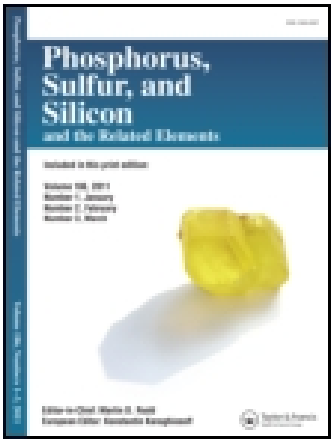

Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gpss20

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Published online: 12 Jul 2011.

To cite this article: Jih Ru Hwu, Dr Shu-Yu Lin, Professor Shwu-Chen Tsay, Dr Raghunath Singha, Dr Benoy Kumar Pal, Dr Pieter Leyssen & Professor Johan Neyts (2011) Development of New Sulfur-Containing Conjugated Compounds as Anti-HCV Agents, Phosphorus, Sulfur, and Silicon and the Related Elements, 186:5, 1144-1152, DOI: 10.1080/10426507.2010.520284

To link to this article: http://dx.doi.org/10.1080/10426507.2010.520284

Phosphorus, Sulfur, and Silicon, 186:1144–1152, 2011

ISSN: 1042-6507 print / 1563-5325 online
DOI: 10.1080/10426507.2010.520284

DEVELOPMENT OF NEW SULFUR-CONTAINING CONJUGATED COMPOUNDS AS ANTI-HCV AGENTS

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Abstract *Various conjugated compounds containing a coumarin moiety and a heterocyclic nucleus were synthesized. Their activity against hepatitis C virus was tested on subgenomic replicon replication in Huh 5-2 cells. Some heterobicycle–coumarin conjugates with the $-S-CH_2-$ linker were found to possess appealing antiviral activities. The sulfur atom in these conjugated compounds was found to be an essential element to their antiviral activity.*

Keywords Coumarin; guanosine; heterocycles; methylenethio linker

INTRODUCTION

In the beginning of the 21st century, many medicinal scientists have been devoting their efforts to the invention of new drugs against hepatitis C virus (HCV).[1] With a size of around 50 nm, the HCV is an enveloped, single-stranded, positive sense RNA virus. Infection by HCV is often asymptomatic, but once established, chronic infection can progress to scarring of the liver and cause fibrosis. Even worse is cirrhosis, which is generally apparent after many years. In some cases, those with cirrhosis will go on to develop liver failure or cancer.[2]

HCV infections occur worldwide; the World Health Organization estimated that about 3% of the world's population has been infected with HCV.[3] Currently the only approved treatment for HCV infection is weekly injection of polyethylene glycol-conjugated interferon-α plus daily oral ribavirin. Interferon is thought to work by stimulating the body's natural defenses against virus infection. The 48-week treatment course is often poorly tolerated, so new approaches are needed that enhance the effectiveness and shorten the duration of therapy.[4]

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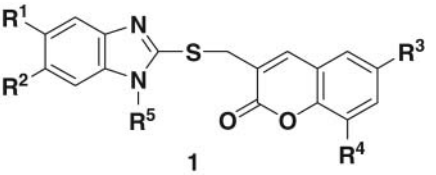
Different types of compounds have been synthesized and found to possess activity against HCV. For examples, Hirashima et al.[5] found that 2-arylbenzimidazole-5-carboxylic acids possess inhibitory activity against HCV NS5B RNA polymerase. Goulet et al.[6] synthesized benzimidazole–diamide derivatives that exhibit potency against HCV NS5B polymerase. Stankiewicz-Drogoń et al.[7] prepared new acridone derivatives that inhibit HCV NS3 helicase. McCauley et al.[8] obtained a new class of macrocyclic isoindolines that show inhibitory activity against hepatitis C virus NS3/4A protease. It can also be inhibited by five- and six-membered cyclic sulfones as reported by Velázquez et al.[9] Recently, our laboratory found that various benzimidazole moieties conjugated with a coumarin moiety by a methylenethio linker (i.e., **1**) exhibit potent inhibitory effects on HCV.[10] Furthermore, the structure–activity relationship toward HCV is established for various coumarins conjugated with imidazopyridine, purine, benzoxazole, and benzothiazole (i.e., **2**).[11] Herein we report our findings on the essential role of the sulfur atom in the conjugated compounds heterocycle—S—CH_2—coumarins for their activity against HCV.

