
3h	170	79	2.2
3i	27	40	0.66
3j	153	153	1.0
3k	13	40	0.32
3l	10	6.9	1.5
3m	118	118	1.0
5	135	105	1.3
7	77	5.5	*14*
9	170	84	2.0
10i	*941*	941	1.0
10n	98	60	1.6
11i	122	110	1.1
11n	127	20	*6.4*

[a] Interferon α-2b was utilized as a reference compound at 10,000 units/well. It reduced the signal in the viral RNA (luciferase) assay to background levels without exhibiting any anti-metabolic activity. All values were obtained as averages of triplicate determinations.
[b] Concentration at which 50% adverse effect was observed on the host cell metabolism as measured using the MTS method.
[c] Concentration at which 50% inhibition of virus replication was observed as measured from luminescence.
[d] Selectivity index (ratio of CC_{50} to EC_{50}).

Among the 20 new synthesized hybrids in the compound library, three with attractive activities were identified. Benzimidazole derivative **3d**, benzocoumarin derivative **7**, and furanoside derivative **11n** inhibited HCV replication in a concentration-dependent manner in Huh 5-2 cells, as revealed by measurements of the luciferase activity. Their EC_{50} values were calculated to be 3.0, 5.5, and 20 μM, respectively (Table 1). When the effect of all of the hybrids on exponentially growing Huh 5-2 cells was assessed, (monomethyl)benzimidazole–coumarin–ribofuranoside **3a** showed the least cytostatic (with CC_{50} = 2.3 μM). However, a selectivity index of 14 was calculated for hybrid **7**, whereas corresponding values for **3d** and **11n** were 7.9 and 6.4, respectively.

4. Discussion

4.1. Structure–activity relationship: essential moieties and functional groups

The hinged scaffold of the hybrid compounds and the various substituents that were attached to the nuclei of benzimidazole, coumarin, and ribofuranose may affect their biological activities. The substituents in the new hybrids were Me, COOH, OMe, OAc, F, Br, I, and NO_2. Their EC_{50} and CC_{50} values shown in Table 1 yield the following SAR:

(1) The attachment of a methyl group to the benzimidazole nucleus generated hybrids with remarkable anti-HCV activity. Successful examples included **3d** and **7** with EC_{50} = 3.0 and 5.5 μM, respectively. The attachment of a second methyl group to the benzimidazole nucleus could increase the SI value by a factor of 7.9 (**3a** *versus* **3d**). The addition of a methyl group to the C-4 position of the coumarin nucleus, however, reduced the HCV inhibition and the SI value (**3d** *versus* **3e** and **11n** *versus* **11i**).
(2) The addition of a Br, OMe, and OAc substituent into the coumarin nucleus of hinged hybrids reduced the cytotoxicity and the anti-HCV activity (**3f** *versus* **3d**, **3c** *versus* **3a**, and **3j** *versus* **3i**).
(3) The incorporation of a fused benzene ring to the coumarin nucleus in the hinged hybrids increased the SI value by a factor of 14 (**3a** *versus* **7**). In contrast, its incorporation to the benzimidazole nucleus (i.e., **5**) resulted in a hybrid with negligible anti-HCV activity.
(4) The introduction of a β-D-ribofuranose moiety to the hinged benzimidazole–coumarin hybrids (such as **11n**) led to a triply conjugated compound, which also exhibited anti-HCV activity (EC_{50} = 20 μM) with a significant SI value (6.4).
(5) The removal of acetyl groups from the β-D-ribofuranose moiety in hybrids enhanced their ability to inhibit HCV by factors of 3.0–8.6 (**10i** *versus* **11i** as well as **10n** *versus* **11n**).

5. Conclusions

Various benzimidazoles were directly connected to different coumarin moieties by chemical synthesis to generate hinged

hybrids as potential anti-viral leads. Substituents on the benzimidazoles included Me, F, and COOH; substituents on the coumarins included Me, OMe, Br, I, OAc, and NO_2. Meanwhile, two new hybrids that contained naphthalene nuclei were prepared by the reaction of a naphthimidazole moiety with a coumarin (**4** + **2a** → **5**) and by the reaction of a benzimidazole moiety with 5,6-benzocoumarin (**1a** + **6** → **7**). In this new compound library, hybrids **3d**, **7**, and **11n** exhibited potent anti-HCV activity with EC_{50} values of 3.0, 5.5, and 20 μM, respectively, as well as with remarkable SI values (>14 for hybrid **7**). The structure–activity relationship is established, disclosing the critical roles of the hinged scaffold, the substituents, and fused moieties in benzimidazole–coumarin hybrids. The mechanism of action between hinged hybrids and HCV-related enzymes will be the subject of subsequent studies. The results will be reported in due course.

6. Experimental section

6.1. General procedure

All reactions were carried out in oven-dried glassware (110 °C) under an atmosphere of nitrogen unless as indicated otherwise. Chloroform, ethanol, ethyl acetate (EtOAc), and hexanes were purchased from Mallinckrodt Chemical Co. Hexanes and acetonitrile were dried and distilled from CaH_2. 1,2-Diaminobenzene, 3,4-diaminobenzoic acid, 1,2-diamino-4,5-dimethylbenzene, 1,2-diamino-4-fluorobenzene, 2,3-diaminonaphthalene, 2,3-diaminopyridine, 3,4-diaminotoluene, and 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose were purchased from Aldrich Chemical Co. Salicylaldehyde and trimethylsilyl trifluoromethanesulfonate (Me_3SiOTf) were purchased from Fluka Chemika. *N*,*O*-Bistrimethylsilylacetamide (BSA) was purchased from Acros Chemical Co. Acetonitrile was purchased from Fischer Scientific Co. Acetic acid, acetic anhydride, and hydrochloric acid were purchased from Riedel-de Haën Chemical Co. Potassium hydroxide was purchased from Merck Inc. 3-Ethoxycarbonylcoumarins was prepared according to the literature method. Analytical thin layer chromatography (TLC) was performed on precoated plates (silica gel 60 F-254), purchased from Merck Inc. Purification by gravity column chromatography was carried out by use of Merck Reagents Silica Gel 60 (particle size 0.063–0.200 mm, 70–230 mesh ASTM).

