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Review Article

New Conjugated Compounds Coming On Stream against Hepatitis C Virus

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Abstract

New conjugated compounds containing the coumarin moiety attached to mono- or bis-heterocyles have been synthesized. In >100 new conjugates of five categories, some of them exhibit significant and appealing activity against Hepatitis C Virus (HCV). The heterocycles therein include adenine, benzimidazole, benzothiazole, benzoxazole, guanine, hypoxanthine, imidazole, imidazopyridine, and purine. Use of the thiomethylene (–SCH_2–) linker to connect a coumarin moiety and a purine or imidazole nucleus leads to the conjugates with greater activity and selectivity than others. Various substituents, including CH_3, F, Cl, Br, OCH_3, OAc, CO_2H, COPh, NO_2, β-D-glucose, and β-D-ribofuranose, are also attached to the core nuclei. Incorporation of a halogen substituent (particularly the Br) onto the coumarin nucleus generally enhances the anti-HCV activity from double-digit to single-digit of μM potency. The structure-activity relationship is established, which is of value to the development of new anti-HCV drugs.

Introduction

Hepatitis C Virus (HCV), belonging to the family Flaviviradae, is a small enveloped (+)-RNA virus. About 150 million individuals have been infected with chronic HCV and about 4 million new cases occur annually [1]. The traditional treatment involves the use of interferon α-2 alone or its combination with Ribavirin [2,3]. In 2011, two HCV protease inhibitors (i.e., boceprevir and telaprevir) were first approved for the treatment of HCV genotype 1 in combination with peg-interferon α and ribavirin. Though they can significantly increase the overall cure rates [4,5], some substantial adverse effects still exist [6]. Lately, Harvoni™ (ledipasvir/sofosbuvir), simeprevir, sofosbuvir, daclatasvir, Viekira Pak™ (ombitasvir/paritaprevir/ritonavirtablet; dasabuvir tablet), and Zepatier™ (elbasvir/grazoprevir) were approved by the U.S. Food and Drug Administration [7-10]. These new drugs, however, are highly costly for the intervention of HCV infections [11]. Thus the development and syntheses of new chemical entities with safer and lower price than the existing drugs to combat the HCV disease is at current need.

Discussion

Since 2008, Hwu, Neyts, Tsay, *et al.* have progressively published a series of articles [12-17] related to heterocycle–coumarin conjugates as new agents against HCV. There are over 100 conjugates designed and synthesized, which can be divided into five categories as shown in Figure 1.

To bring up the conjugates, a direct linkage called "hinged" (i.e., Group 1) and a newly invented thiomethylene (–SCH_2–) linker (i.e., Groups 2–5) between the heterocycle and coumarin nuclei are adopted. The heterocyclic rings therein include benzimidazole (**1** and **2**), benzoxazole (**3**, X = O), benzothiazole (**3**, X = S), imidazopyridine (**4**, X = CH, Y = Z = H), purine (**4**, X = N, Y = Z = H), adenine (**4**, X = N, Y = NH_2, Z = H), hypoxanthine (**4**, X = N, Y = OH, Z = H), guanine (**4**, X = N, Y = OH, Z = NH_2), and imidazole **(5).** Moreover, different substituents, such as CH_3, halogen, OCH_3, OAc, CO_2H, COPh, NO_2, β-D-glucose, and β-D-ribofuranose, are attached to the core nuclei. On the basis of their molecular frameworks and anti-viral activities, conclusive structure–activity relationship has been established for the betterment of heterocycle–coumarin type conjugates as anti-HCV therapeutics. The valuable information will contribute to the development of new drugs for a large number of patients.

Out of these ~100 compounds in five compound libraries, the conjugates with SI values >10 are picked up to list in Table in comparison with their parent conjugates and corresponding nucleoside derivatives. In Group 1, the conjugated compound having $R^1 = R^2 = CH_3$, $R^3 = R^4 = R^5 = R^6 = R^7 = H$ exhibits the most prominent anti-viral activity (EC_{50}= 3.0 μM) with a relatively poor SI value of 7.9. Addition of a substituent such as CH_3 or Br into the coumarin nucleus or introduction of a β-D-ribofuranose moiety to the benzimidazole ring does not improve their anti-HCV profile. Thus none of the hinged conjugates **1** is enrolled into the campaign for future development. Replacement of the hinged joint with a –SCH_2– linker to form conjugated compounds in Group 2 can significantly enhance the SI values. Successful examples include conjugates **2b–e** with EC_{50}= 2.3~4.1 μM and