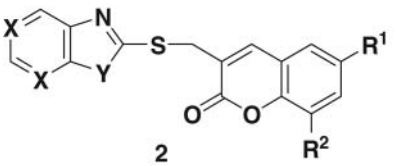


1

R^1, R^2, R^3, R^4 = H, Me, OMe, F, Cl, Br

R^5 = H, sugar

Benzimidazole–Coumarin Conjugates



2

R^1, R^2 = H, Br, OMe

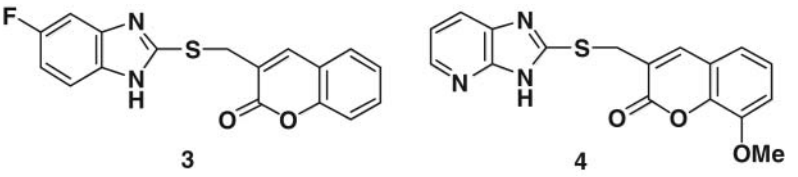
X = CH, N

Y = O, S, NH, N–sugar

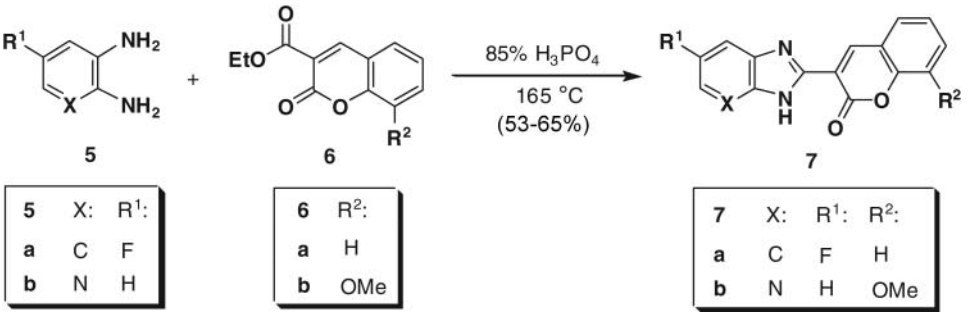
Heterobicycle–Coumarin Conjugates

RESULTS AND DISCUSSION

To compare with conjugated compounds **3**[10] and **4**[11] containing an —S—CH_2— linker, we synthesized two closely related "hinged" heterobicycle–coumarin conjugates as shown in Scheme 1. Treatment of arylenediamines **5** with 3-(ethoxycarbonyl)coumarins[12] **6** in 85% *o*-phosphoric acid at 165°C provided heterobicycle–coumarin conjugates **7** in 53–65% yields.



Recently, several research groups have reported their efforts on the syntheses of guanosine analogues, which exhibit antiviral activities.[13] Accordingly, we synthesized a series of guanosine−SCH_2−coumarin conjugates **11** and performed their bioassays. Treatment of the commercially available 8-bromoguanosine (**8**) with thiourea in ethanol gave 8-mercaptoguanosine[14] (**9**). We then coupled **9** with different 3-(chloromethyl)coumarins **10**[15] in the presence of 35% NH_4OH in water and acetonitrile (Scheme 2). Upon workup and purification by chromatography, the desired guanosine−SCH_2−coumarin conjugates **11a**–**e** were produced in 74–91% yields.



5	X:	R^1:
a	C	F
b	N	H

6	R^2:
a	H
b	OMe

7	X:	R^1:	R^2:
a	C	F	H
b	N	H	OMe

Scheme 1 Synthesis of hinged heterobicycle–coumarin derivatives.

We confirmed the structures of conjugates **11** on the basis of their spectroscopic characteristics. For example, the mass spectrum of **11a** in the positive ion mode by fast atom bombardment–mass spectrometry (FAB-MS) technique exhibited at 474.1091 for species $[M + H]^+$, which indicates the molecular formula to be $C_{20}H_{19}N_5O_7S + H^+$ with the theoretical value of 474.1083. Its infrared (IR) spectrum showed one strong absorption band at 1723 cm^{-1}, which was attributed to the carbonyl stretching vibration of the coumarin moiety.[16] Its ^{13}C NMR spectrum had resonance at 32.67 and 160.12 ppm for the SCH_2 and the O=C−O carbons, respectively, in the coumarin moiety. On the other hand, two diastereotopic SCH_2 protons appeared at 4.25 and 4.20 ppm as two doublets with $J = 13.8$ Hz in its ^1H NMR spectrum. The glycosidic proton resonated at the 5.70 ppm as a doublet with $J = 6.4$ Hz.

Evaluation on the Antiviral Activities in the HCV Genotype 1b Subgenomic Replicon

All compounds were evaluated in the HCV subgenomic replicon system in Huh 5–2 cells.[17] The antiviral assays and cytostatic determination assays have been described in detail before.[18] The 50% inhibitory concentrations for virus replication (EC_{50}) and host cell growth (CC_{50}) of conjugates **3**, **4**, and **7a**,**b** are shown in Table 1.