Infrared (IR) spectra were measured on a Bomem Michelson Spectrometer FT–IR. Absorption intensities are recorded by the following abbreviations: s, strong; m, medium; w, weak; br, broad. High-resolution mass spectra were obtained by means of a JEOL JMS-HX110 mass Spectrometer. Proton NMR spectra were obtained on a Varian Mercury-400 (400 MHz) spectrometer by use of chloroform-*d*, and dimethylsulfoxide-d_6 as solvents and tetramethylsilane as an internal standard. Carbon-13 NMR spectra were performed on a Varian Mercury-400 (100 MHz) spectrometer by use of chloroform-*d* and dimethylsulfoxide-d_6 as solvents. Carbon-13 chemical shifts are referenced to the center of the $CDCl_3$ triplet (δ 77.0 ppm) and DMSO-d_6 pentet (δ 39.54 ppm). Multiplicities are recorded by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; *J*, coupling constant (Hz).

6.2. Standard procedure 1 for the preparation of 3-(benzimidazol-2′-yl)coumarins (**3a**–**m**)

To a solution containing of 1,2-phenylenediamines **1** (1.0 equiv) and 3-ethoxycarbonylcoumarins **2** (1.0 equiv) were added *o*-phosphoric acid (85%, 15–16 equiv). The reaction mixture was stirred at room temperature for 5.0 min and at 165 °C for another 8.0 h. Then the solution was permitted to cool down to 100 °C and poured in a large volume (500 mL for 0.20 mol of coumarin ester) of stirred water. The solution was neutralized with saturated aqueous $NaHCO_3$ solution and extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with brine (15 mL), dried over $MgSO_4$ (s), filtered, and concentrated under reduced pressure to provide crude solids, which were then recrystallized in EtOH to give the pure coumarins **3a**–**m** in 40–67% yields with purity >99.6%, as checked by GC.

6.2.1. 3-(5′-Methylbenzimidazol-2′-yl)coumarin (**3a**)

The Standard procedure 1 was followed by use of 3, 4-diaminotoluene (**1a**, 1.23 g, 10.1 mmol, 1.0 equiv), 3-ethoxycarbonylcoumarin (**2a**, 2.17 g, 9.95 mmol, 1.0 equiv), and *o*-phosphoric acid (15.11 g, 0.1531 mol, 15 equiv). After removal of the solvent under reduced pressure, the remaining crude solids were purified by crystallization to give **3a** (2.08 g, 7.54 mmol) in 76% yield as yellow solids: mp (recrystallized from EtOH, 54% yield) 312.8–313.3 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.03 (s, 1H, O═C–C═CH), 7.94 (d, J = 8.0 Hz, 1H, ArH), 7.85 (t, J = 8.0 Hz, 1H, ArH), 7.74 (d, J = 8.0 Hz, 1H, ArH), 7.63–7.59 (m, 2H, 2 × ArH), 7.53 (t, J = 8.0 Hz, 1H, ArH), 7.38 (d, J = 8.0 Hz, 1H, ArH), 2.49 (s, 3H CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) 157.65 (C═O), 153.70, 146.12, 143.12, 136.35, 135.19, 132.72, 131.91, 130.21, 127.80, 125.77, 117.91, 116.64, 114.09, 113.65, 111.63, 21.22 (CH_3); IR (KBr) 3350 (br, NH), 1705 (s, C═O), 1600 (m), 1110 (m), 740 (m) cm^{-1}; HRMS *m*/*z* calcd for $C_{17}H_{12}N_2O_2$: 276.0899, found: 276.0898.

6.2.2. 3-(5′-Methylbenzimidazol-2′-yl)-4-methylcoumarin (**3b**)

The Standard procedure 1 was followed by use of 3,4-diaminotoluene (**1a**, 132.1 mg, 1.081 mmol, 1.0 equiv), 3-ethoxycarbonyl-4-methylcoumarin (**2b**, 250.8 mg, 1.080 mmol, 1.0 equiv), and *o*-phosphoric acid (1.59 g, 16.3 mmol, 15 equiv). After removal of the solvent under reduced pressure, the remaining crude solids were purified by crystallization to give **3b** (239.5 mg, 0.8259 mmol) in 76% yield as yellow solids: mp (recrystallized from EtOH, 54% yield) 205.7–206.5 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.86 (d, J = 1.6 Hz, 1H, ArH), 7.58–7.54 (m, 2H, 2 × ArH), 7.39–7.34 (m, 3H, 3 × ArH), 7.09 (d, J = 8.0 Hz, 1H, ArH), 3.17 (s, 3H, O═C–C═CCH_3), 2.46 (s, 3H, ArCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) 159.08 (C═O), 153.21, 152.12, 145.54, 132.86, 131.38, 130.52, 126.50, 126.14, 124.93, 123.68, 123.02, 121.24, 119.68, 116.73, 116.47, 21.22 (CH_3), 16.56 (CH_3); IR (KBr) 3363 (br, NH), 1702 (s, C═O), 1326 (m), 1100 (m), 735 (m) cm^{-1}; HRMS *m*/*z* calcd for $C_{18}H_{14}N_2O_2$: 290.1055, found: 290.1053.