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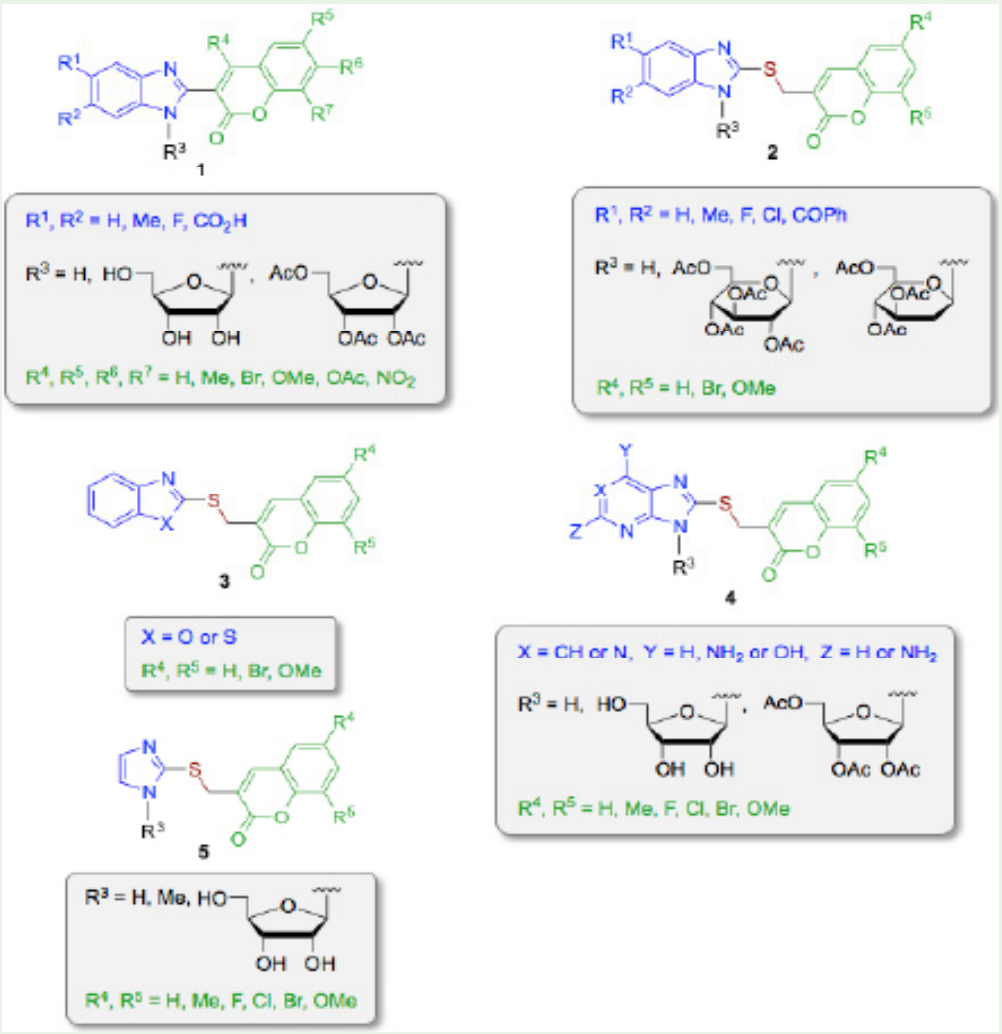

Figure 1: General structure of five categories of heterocycle–coumarin conjugates.

SI = 10~19, which display greater activity and selectivity than their parent compound **2a**. Introduction of a peracetyl β-D-glucose moiety onto the benzimidazole nucleus to give the resultant glycoside-type conjugates maintains the same order of activity and selectivity as their kin (**2b** versus **2d** and **2c** versus **2e**).

For investigation of the influence resulting from heteroatoms in a five-membered nucleus (i.e., the imidazole part), one nitrogen atom in benzimidazole nucleus is replaced with an oxygen or a sulfur atom. The resultant benzoxazole–containing conjugate **3b** possesses an appealing selectivity index value (SI =11) by addition of a Br substituent onto the coumarin nucleus of parent conjugate **3a**. The enhancement of the SI value comes from the reduction of cytotoxicity of the compound. Furthermore, one or two carbon atoms in the aromatic ring of benzimidazole nucleus are replaced with one or two nitrogen atoms to yield imidazopyridine–containing conjugates (**4a** and **4b**) as well as purine–containing conjugate (**4c**), respectively. The conjugated compound with the purine nucleus (**4c**, EC_{50} = 2.0 μM, SI = 54) has a much higher selectivity than those with a benzimidazole, benzoxazole, benzothiazole, or imidazopyridine nucleus due to lower cytotoxicity. On the other hand, addition of a Br substituent onto the

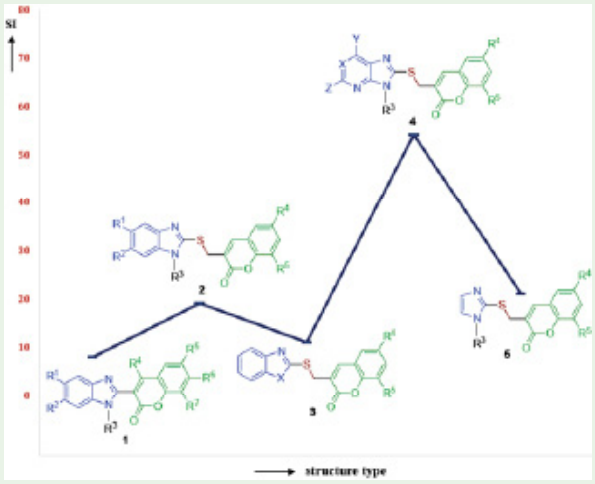

Figure 2: Relative relationship between heterocycle–coumarin conjugates **1**–**5** and their associated selectivity index values (SI = selectivity index, ratio of CC_{50} to EC_{50}).