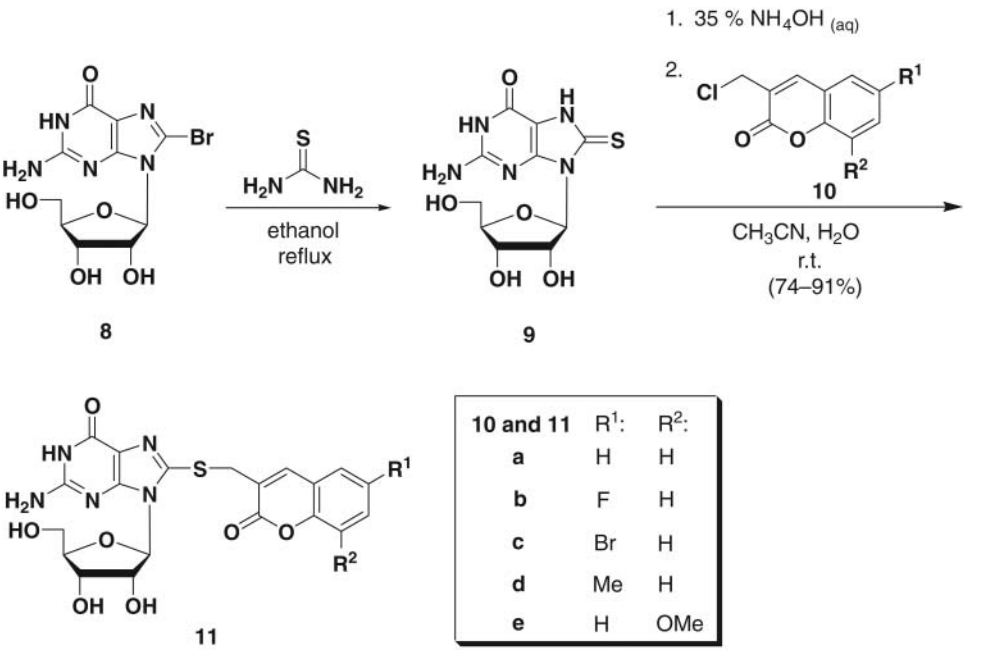
Table 1 Inhibitory effects of conjugated compounds on HCV subgenomic replicon replication in Huh 5–2 cells

Compound[a]	CC_{50}[b] (μM)	7a/3	7b/4	EC_{50}[c] (μM)	7a/3	7b/4
3	45			11		
		0.47	—			—
7a	21			131	12	
4	30			11		
		—	5.7			7.4
7b	>170			81	—	

[a]Interferon α-2b was used as a (positive) reference compound at 10,000 units/well and reduced the signal in the viral RNA (luciferase) assay to background levels without any cytotoxic activity. The values were obtained as the average of triplicate determinations.

[b]Minimum cytotoxic concentration required to cause a microscopically detectable alteration of normal cell morphology.

[c]Minimum inhibitory concentration required to reduce virus-induced cytopathogenicity by 50%.



Scheme 2 Synthesis of guanosine−SCH_2−coumarin derivatives.

For the conjugates **11a–e**, we found that their HCV replication occurred at EC_{50} values above 91 μM without apparent cytostatic activity. Therefore, the guanosine moiety made a limited contribution to its HCV inhibition.

Structure–Activity Relationship

We deduce the following structure–activity relationship (SAR) by scrutinizing their EC_{50} and CC_{50} values shown in Table 1.

1. Attachment of a coumarin moiety to the benzimidazole nucleus (e.g., **3**) with an $-S-CH_2-$ linker is the key to gain appealing anti-HCV activity. Addition of the $-S-CH_2-$ linker between the coumarin moiety and the benzimidazole nucleus enhanced the HCV inhibitory activity by 12-fold (cf. **3** versus **7a**).
2. Attachment of an imidazopyridine (e.g., **4**) nucleus to the thio terminal of the coumarin conjugates may offer a convenient approach to enhance the HCV inhibition by a factor of 7.4-fold (cf. **4** versus **7b**). The $-S-CH_2-$ linker therein is essential and should not be removed.
3. Replacement of the benzimidazole moiety with a guanosine moiety in conjugated coumarins (cf. **3** versus **11a**) displayed no notable activity on HCV.