6.2.3. 8-Methoxy-3-(5′-methylbenzimidazol-2′-yl)coumarin (**3c**)

The Standard procedure 1 was followed by use of 3,4-diaminotoluene (**1a**, 510.1 mg, 4.106 mmol, 1.0 equiv), 3-ethoxycarbonyl-8-methoxycoumarin (**2g**, 1.02 g, 4.11 mmol, 1.0 equiv), and *o*-phosphoric acid (5.93 g, 60.6 mmol, 15 equiv). After removal of the solvent under reduced pressure, the remaining crude solids were purified by crystallization to give **3c** (1.038 g, 3.391 mmol) in 83% yield as light brown solids: mp (recrystallized from EtOH, 64% yield) 262.2–262.8 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.96 (s, 1H, O═C–C═CH), 7.14–7.26 (m, 6H, 6 × ArH), 3.94 (s, 3H, OCH_3), 2.42 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) 159.17 (C═O), 146.31, 145.49, 143.25, 142.08, 135.08, 132.91, 131.17, 124.97, 124.38, 123.88, 120.58, 119.99, 116.83, 114.94, 112.33, 56.15 (OCH_3), 21.48 (CH_3); IR (KBr) 3340 (br, NH), 1697 (s, C═O), 1574 (m), 1273 (m), 1103 (m) cm^{-1}; HRMS *m*/*z* calcd for $C_{18}H_{14}N_2O_3$: 306.1004, found: 306.1006.

6.2.4. 3-(5′,6′-Dimethylbenzimidazol-2′-yl)coumarin (**3d**)

The Standard procedure 1 was followed by use of 1,2-diamino-4,5-dimethylbenzene (**1b**, 762.1 mg, 5.595 mmol, 1.0 equiv), 3-ethoxycarbonylcoumarin (**2a**, 1.22 g, 5.60 mmol, 1.0 equiv), and

o-phosphoric acid (8.49 g, 86.7 mmol, 16 equiv). After removal of the solvent under reduced pressure, the remaining crude solids were purified by crystallization to give **3d** (1.183 g, 4.078 mmol) in 73% yield as yellow solids: mp (recrystallized from EtOH, 50% yield) 283.6–284.2 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.07 (s, 1H, O=C–C=CH), 7.98 (d, J = 7.6 Hz, 1H, ArH), 7.69–7.67 (m, 1H, ArH), 7.51 (d, J = 8.4 Hz, 1H, ArH), 7.45–7.42 (m, 3H, 3 × ArH), 2.31 (s, 6H, 2 × CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) 159.29 (C=O), 153.13, 144.80, 141.67, 141.51, 133.42, 132.72, 131.59, 130.67, 129.47, 125.06, 119.13, 118.44, 116.87, 116.11, 112.60, 20.20 (CH_3), 20.08 (CH_3); IR (KBr) 3350 (br, NH), 1705 (s, C=O), 1600 (m), 1280 (m), 1130 (m), 1090 (m) cm^{-1}; HRMS m/z calcd for $C_{18}H_{14}N_2O_2$: 290.1055, found: 290.1056.

*6.2.5. 3-(5′,6′-Dimethylbenzimidazol-2′-yl)-4-methylcoumarin (**3e**)*

The Standard procedure 1 was followed by use of 1,2-diamino-4,5-dimethylbenzene (**1b**, 171.6 mg, 1.260 mmol, 1.0 equiv), 3-ethoxycarbonyl-4-methylcoumarin (**2b**, 292.1 mg, 1.258 mmol, 1.0 equiv), and *o*-phosphoric acid (1.71 g, 19.3 mmol, 15 equiv). After removal of the solvent under reduced pressure, the remaining crude solids were purified by crystallization to give **3e** (296.8 mg, 0.9763 mmol) in 78% yield as yellow solids: mp (recrystallized from EtOH, 56% yield) 230.2–230.8 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.81 (d, J = 1.6 Hz, 1H, ArH), 7.59–7.55 (m, 1H, ArH), 7.42–7.38 (m, 2H, 2 × ArH), 7.36–7.24 (m, 2H, 2 × ArH), 3.17 (s, 3H, O=C–C=CCH_3), 2.35 (s, 6H, 2 × ArCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) 158.64 (C=O), 155.11, 152.38, 143.46, 141.32, 139.46, 133.90, 132.53, 131.12, 130.74, 129.12, 125.35, 119.30, 118.27, 116.70, 114.64, 20.03 (CH_3), 19.77 (CH_3),16.87 (CH_3); IR (KBr) 3363 (br, NH), 1702 (s, C=O), 1100 (m), 756 (m) cm^{-1}; HRMS m/z calcd for $C_{19}H_{16}N_2O_2$: 304.1212, found: 304.1214.