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Table 1: Antiviral effect of conjugated compounds on HCV 1b subgenomic replicon replication in Huh 5-2 cells.

Compound	R^1	R^2	R^3	R^4	R^5	X	Y	Z	CC_{50}[a] (μM)	EC_{50}[b](μM)	SI[c]
2a	H	H	H	H	H	–	–	–	90	27	3.4
2b	H	H	H	Br	H	–	–	–	42	4	10
2c	Cl	Cl	H	Br	H	–	–	–	44	2.3	19
2d	H	H	peracetyl β-D-glucose	Br	H	–	–	–	43	4.1	10
2e	Cl	Cl	peracetyl β-D-glucose	Br	H	–	–	–	>64.8	4.1	>16
3a	H	H	–	H	H	O	–	–	45	12	3.9
3b	H	H	–	Br	H	O	–	–	131	12	11
4a	–	–	H	H	H	CH	H	H	95	59	1.6
4b	–	–	H	Br	H	CH	H	H	128	6.8	19
4c	–	–	H	H	H	N	H	H	109	2.0	54
4d	–	–	β-D-ribofuranose	H	H	N	H	H	50	5.5	9.1
4e	–	–	β-D-ribofuranose	H	H	N	NH_2	H	49	28	1.7
4f	–	–	β-D-ribofuranose	H	H	N	OH	H	109	61	1.8
5a	–	–	H	H	H	–	–	–	122	30	4.1
5b	–	–	H	F	H	–	–	–	83	7.2	12
5c	–	–	H	Br	H	–	–	–	75	5.1	15
5d	–	–	H	H	OCH_3	–	–	–	173	8.4	21
5e	–	–	β-D-ribofuranose	H	H	–	–	–	128	59	2.2

[a]The concentration of a compound with an adverse effect of 50% was observed on the host cell metabolism, as determined by the MTS method. [b]The concentration of a compound at which virus replication was inhibited by 50% was observed, as determined by real-time quantitative RT–PCR. [c]Selectivity index (ratio of CC_{50} to EC_{50}).

coumarin nucleus of parent imidazopyridine–containing conjugate **4a** gives the conjugate **4b**, which gains significant increment of anti-HCV activity with EC_{50} = 6.8 μM and SI = 19. In contrast, incorporation of a β-D-ribofuranose moiety onto the purine nucleus reduces the selectivity (i.e., **4d**, EC_{50} = 5.5 μM, SI = 9.1). It is due to the increment of cytotoxicity. Furthermore, introduction of an NH_2 or OH substituent onto the purine nucleus to form adenosine–, inosine–, or guanosine–coumarin conjugates (e.g., **4e** and **4f**) does not increase their SI values owing to the lower potency they exhibit.

Finally, the heterobicycle nucleus of conjugates is simplified to monocyclic nucleus by elimination of the aromatic ring. The resultant imidazole–coumarin conjugates **5b–d** with EC_{50} = 5.1~8.4 μM and SI = 12~21 can acquire increasing anti-viral activity and selectivity through incorporation of an F, Br, or OCH_3 substituent onto the parent conjugate **5a.** Nevertheless, modification of the imidazole nucleus with a β-D-ribofuranose moiety cannot better the anti-HCV potency (cf. **5e**).

The conjugated compounds in this report primarily involve three elements: linkers, coumarin, and heterocycles. Through systematic modifications and derivatization of these key elements, the heterocycle–coumarin conjugates with the optimized selectivity for every category are obtained. Accordingly, their representative scaffold versus SI value is plotted in Figure 2. The purine–coumarin conjugates **4** have a higher position than other conjugates and could be held an ideal scaffold in the development of anti-HCV therapeutics. Furthermore, we draw the following structure–activity relationship on the basis of their EC_{50}, CC_{50}, and SI values.

1. Adoption of the thiomethylene linker (–SCH_2–) as a joint to construct heterocycle–coumarin conjugates is a better choice than the hinged connection.
2. Incorporation of a halogen substituent (particularly the Br atom) onto the coumarin nucleus generally enhances the anti-HCV activity from double-digit to single-digit of μM potency.
3. Enhancement of the selectivity index values resulting from the heterocycle nucleus follows the order: purine > imidazole > benzimidazole, imidazopyridine, benzoxazole > adenine, hypoxanthine, guanine, benzothiazole.
4. Replacement of the hydrogen atom in the heterocyclic ring with a β-D-glucose or β-D-ribofuranose displays a slim or no effect on the HCV inhibition.

Conclusion

Systematic investigation of the structure–activity relationship on various heterocycle–coumarin conjugates against HCV is illustrated. In these compound libraries, conjugates bearing a simple purine or imidazole nucleus exhibit better anti-HCV activity and selectivity than their derivatives. Moreover, a –SCH_2– linker used as the joint between the heterocycle and coumarin nuclei is crucial to the design. Use of these findings leads a legitimate way to optimize the anti-HCV profile of these conjugated compounds.

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