EXPERIMENTAL

General Experimental

All reactions were carried out in oven-dried glassware (120°C) under an atmosphere of nitrogen unless as indicated otherwise. Dichloromethane and methanol were purchased from Mallinckrodt Chemical Co. (Phillipsburg, NJ). Acetonitrile was purchased from Fisher Scientific Co. (Fairlawn, NJ). Ethyl acetate (EtOAc) and hexanes from Mallinckrodt Chemical Co. were dried and distilled from CaH_2. Aqueous ammonium hydroxide was purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ). 1,2-Diamino-4-fluorobenzene and 2,3-diaminopyridine were purchased from Sigma-Aldrich Chemical Co. (Steinheim, Germany). 2-(Coumarin-3′-yl)methylthio-5-fluorobenzimidazole[10] (**3**), 2-(8′-methoxycoumarin-3′-yl)methylthio-1*H*-imidazo[4,5-*b*]pyridine[11] (**4**), 3-(ethoxycarbonyl)coumarins[12] **6a**,**b**, 8-mercaptoguanosine[14] (**9**), and 3-(chloromethyl)coumarins[15] **10a**–**e** were prepared according to the reported methods.

Analytical thin-layer chromatography (TLC) was performed on precoated plates (silica gel 60 F-254) purchased from Merck Inc. (Darmstadt, Germany). Purification by gravity column chromatography was carried out using Silicycle ultra pure silica gel (particle size 40–63 μm, 230–400 mesh). High-performance liquid chromatography (HPLC) was performed on two Waters (Québec City, Québec) 515 HPLC pumps equipped with a Waters 2489 UV-Visible Detector and a Thermo 5 μm Hypersil ODS (250 $\times$ 4.6 mm D.I.). Purity of all compounds was >98.0%, as checked by HPLC.

IR spectra were measured on a Perkin-Elmer model spectrum one B spectrophotometer and a Bomem Michelson Fourier transform infrared spectrometer (FTIR). Absorption intensities are recorded by the following abbreviations: s, strong; m, medium; w, weak. High-resolution mass spectra were obtained by means of a JEOL JMS-700 mass spectrometer. Proton nuclear magnetic resonance (NMR) spectra were obtained on a Varian Mercury-400 (400 MHz) spectrometer or Bruker AC-400 (400 MHz) spectrometer by use of chloroform-*d*, dimethylsulfoxide-d_6, and methanol-d_4 as solvents. Proton NMR chemical shifts are referenced to the center of DMSO-d_6 quintet (δ 2.49 ppm). ^{13}C NMR spectra were performed on a Varian Mercury-400 (100 MHz) spectrometer or Bruker AC-400 (400 MHz) spectrometer by use of dimethylsulfoxide-d_6 as solvents. ^{13}C chemical shifts are referenced to the center of the DMSO-d_6 septet (δ 39.5 ppm). Multiplicities are recorded by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; *J*, coupling constant (Hertz).

Standard Procedure 1 for the Preparation of 3-(Benzimidazol-2′-yl)coumarins (7)

To a solution containing a diamine **5** (1.0 equiv.) and 3-(ethoxycarbonyl)coumarins **6** (1.0 equiv.) was added *o*-phosphoric acid (85%, 15 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 of stirred water (500 mL). 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. The resultant solids were recrystallized with EtOH to give the desired products with purity >99.6%, as checked by gas chromatography (GC).

3-(5′-Fluorobenzimidazol-2′-yl)coumarin (7a). Standard procedure 1 was followed by the use of 1,2-diamino-4-fluorobenzene (**5a**, 750.1 mg, 5.948 mmol, 1.0 equiv.), 3-(ethoxycarbonyl)coumarin (**6a**, 1.29 g, 5.91 mmol, 1.0 equiv.), and *o*-phosphoric acid (9.09 g, 92.8 mmol, 16 equiv.). After workup, the solids were recrystallized with EtOH to give **7a** (1.076 g, 3.839 mmol) in 65% yield as greenish yellow solids: ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.09 (s, 1 H, CH=C−COO), 7.98 (d, J = 8.0 Hz, 1 H, ArH), 7.72–7.65 (m, 2 H, 2 × ArH), 7.52–7.43 (m, 4 H, 4 × ArH), 7.07 (bs, 1 H, 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 $(M)^+$; high-resolution mass spectrometry (HRMS) m/z Anal. Calcd. for $C_{16}H_9FN_2O_2$: 280.0648; Found: 280.0646.

3-(Imidazopyridin-2′-yl)-8-methoxycoumarin (7b). Standard procedure 1 was followed by the use of 2,3-diaminopyridine (**5b**, 217.9 mg, 1.997 mmol, 1.0 equiv.), 3-ethoxycarbonyl-8-methoxycoumarin (**6b**, 494.5 mg, 1.994 mmol, 1.0 equiv.), and *o*-phosphoric acid (2.99 g, 30.5 mmol, 15 equiv.). After workup, the solids were recrystallized with EtOH to give **7b** (308.8 mg, 1.053 mmol) in 53% yield as light brown solids: ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.98 (bs, 1 H, NH), 8.89 (s, 1 H, CH=C−COO), 8.46 (d, J = 1.6 Hz, 1 H, ArH), 8.16–8.12 (m, 1 H, ArH), 7.39–7.32 (m, 4 H, 4 × ArH), 3.94 (s, 3 H, 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 (OCH_3); IR (KBr) 3379 (br, NH), 1705 (s, C=O), 1543 (m), 1466 (m) cm^{-1}; HRMS m/z Anal. Calcd. for $C_{16}H_{11}N_3O_3$: 293.0800; Found: 293.0798.