*6.2.6. 6-Bromo-3-(5′,6′-dimethylbenzimidazol-2′-yl)coumarin (**3f**)*

The Standard procedure 1 was followed by use of 1,2-diamino-4,5-dimethylbenzene (**1b**, 136.5 mg, 1.002 mmol, 1.0 equiv), 6-bromo-3-ethoxycarbonylcoumarin (**2c**, 297.3 mg, 1.001 mmol, 1.0 equiv), and *o*-phosphoric acid (1.48 g, 15.2 mmol, 15 equiv). After removal of the solvent under reduced pressure, the remaining crude solids were purified by crystallization to give **3f** (307.1 mg, 0.8322 mmol) in 83% yield as yellow solids: mp (recrystallized from EtOH, 64% yield) 300.9–301.6 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.09 (s, 1H, O=C–C=CH), 7.69 (d, J = 6.8 Hz, 1H, ArH), 7.64–7.60 (m, 1H, ArH), 7.42–7.34 (m, 3H, 3 × ArH), 2.41 (s, 6H, 2 × ArCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) 158.31 (C=O), 154.63, 152.27, 145.13, 140.71, 135.53, 133.52, 131.92, 126.18, 125.29, 123.81, 120.56, 119.08, 118.21, 117.31, 116.90, 20.83 (CH_3), 20.49 (CH_3); IR (KBr) 3309 (br, NH), 1705 (s, C=O), 1250 (m), 1142 (m) cm^{-1}; MS (ESI) m/z (M + H)$^+$ 369.0, 371.0; HRMS m/z calcd for $C_{18}H_{13}BrN_2O_2$: 370.0140, found: 370.0128.

*6.2.7. 3-(5′-Fluorobenzimidazol-2′-yl)coumarin (**3g**)*

The Standard procedure 1 was followed by use of 1,2-diamino-4-fluorobenzene (**1c**, 750.1 mg, 5.948 mmol, 1.0 equiv), 3-ethoxycarbonylcoumarin (**2a**, 1.29 g, 5.91 mmol, 1.0 equiv), and *o*-phosphoric acid (9.09 g, 92.8 mmol, 16 equiv). After removal of the solvent under reduced pressure, the remaining crude solids were purified by crystallization to give **3g** (1.262 g, 4.908 mmol) in 83% yield as greenish yellow solids: mp (recrystallized from EtOH, 59% yield) 234.2–234.9 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.09 (s, 1H, O=C–C=CH), 7.98 (d, J = 8.0 Hz, 1H, ArH), 7.72–7.65 (m, 2H, 2 × ArH), 7.52–7.43 (m, 4H, 4 × ArH), 7.07 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 164.00 (C=O), 159.23, 156.71, 154.48, 153.29, 148.38, 134.28, 133.11, 130.28, 130.19, 125.13, 124.82, 118.99, 118.35, 117.99, 116.18; IR (KBr) 3354 (br, NH), 1725 (s, C=O), 1607 (m), 1483 (m), 1437 (m), 1412 (m), 1314 (m), 1279 (m), 1042 (s) cm^{-1}; MS (LC): 280.00 (M)$^+$; HRMS m/z calcd for $C_{16}H_9FN_2O_2$: 280.0648, found: 280.0646.

*6.2.8. 3-(5′-Fluorobenzimidazol-2′-yl)-4-methylcoumarin (**3h**)*

The Standard procedure 1 was followed by use of 1,2-diamino-4-fluorobenzene (**1c**, 127.2 mg, 1.009 mmol, 1.0 equiv), 3-ethoxycarbonyl-4-methylcoumarin (**2b**, 233.8 mg, 1.007 mmol, 1.0 equiv), and *o*-phosphoric acid (1.49 g, 15.3 mmol, 15 equiv). After removal of the solvent under reduced pressure, the remaining crude solids were purified by crystallization to give **3h** (205.7, 0.7000 mmol) in 70% yield as greenish yellow solids: mp (recrystallized from EtOH, 46% yield) 230.2–230.8 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.79 (d, J = 8.0 Hz, 1H, ArH), 7.75–7.71 (m, 2H, 2 × ArH), 7.52–7.45 (m, 3H, 3 × ArH), 7.16–7.02 (m, 1H, ArH), 2.66 (s, 3H, O=C–C=CCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 164.00 (C=O), 159.23, 156.71, 154.48, 153.29, 148.38, 134.28, 133.11, 130.28, 130.19, 125.13, 124.82, 118.99, 118.35, 117.99, 116.18, 16.53; IR (KBr) 3362 (s, NH), 1693 (s, C=O), 1592 (m), 1169 (m) cm^{-1}; HRMS m/z calcd for $C_{17}H_{11}FN_2O_2$: 294.0805, found: 294.0802.

*6.2.9. 3-(Benzimidazol-2′-yl)-4-methylcoumarin (**3i**)*

The Standard procedure 1 was followed by use of 1,2-diaminobenzene (**1d**, 117.2 mg, 1.084 mmol, 1.0 equiv), 3-ethoxycarbonyl-4-methylcoumarin (**2b**, 251.2 mg, 1.081 mmol, 1.0 equiv), and *o*-phosphoric acid (1.61 g, 16.5 mmol, 15 equiv). After removal of the solvent under reduced pressure, the remaining crude solids were purified by crystallization to give **3i** (260.1 mg, 0.9423 mmol) in 87% yield as yellow solids: mp (recrystallized from EtOH, 67% yield) 223.6–224.4 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.45 (bs, 1H, NH), 7.91 (d, 1H, J = 8.0 Hz, 1H, ArH), 7.74–7.68 (m, 1H, ArH), 7.64–7.57 (m, 2H, 2 × ArH), 7.46–7.39 (m, 2H, 2 × ArH), 7.36–7.29 (m, 2H, 2 × ArH), 3.22 (s, 3H, O=C–C=CCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) 161.26 (C=O), 154.63, 151.87, 146.78, 132.63, 131.73, 126.09, 125.02, 124.18, 124.72, 123.20, 120.83, 117.07, 116.83, 115.08, 114.11, 17.62 (CH_3); IR (KBr) 3350 (br, NH), 1686 (s, C=O), 1542 (m), 1505 (m), 1310 (m), 1155 (m) cm^{-1}; HRMS m/z calcd for $C_{17}H_{12}N_2O_2$: 276.0899, found: 276.0894.

*6.2.10. 7-Acetoxy-3-(benzimidazol-2′-yl)-4-methylcoumarin (**3j**)*

The Standard procedure 1 was followed by use of 1,2-diaminobenzene (**1d**, 108.8 mg, 1.006 mmol, 1.0 equiv), 7-acetoxy-3-ethoxycarbonyl-4-methylcoumarin (**2d**, 290.5 mg, 1.001 mmol, 1.0 equiv), and *o*-phosphoric acid (1.44 g, 14.7 mmol, 15 equiv). After removal of the solvent under reduced pressure, the remaining crude solids were purified by crystallization to give **3j** (260.7 mg, 0.7806 mmol) in 78% yield as gray solids: mp (recrystallized from EtOH, 56% yield) 129.1–129.6 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.58–7.52 (m, 2H, ArH), 7.43 (d, J = 8.8 Hz, 1H, ArH), 7.26–7.20 (m, 2H, 2 × ArH), 6.90–6.83 (m, 2H, 2 × ArH), 6.07 (s, 1H, NH), 2.62 (s, 3H, ArCH_3), 2.37 (s, 3H, O=C–CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) 162.40 (C=O), 161.69 (C=O), 157.23, 155.12, 153.69, 150.96, 135.96, 135.52, 126.58, 125.71, 123.24, 121.00, 114.13, 113.65, 113.15, 112.50, 110.52, 18.64 (CH_3), 13.88 (CH_3); IR (KBr) 3502 (br, NH), 1716 (s, C=O), 1671 (s, C=O), 1605 (m), 1453 (m), 1391 (m), 1274 (m), 1136 (m), 1073 (m) cm^{-1}; HRMS m/z calcd for $C_{19}H_{14}N_2O_4$: 334.0954, found: 334.0952.

*6.2.11. 3-(5′-Carboxybenzimidazol-2′-yl)coumarin (**3k**)*

The Standard procedure 1 was followed by use of 3,4-diaminobenzoic acid (**1e**, 500.4 mg, 3.288 mmol, 1.0 equiv), 3-ethoxycarbonylcoumarin (**2a**, 716.3 mg, 3.286 mmol, 1.0 equiv), and *o*-phosphoric acid (4.79 g, 48.9 mmol, 15 equiv). After removal of the solvent under reduced pressure, the remaining crude solids were purified by crystallization to give **3k** (815.4 mg, 3.017 mmol) in 81% yield as yellow solids: mp (recrystallized from EtOH, 62%

yield) 254.5–255.3 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.24 (s, 1H, COOH), 8.81 (s, 1H, O═C–C═CH), 7.68–7.61 (m, 3H, 3 × ArH), 7.47–7.38 (m, 4H, 4 × ArH), 5.27 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) 162.18 (C═O), 160.84 (C═O), 153.59, 151.34, 145.88, 142.45, 135.65, 133.18, 130.42, 129.21, 126.16, 125.46, 123.63, 119.06, 118.42, 116.70, 115.76; IR (KBr) 3337 (br, NH), 1748 (m, C═O), 1710 (s, C═O), 1606 (m), 1569 (m), 1455 (m), 1401 (m), 1316 (m), 1257 (m), 1123 (m) cm^{-1}; HRMS *m*/*z* calcd for $C_{17}H_{10}N_2O_4$: 306.0641, found: 306.0636.

*6.2.12. 3-(5′-Carboxybenzimidazol-2′-yl)-6-nitrocoumarin (**3l**)*

The Standard procedure 1 was followed by use of 3,4-diaminobenzoic acid (**1e**, 116.1 mg, 0.7633 mmol, 1.0 equiv), 3-ethoxycarbonyl-6-nitrocoumarin (**2e**, 199.8 mg, 0.7592 mmol, 1.0 equiv), and *o*-phosphoric acid (1.16 g, 11.9 mmol, 16 equiv). After removal of the solvent under reduced pressure, the remaining crude solids were purified by crystallization to give **3l** (173.8 mg, 0.4952 mmol) in 73% yield as yellow solids: mp (recrystallized from EtOH, 48% yield) 278.8–279.5 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.67 (s, 1H, O═C–C═CH), 8.59–8.52 (m, 2H, 2 × ArH), 8.40–8.33 (m, 2H, 2 × ArH), 7.51–7.44 (m, 2H, 2 × ArH); ^{13}C NMR ($CDCl_3$, 100 MHz) 160.03 (C═O), 159.17 (C═O), 157.67, 153.30, 147.40, 143.21, 142.65, 139.56, 134.98, 133.15, 129.68, 125.12, 118.99, 116.34, 113.57, 111.21, 103.82; IR (KBr) 3449 (br, NH), 1725 (s, C═O), 1618 (s, C═C), 1535 (m), 1523 (m), 1353 (m), 1235 (m), 1208 (s) cm^{-1}; HRMS *m*/*z* calcd for $C_{17}H_9N_3O_6$: 351.0491, found: 351.0494.