Standard Procedure 2 for the Preparation of Conjugated Compounds

To a solution containing 8-mercaptoguanosine (**9**; 1.0 equiv.) in water (5.0 mL) and acetonitrile (3.0 mL) was added aqueous ammonium hydroxide (0.25 mL). After the solution was stirred at room temperature for 10 min, a 3-(chloromethyl)coumarin (**10**, 1.2 equiv.) was added and stirring was continued at room temperature for 15 min to 1.0 h. Acetonitrile therein was removed under reduced pressure and water was further removed under reduced pressure with Kügelrohr GKR-51 containing P_2O_5. The residue was purified by use of column chromatography packed with silica gel to give the desired products with purity >98.0%, as determined by HPLC.

8-[(Coumarin-3′-yl)methylthio]guanosine (11a). Standard procedure 2 was followed by use of **9** (65.6 mg, 0.208 mmol, 1.0 equiv.) and 3-(chloromethyl)coumarin

(**10a**, 48.7 mg, 0.250 mmol, 1.2 equiv.). After the solution was stirred at room temperature for 15 min and then worked up, the residue was purified by use of column chromatography (15% methanol in CH_2Cl_2 as the eluant) to give **11a** (81.4 mg, 0.172 mmol) in 83% yield as white solids: ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.63 (br, 1 H, NH), 7.95 (s, 1 H, CH=C−COO), 7.62–7.56 (m, 2 H, 2 × ArH), 7.40 (d, J = 8.0 Hz, 1 H, ArH), 7.32 (dd, J = 7.6, 7.2 Hz, 1 H, ArH), 6.40 (s, 2 H, NH_2), 5.70 (d, J = 6.4 Hz, 1 H, H-1″), 5.35 (d, J = 6.4 Hz, 1 H, OH), 5.05 (d, J = 4.8 Hz, 1 H, OH), 4.93 (dd, J = 6.4, 5.2 Hz, 1 H, OH), 4.90–4.84 (m, 1 H, H-2″), 4.25 (d, J = 13.8 Hz, 1 H, SCH), 4.20 (d, J = 13.8 Hz, 1 H, SCH), 4.09–4.06 (m, 1 H, H-3″), 3.80–3.77 (m, 1 H, H-4″), 3.62–3.57 (m, 1 H, H-5″), 3.50–3.44 (m, 1 H, H-5″); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.12 (C=O), 155.62, 153.11, 152.91, 152.52, 141.69, 140.97, 131.74, 128.31, 124.69, 124.16, 118.85, 117.37, 116.09, 88.23, 85.75, 70.56, 70.54, 61.99, 32.67 (SCH_2); IR (ATR) 3140 (br, OH), 2924 (w), 1711 (m), 1688 (s), 1608 (s), 1073 (s), 923 (m) cm^{-1}; MS (FAB$^+$) m/z 474 (MH^+, 16), 342 (32), 219 (29), 105 (18), 97 (100); HRMS (FAB) Anal. Calcd. for $(C_{20}H_{19}N_5O_7S + H)^+$: 474.1083; Found 474.1091.