*6.2.13. 3-(5′-Carboxybenzimidazol-2′-yl)-7-iodocoumarin (**3m**)*

The Standard procedure 1 was followed by use of 3,4-diaminobenzoic acid (**1e**, 115.2 mg, 0.7569 mmol, 1.0 equiv), 3-ethoxycarbonyl-7-iodocoumarin (**2f**, 260.4 mg, 0.7568 mmol, 1.0 equiv), and *o*-phosphoric acid (1.14 g, 11.7 mmol, 16 equiv). After removal of the solvent under reduced pressure, the remaining crude solids were purified by crystallization to give **3m** (201.5 mg, 0.4663 mmol) in 65% yield as yellow solids: mp (recrystallized from EtOH, 42% yield) 277.2–277.9 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.05 (s, 1H, O═C–C═CH), 8.40 (s, 1H, ArH), 7.96 (d, J = 8.4 Hz, 1H, ArH), 7.73–7.66 (m, 2H, 2 × ArH), 7.27–7.21 (m, 2H, 2 × ArH); ^{13}C NMR (DMSO-d_6, 100 MHz) 159.94 (C═O), 158.84 (C═O), 153.24, 146.67, 142.58, 142.14, 139.05, 133.05, 129.60, 125.04, 122.56, 121.35, 119.41, 118.93, 116.18, 103.78, 98.62; IR (KBr) 3384 (br, NH), 1717 (s, C═O), 1388 (m), 1240 (m), 1126 (s), 947 (m) cm^{-1}; HRMS *m*/*z* calcd for $C_{17}H_9N_2O_4I$: 431.9607, found: 431.9604.

*6.2.14. 3-(Naphthimidazol-2′-yl)coumarin (**5**)*

The Standard procedure 1 was followed by use of 2,3-diaminonaphthalene (**4**, 298.6 mg, 1.887 mmol, 1.0 equiv), 3-ethoxycarbonylcoumarin (**2a**, 411.8 mg, 1.889 mmol, 1.0 equiv), and *o*-phosphoric acid (2.81 g, 28.7 mmol, 15 equiv). After removal of the solvent under reduced pressure, the remaining crude solids were purified by crystallization to give **5** (340.3 mg, 1.090 mmol) in 68% yield as yellow solids: mp (recrystallized from EtOH, 45% yield) 251.7–252.5 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.90 (bs, 1H, NH), 9.28 (s, 1H, O═C–C═CH), 8.17 (bs, 2H, 2 × ArH), 8.08–7.97 (m, 2H, 2 × ArH), 7.76–7.72 (m, 1H, ArH), 7.53–7.44 (m, 3H, 3 × ArH), 7.10–7.02 (m, 2H, 2 × ArH); ^{13}C NMR (DMSO-d_6, 100 MHz) 159.22 (C═O), 153.60, 150.12, 146.13, 144.06, 138.10, 133.49, 130.16, 129.93, 127.84, 125.44, 125.17, 124.62, 123.57, 122.73, 121.34, 119.00, 116.43, 108.07, 107.09; IR (KBr) 3350 (br, NH), 1705 (s, C═O), 1600 (m), 1510 (m), 1410 (m), 1100 (m) cm^{-1}; HRMS *m*/*z* calcd for $C_{20}H_{12}N_2O_2$: 312.0899, found: 312.0897.

*6.2.15. 3-(5′-Methylbenzimidazol-2′-yl)benzocoumarin (**7**)*

The Standard procedure 1 was followed by use of 3,4-diaminotoluene (**1a**, 123.5 mg, 1.011 mmol, 1.0 equiv), 3-carboeth oxybenzocoumarin (**6**, 270.4 mg, 1.009 mmol, 1.0 equiv), and *o*-phosphoric acid (1.49 g, 15.2 mmol, 15 equiv). After removal of the solvent under reduced pressure, the remaining crude solids were purified by crystallization to give **7** (222.4 mg, 0.6823 mmol) in 68% yield as yellow solids: mp (recrystallized from EtOH, 45% yield) 210.6–211.3 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.67 (s, 1H, O═C–C═CH), 8.43 (d, J = 8.4 Hz, 1H, ArH), 8.16 (d, J = 8.8 Hz, 1H, ArH), 7.93 (d, J = 8.0 Hz, 1H, ArH), 7.66–7.62 (m, 1H, ArH), 7.53–7.49 (m, 1H, ArH), 7.45–7.43 (d, J = 9.2 Hz, 1H, ArH), 7.20–7.18 (d, J = 8.0 Hz, 1H, ArH), 7.12 (s, 1H, ArH), 6.99–6.97 (m, 1H, ArH), 2.48 (s, 3H, $ArCH_3$); ^{13}C NMR (DMSO-d_6, 100 MHz) 164.15 (C═O), 157.27, 156.34, 145.18, 137.25, 136.21, 133.49, 132.15, 131.38, 131.36, 129.83, 128.99, 126.34, 124.63, 123.51, 123.53, 122.37, 121.76, 118.97, 117.65, 20.5 ($ArCH_3$); IR (KBr) 3355 (br, NH), 1751 (s, C═O), 1566 (m), 1211 (m) cm^{-1}; HRMS *m*/*z* calcd for $C_{21}H_{14}N_2O_2$: 326.1055, found: 326.1053.

*6.2.16. 3-(Imidazopyridin-2′-yl)-8-methoxycoumarin (**9**)*

The Standard procedure 1 was followed by use of 2,3-diaminopyridine (**8**, 217.9 mg, 1.997 mmol, 1.0 equiv), 3-ethoxycarbonyl-8-methoxycoumarin (**2g**, 494.5 mg, 1.994 mmol, 1.0 equiv), and *o*-phosphoric acid (2.99 g, 30.5 mmol, 15 equiv). After removal of the solvent under reduced pressure, the remaining crude solids were purified by crystallization to give **9** (408.5 mg, 1.3940 mmol) in 69% yield as light brown solids: mp (recrystallized from EtOH, 46% yield) decomposed at 210.2 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.98 (bs, 1H, NH), 8.89 (s, 1H, O═C–C═CH), 8.46 (d, J = 1.6 Hz, 1H, ArH), 8.16–8.12 (m, 1H, ArH), 7.39–7.32 (m, 4H, 4 × ArH), 3.94 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) 160.45 (C═O), 160.16, 153.70, 147.47, 146.33, 144.90, 143.16, 131.88, 125.27, 121.21, 119.91, 119.00, 117.96, 116.25, 112.68, 56.29 (CH_3); IR (KBr) 3379 (br, NH), 1705 (s, C═O), 1543 (m), 1466 (m) cm^{-1}; HRMS *m*/*z* calcd for $C_{16}H_{11}N_3O_3$: 293.0800, found: 293.0798.

*6.3. Standard procedure **2** for the preparation of N-nucleosides of 3-(benzimidazol-2′-yl)coumarins **10i** and **10n***

To a solution of coumarins **3** (1.0 equiv) in acetonitrile (35 mL) was added *N*,*O*-bistrimethylsilylacetamide (BSA, 1.5 equiv) in nitrogen atmosphere. After the solution was stirred at room temperature for 15 min, 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (1.0 equiv) in acetonitrile (2.0 mL) was added to the reaction mixture followed by addition of Me_3SiOTf (1.5 equiv). The reaction mixture was heated to 80 °C in an oil bath for 18 h. After the solvent was removed under reduced pressure and the residue was treated with 20% aqueous $NaHCO_3$ solution. The organic layers extracted with chloroform were collected to provide a crude thick mass, which was purified by column chromatography on silica gel to afford the desired *N*-nucleosides with purity >99.7%, as checked by HPLC.

*6.3.1. 4-Methyl-3-[1′-(2″,3″,5″-tri-O-acetyl-β-D-ribofuranos-1″-yl) benzimidazol-2′-yl] coumarin (**10i**)*

The Standard procedure 2 was followed by use of **3i** (96.9 mg, 0.351 mmol, 1.0 equiv), BSA (107.2 mg, 0.5271 mmol, 1.5 equiv), 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (112.3 mg, 0.3528 mmol, 1.0 equiv) and Me_3SiOTf (117.2 mg, 0.5273 mmol, 1.5 equiv). After the reaction mixture was worked up, the residue was purified by use of column chromatography (EtOAc as eluant) to give **10i** (159.6 mg, 0.2989 mmol) in 85% yield as white solid foams: mp (recrystallized from EtOH, 73% yield) 193.1–193.8 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.82–7.68 (m, 4H, 4 × ArH), 7.45–7.29 (m, 4H, 4 × ArH), 5.85 (d, 1H, J = 6.8 Hz, H-1′), 5.78–5.74 (m, 1H, H-2′), 5.55–5.51 (m, 1H, H-3′), 4.42–4.28 (m, 3H, H-4′ + 2 × H-5′), 2.36 (s, 3H, C═C–CH_3), 2.19 (s, 3H, O═C–CH_3), 2.13 (s, 3H, O═C–CH_3), 2.11 (s, 3H, O═C–CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) 170.08 (C═O),

169.39 (C=O), 169.11 (C=O), 159.08 (C=O), 155.48, 153.33, 146.76, 143.63, 133.05, 132.15, 125.98, 125.28, 124.71, 123.69, 120.44, 119.47, 117.05, 115.41, 111.84, 87.51 (C-1′), 79.47 (C-4′), 70.29 (C-2′), 68.99 (C-3′), 62.64 (C-5′), 20.55 (CH_3), 20.33 (CH_3), 20.07 (CH_3), 16.58 (CH_3); IR (KBr) 1745 (s, C=O), 1723 (s, C=O),1609 (m), 1454 (m), 1373 (s), 1233 (s), 1107 (m), 1074 (m), 750 (m) cm^{-1}; HRMS *m*/*z* calcd for $C_{28}H_{26}N_2O_9$: 534.1638, found: 534.1635.

6.3.2. 3-[1′-(2″,3″,5″-Tri-O-acetyl-β-D-ribofuranos-1″-yl) benzimidazol-2′-yl]coumarins (**10n**)

The Standard procedure 2 was followed by use of 3-(benzimidazol-2′-yl)coumarin (**3n**, 499.7 mg, 1.905 mmol, 1.0 equiv), BSA (582.2 mg, 2.862 mmol, 1.5 equiv), 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (607.2 mg, 1.908 mmol, 1.0 equiv) and Me_3SiOTf (636.1 mg, 2.862 mmol, 1.5 equiv). After the reaction mixture was worked up, the residue was purified by use of column chromatography (80% EtOAc in hexanes as eluant) to give **10n** (705.6 mg, 1.356 mmol) in 81% yield as white solid foams: mp (recrystallized from EtOH, 62% yield) 187.7–188.5 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.27 (s, 1H, O=C–C=CH), 7.83–7.78 (m, 1H, ArH), 7.77–7.73 (m, 1H, ArH), 7.64–7.59 (m, 2H, 2 × ArH), 7.41–7.27 (m, 4H, 4 × ArH), 5.97 (d, J = 6.8 Hz, 1H, H-1′), 5.73 (t, J = 6.8 Hz, 1H, H-2′), 5.48 (dd, J = 5.2 Hz, J = 6.8 Hz, 1H, H-3′), 4.43–4.39 (m, 2H, 2 × H-5′), 4.37–4.33 (m, 1H, H-4′), 2.15 (s, 3H, O=C–CH_3), 2.06 (s, 3H, O=C–CH_3), 2.05 (s, 3H, O=C–CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) 170.21 (C=O), 169.49 (C=O), 169.05 (C=O), 159.02 (C=O), 154.55, 147.48, 142.83, 133.48, 133.14, 129.02, 125.18, 124.30, 123.78, 120.45, 118.53, 118.36, 116.85, 115.27, 112.40, 88.46 (C-1′), 79.67 (C-4′), 71.21 (C-2′), 69.19 (C-3′), 62.76 (C-5′), 20.70 (CH_3), 20.45 (CH_3), 20.21 (CH_3); IR (KBr) 1746 (s, C=O), 1609 (m), 1455 (m), 1377 (m), 1239 (m), 1105 (m) cm^{-1}; HRMS *m*/*z* calcd for $C_{27}H_{24}N_2O_9$: 520.1482, found: 520.1478.