8-[(6′-Fluorocoumarin-3′-yl)methylthio]guanosine (11b). Standard procedure 2 was followed by use of **9** (65.2 mg, 0.207 mmol, 1.0 equiv.) and 3-chloromethyl-6-fluorocoumarin (**10b**, 52.8 mg, 0.248 mmol, 1.2 equiv). After the solution was stirred at room temperature for 15 min and then worked up, the residue was purified by use of column chromatography (15% methanol in CH_2Cl_2 as the eluant) to give **11b** (92.4 mg, 0.188 mmol) in 91% yield as white solids: 1H NMR (DMSO-d_6, 400 MHz) δ: 10.68 (br, 1 H, NH), 7.90 (s, 1 H, CH=C−COO), 7.53–7.42 (m, 3 H, 3 × ArH), 6.41 (s, 2 H, NH_2), 5.70 (d, J = 6.8 Hz, 1 H, H-1″), 5.35 (d, J = 6.0 Hz, 1 H, OH), 5.07 (d, J = 4.8 Hz, 1 H, OH), 4.94 (dd, J = 6.8, 5.2 Hz, 1 H, OH), 4.89–4.85 (m, 1 H, H-2″), 4.24 (d, J = 14.0 Hz, 1 H, SCH), 4.19 (d, J = 14.0 Hz, 1 H, SCH), 4.10–4.06 (m, 1 H, H-3″), 3.80–3.76 (m, 1 H, H-4″), 3.62–3.56 (m, 1 H, H-5″), 3.49–3.43 (m, 1 H, H-5″); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.83 (C=O), 158.07 (d), 155.60, 153.10, 152.47, 149.25, 141.48, 139.84, 125.44, 119.81 (d), 118.83 (d), 118.00 (d), 117.42, 113.47 (d), 88.27, 85.74, 70.53, 70.50, 61.96, 32.77 (SCH_2); IR (ATR) 3146 (br, OH), 2922 (w), 1723 (m), 1681 (s), 1626 (s), 1071(s) cm^{-1}; MS (FAB$^+$) m/z 492 (MH^+, 35), 360 (62), 307 (98), 219 (23), 156 (100), 107 (100); HRMS (FAB) Anal. Calcd. for $(C_{20}H_{18}FN_5O_7S + H)^+$: 492.0989; Found 492.0996.

8-[(6′-Bromocoumarin-3′-yl)methylthio]guanosine (11c). Standard procedure 2 was followed by use of **9** (62.2 mg, 0.197 mmol, 1.0 equiv.) and 6-bromo-3-(chloromethyl)coumarin (**10c**, 64.7 mg, 0.237 mmol, 1.2 equiv.). After the solution was stirred at room temperature for 15 min and then worked up, the residue was purified by use of column chromatography (15% methanol in CH_2Cl_2 as the eluant) to give **11c** (85.7 mg, 0.155 mmol) in 79% yield as white solids: 1H NMR (DMSO-d_6, 400 MHz) δ: 10.66 (br, 1 H, NH), 7.89 (s, 1 H, CH=C−COO), 7.88 (d, J = 2.4 Hz, 1 H, ArH), 7.73 (dd, J = 8.8, 2.4 Hz, 1 H, ArH), 7.38 (d, J = 8.8 Hz, 1 H, ArH), 6.40 (s, 2 H, NH_2), 5.70 (d, J = 6.8 Hz, 1 H, H-1″), 5.34 (d, J = 6.4 Hz, 1 H, OH), 5.06 (d, J = 4.8 Hz, 1 H, OH), 4.93 (dd, J = 6.8, 5.2 Hz, 1 H, OH), 4.89–4.85 (m, 1 H, H-2″), 4.23 (d, J = 14.0 Hz, 1 H, SCH), 4.19 (d, J = 14.0 Hz, 1 H, SCH), 4.10–4.06 (m, 1 H, H-3″), 3.80–3.76 (m, 1 H, H-4″), 3.62–3.56 (m, 1 H, H-5″), 3.49–3.43 (m, 1 H, H-5″); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 159.65 (C=O), 155.70, 153.18, 152.52, 151.95, 141.46, 139.52, 134.03, 130.29, 125.50, 120.78, 118.39, 117.43, 116.24, 88.23, 85.77, 70.57, 70.54, 61.99, 32.80 (SCH_2); IR (ATR) 3334 (br, OH), 2920 (w), 1709 (s), 1692 (s), 1599 (s), 1066 (s) cm^{-1}; MS (FAB$^+$) m/z 552 (MH^+, 12), 307 (36), 289 (25), 154 (100), 136 (74); HRMS (FAB) Anal. Calcd. for $(C_{20}H_{18}BrN_5O_7S + H)^+$: 552.0189; Found 552.0180.

8-[(6′-Methylcoumarin-3′-yl)methylthio]guanosine (11d). Standard procedure 2 was followed by use of **9** (52.3 mg, 0.166 mmol, 1.0 equiv.) and 3-chloromethyl-6-methylcoumarin (**10d**, 41.5 mg, 0.199 mmol, 1.2 equiv.). After the solution was stirred at room temperature for 30 min and then worked up, the residue was purified by use of column chromatography (15% methanol in CH_2Cl_2 as the eluant) to give **11d** (59.6 mg, 0.122 mmol) in 74% yield as white solids: 1H NMR (DMSO-d_6, 400 MHz) δ: 10.72 (br, 1 H, NH), 7.90 (s, 1 H, CH═C─COO), 7.40–7.38 (m, 2 H, 2 × ArH), 7.30 (d, $J = 8.8$ Hz, 1 H, ArH), 6.46 (s, 2 H, NH_2), 5.69 (d, $J = 6.4$ Hz, 1 H, H-1″), 5.38 (d, $J = 6.4$ Hz, 1 H, OH), 5.09 (d, $J = 4.8$ Hz, 1 H, OH), 4.95 (dd, $J = 6.0$, 6.0 Hz, 1 H, OH), 4.88–4.84 (m, 1 H, H-2″), 4.24 (d, $J = 13.8$ Hz, 1 H, SCH), 4.19 (d, $J = 13.8$ Hz, 1 H, SCH), 4.11–4.06 (m, 1 H, H-3″), 3.81–3.78 (m, 1 H, H-4″), 3.63–3.57 (m, 1 H, H-5″), 3.50–3.43 (m, 1 H, H-5″), 2.33 (s, 3 H, $ArCH_3$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 160.31 (C═O), 155.64, 153.25, 152.53, 151.08, 141.71, 140.95, 134.00, 132.61, 127.96, 124.08, 118.61, 117.38, 115.87, 88.29, 85.77, 70.60, 70.58, 62.03, 32.65 (SCH_2), 20.23 ($ArCH_3$); IR (ATR) 3301 (br, OH), 2924 (w), 1720 (m), 1644 (s), 1566 (m), 1081 (s) cm^{-1}; MS (FAB$^+$) *m/z* 487 (M^+, 1), 185 (9), 97 (31), 55 (100); HRMS (FAB) Anal. Calcd. for $(C_{21}H_{21}N_5O_7S)^+$: 487.1162; Found 487.1164.

8-[(8′-Methoxycoumarin-3′-yl)methylthio]guanosine (11e). Standard procedure 2 was followed by use of **9** (63.7 mg, 0.202 mmol, 1.0 equiv.) and 3-chloromethyl-8-methoxycoumarin (**10e**, 54.0 mg, 0.243 mmol, 1.2 equiv.) was added. After the solution was stirred at room temperature for 1.0 h and then worked up, the residue was purified by use of column chromatography (10% methanol in CH_2Cl_2 as the eluant) to give **11e** (84.3 mg, 0.167 mmol) in 83% yield as white solids: ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.66 (br, 1 H, NH), 7.93 (s, 1 H, CH═C─COO), 7.28–7.23 (m, 2 H, 2 × ArH), 7.16–7.14 (m, 1 H, ArH), 6.42 (s, 2 H, NH_2), 5.69 (d, $J = 6.8$ Hz, 1 H, H-1″), 5.38 (d, $J = 6.4$ Hz, 1 H, OH), 5.09 (d, $J = 5.2$ Hz, 1 H, OH), 4.95 (dd, $J = 6.0$, 5.6 Hz, 1 H, OH), 4.88–4.84 (m, 1 H, H-2″), 4.24 (d, $J = 13.8$ Hz, 1 H, SCH), 4.19 (d, $J = 13.8$ Hz, 1 H, SCH), 4.09–4.05 (m, 1 H, H-3″), 3.89 (s, 3 H, OCH_3), 3.80–3.77 (m, 1 H, H-4″), 3.61–3.56 (m, 1 H, H-5″), 3.49–3.43 (m, 1 H, H-5″); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 159.87 (C═O), 155.68, 153.14, 152.55, 146.41, 142.25, 141.73, 141.24, 124.66, 124.34, 119.47, 119.41, 117.38, 114.05, 88.26, 85.78, 70.55, 70.52, 62.01, 56.16 (OCH_3), 32.63 (SCH_2); IR (ATR) 3142 (br, OH), 2923 (w), 1714 (m), 1682 (s), 1274 (m), 1092 (m) cm^{-1}; MS (FAB$^+$) *m/z* 503 (M^+, 2), 372 (10), 232 (11), 154 (45), 79 (100); HRMS (FAB) Anal. Calcd. for $(C_{21}H_{21}N_5O_8S)^+$: 503.1107; Found 503.1111.

CONCLUSIONS

New substituted heterobicycle–coumarin conjugates were synthesized by chemical methods and their biological activities were evaluated by the HCV subgenomic replicon system in Huh 5–2 cells. Through analysis of their anti-HCV data, structure–activity relationships including three guidelines were illustrated. The most important finding is that the lack of the sulfur atom–containing linker in these conjugated compounds would lead to poor biological activities. These new findings provide a rational approach for development of new anti-HCV drugs in the future.

REFERENCES

1. De Clercq, E. *Nat. Rev. Drug Discovery* **2007,** *6*, 1001–1018.
2. Moradpour, D.; Penin, F.; Rice, C. M. *Nat. Rev. Microb.* **2007,** *5*, 453–463.