6.4. Standard procedure 3 for deprotection of O-acetyl N-ribosides of 3-(benzimidazol-2′- yl)coumarins **11i** and **11n**

The acetyl protected nucleosides **10i** and **10n** (1.0 equiv) were treated with saturated methanolic ammonia at room temperature for 18 h. The mixture was concentrated to dryness under reduced pressure and triturated with chloroform. The formed precipitate was collected by vacuum filtration to give unprotected *N*-ribosides with purity >99.5%, as checked by HPLC.

6.4.1. 4-Methyl-3-[1′-(β-D-ribofuranos-1″-yl)benzimidazol-2′-yl] coumarin (**11i**)

The Standard procedure 3 was followed by use of **10i** (50.4 mg, 0.0943 mmol, 1.0 equiv) and methanol saturated with ammonia (30.0 mL). After the reaction mixture was worked up, crude solids were purified by crystallization from 10% methanol in chloroform to give **11i** (32.8 mg, 0.0802 mmol) in 85% yield as white solids: mp (recrystallized from EtOH, 65% yield) 156.7–156.2 °C; ^{1}H NMR (CD_3CN, 400 MHz) δ 7.95–7.83 (m, 4H, 4 × ArH), 7.38–7.29 (m, 4H, 4 × ArH), 5.47 (d, J = 7.2 Hz, 1H, H-1′), 4.47–4.42 (m, 1H, H-2′), 4.17–4.12 (m, 2H, H-3′ + H-4′), 3.82–3.76 (m, 2H, 2 × H-5′), 3.47 (bs, 3H, 3 × OH), 2.27 (s, 3H, CH_3); ^{13}C NMR (CD_3OD, 100 MHz) 161.10 (C=O), 155.74, 149.43, 149.10, 143.85, 134.77, 134.43, 130.59, 126.38, 125.04, 124.49, 120.22, 119.95, 119.40, 117.62, 115.79, 91.61 (C-1′), 87.06 (C-4′), 73.36 (C-2′), 71.12 (C-3′), 62.78 (C-5′), 24.14 (CH_3); IR (KBr) 3408 (br, OH), 1707 (s, C=O), 1604 (m), 1460 (m), 1394 (m), 1272 (m), 1057 (m) cm^{-1}; HRMS *m*/*z* calcd for $C_{22}H_{20}N_2O_6$: 408.1321, found: 408.1320.

6.4.2. 3-[1′-(β-D-Ribofuranos-1″-yl)benzimidazol-2′-yl]coumarin (**11n**)

The Standard procedure 3 was followed by use of **10n** (50.1 mg, 0.0963 mmol, 1.0 equiv) and saturated methanolic ammonia (30.0 mL). After the reaction mixture was worked up, crude solids were purified by crystallization from 10% methanol in chloroform to give **11n** (33.5 mg, 0.0850 mmol) in 88% yield as white solids: mp (recrystallized from EtOH, 79% yield) 154.1–151.9 °C; ^{1}H NMR (CD_3OD, 400 MHz) δ 8.38 (s, 1H, O=C–C=CH), 8.11–8.03 (m, 1H, ArH), 7.79–7.68 (m, 3H, 3 × ArH), 7.52–7.42 (m, 2H, 2 × ArH), 7.28–7.25 (m, 2H, 2 × ArH), 5.78 (d, J = 7.2 Hz, 1H, H-1′), 4.58 (t, J = 6.8 Hz, 1H, H-2′), 4.24–4.20 (m, 1H, H-3′), 4.02–3.97 (m, 1H, H-4′), 3.81–3.86 (m, 2H, 2 × H-5′), 3.34 (bs, 3H, 3 × OH); ^{13}C NMR (CD_3OD, 100 MHz) 160.96 (C=O), 155.83, 149.48, 148.96, 143.91, 134.70, 134.50, 130.53, 126.32, 125.00, 124.47, 120.21, 120.00, 119.57, 117.64, 115.11, 91.67 (C-1′), 87.15 (C-4′), 73.44 (C-2′), 71.18 (C-3′), 62.78 (C-5′); IR (KBr) 3510 (br, OH), 1719 (s, C=O), 1607 (s), 1531 (m), 1260 (m), 1170 (m), 1026 (m) cm^{-1}; HRMS *m*/*z* calcd for $C_{21}H_{18}N_2O_6$: 394.1165, found: 394.1163.

Author contributions

Shwu-Chen Tsay: prepared the manuscript.

Jih Ru Hwu: the group leader, conceived the project and developed the concept on which it is based.

Raghunath Singha: synthesized compounds.

Yung Hsiung Chang: synthesized compounds.

Wen-Chien Huang: analyzed the purity and the data concerning the compounds.

Ming-Hua Hsu: provided and explained the results.

Fa-kuen Shieh: analyzed the results.

Kuo Chu Hwang: analyzed the results.

Jia-Cherng Horng: analyzed the results.

Erik De Clercq: interpreted the results concerning HCV inhibition.

Inge Vliegen: performed biological testing.

Johan Neyts: interpreted the results concerning HCV inhibition.

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Appendix A. Supplementary data

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