3. Tramontano, E. *Mini-Rev. Med. Chem.* **2008,** *8*, 1298–1310.
4. Iadonato, S. P.; Katze, M. G. *Nature* **2009,** *461*, 357–358.
5. Hirashima, S.; Suzuki, T.; Ishida, T.; Noji, S.; Yata, S.; Ando, I.; Komatsu, M.; Ikeda, S.; Hashimoto, H. *J. Med. Chem.* **2006,** *49*, 4721–4736.
6. Goulet, S.; Poupart, M.-A.; Gillard, J.; Poirier, M.; Kukolj, G.; Beaulieu, P. L. *Bioorg. Med. Chem. Lett.* **2010,** *20*, 196–200.
7. Stankiewicz-Drogoń, A.; Dörner, B.; Erker, T.; Boguszewsk-Chachulska, A. M. *J. Med. Chem.* **2010,** *53*, 3117–3126.
8. McCauley, J. A.; McIntyre, C. J.; Rudd, M. T.; Nguyen, K. T.; Romano, J. J.; Butcher, J. W.; Gilbert, K. F.; Bush, K. J.; Holloway, M. K.; Swestock, J.; Wan, B.-L.; Carroll, S. S.; DiMuzio, J. M.; Graham, D. J.; Ludmerer, S. W.; Mao, S.-S.; Stahlhut, M. W.; Fandozzi, C. M.; Trainor, N.; Olsen, D. B.; Vacca, J. P.; Liverton, N. J. *J. Med. Chem.* **2010,** *53*, 2443–2463.
9. Velázquez, F.; Sannigrahi, M.; Bennett, F.; Lovey, R. G.; Arasappan, A.; Bogen, S.; Nair, L.; Venkatraman, S.; Blackman, M.; Hendrata, S.; Huang, Y.; Huelgas, R.; Pinto, P.; Cheng, K.-C.; Tong, X.; McPhail, A. T.; Njoroge, F. G. *J. Med. Chem.* **2010,** *53*, 3075–3085.
10. Hwu, J. R.; Singha, R.; Hong, S. C.; Chang, Y. H.; Das, A. R.; Vliegen, I.; De Clercq, E.; Neyts, J. *Antivir. Res.* **2008,** *77*, 157–162.
11. Neyts, J.; De Clercq, E.; Singha, R.; Chang, Y. H.; Das, A. R.; Chakraborty, S. K.; Hong, S. C.; Tsay, S.-C.; Hsu, M.-H.; Hwu, J. R. *J. Med. Chem.* **2009,** *52*, 1486–1490.
12. Kirkiacharian, B. S.; Danan, A. *Synthesis* **1986,** 383–385.
13. For the recent reports on the guanosine derivatives that possess anti-HCV activity, see: (a) Chung, D.-H.; Strouse, J. J.; Sun, Y.; Arterburn, J. B.; Parker, W. B.; Jobsson, C. B. *Antivir. Res.* **2009,** *83*, 80–85; (b) Narayanasamy, J.; Pullagurla, M. R.; Sharon, A.; Wang, J.; Schinazi, R. F.; Chu, C. K. *Antivir. Res.* **2007,** *75*, 198–209; (c) Seio, K.; Sasaki, T.; Yanagida, K.; Baba, M.; Sekine, M. *J. Med. Chem.* **2004,** *47*, 5265–5275; (d) McGuigan, C.; Gilles, A.; Madela, K.; Aljarah, M.; Holl, S.; Jones, S.; Vernachio, J.; Hutchins, J.; Ames, B.; Bryant, K. D.; Gorovits, E.; Ganguly, B.; Hunley, D.; Hall, A.; Kolykhalov, A.; Liu, Y.; Muhammad, J.; Raja, N.; Walters, R.; Wang, J.; Chamberlain, S.; Henson, G. *J. Med. Chem.* **2010,** *53*, 4949–4957.
14. Lin, T.-S.; Cheng, J.-C.; Ishiguro, K.; Sartorelli, A. C. *J. Med. Chem.* **1985,** *28*, 1194–1198.
15. Kaye, P. T.; Musa, M. A.; Nocanda, X. W. *Synthesis* **2003**, 531–534.
16. Silverstein, R. M.; Webster, F. X.; Kiemle, D. J. *Spectrometric Identification of Organic Compounds*, 7th ed.; John Wiley & Sons: New York, 2005; p. 98.
17. Lohmann, V.; Korner, F.; Koch, J.; Herian, U.; Theilmann, L.; Bartenschlager, R. *Science* **1999,** *285*, 110–113.
18. Paeshuyse, J.; Kaul, A.; De Clercq, E.; Rosenwirth, B.; Dumont, J.-M.; Scalfaro, P.; Bartenschlager, R.; Neyts, J. *Hepatology* **2006,** *43*, 761–770.