Bioorganic & Medicinal Chemistry Letters 23 (2013) 2679–2682

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Evaluation of anti-HCV activity and SAR study of (+)-lycoricidine through targeting of host heat-stress cognate 70 (Hsc70)

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ARTICLE INFO

Article history:
Received 2 December 2012
Revised 15 February 2013
Accepted 20 February 2013
Available online 1 March 2013

Keywords:
(+)-Lycoricidine
Heat-stress cognate 70 (Hsc70)
Down-regulators
Structure–activity relationship (SAR)
Anti-HCV activity

ABSTRACT

The anti hepatitis C virus (HCV) activity of (+)-lycoricidine (**1**) was evaluated for the first time in this letter, yielding an EC_{50} value of 0.55 nmol/mL and an selection index (SI) value of 12.72. Further studies indicated that **1** induced this effect by down-regulating host heat-stress cognate 70 (Hsc70) expression. In addition, 20 derivatives were designed and synthesised to investigate the basic structure–activity relationship (SAR) of the title compound. Several of these derivatives exhibit a good inhibition of HCV, such as compound **3** (EC_{50} = 0.68 nmol/mL, SI = 33.86), compound **2d** (EC_{50} = 15 nmol/mL, SI = 12) and compound **5** (EC_{50} = 33 nmol/mL, SI >10.91). Meanwhile, the experimental data suggest that the modification of certain groups of (+)-lycoricidine can reduce the cytotoxicity of the compounds.

There are 150 million people infected with the hepatitis C virus (HCV) worldwide, and more than 80% of them will progress into chronic hepatitis.[1–3] At present, typical treatment for hepatitis C focuses on the inhibition of NS protease,[4–7] which involves PEGylated interferon-α (IFN-α) plus the nucleoside analogue ribavirin. However, this treatment also carries side effects and yields a poor sustained virological response (SVR).[8–10] Therefore, the exploration of new drugs, especially ones that involve new mechanisms by which to inhibit HCV proliferation, strongly attracts many researchers.

Previous studies implied that human heat shock cognate 70 (Hsc70, or heat shock protein A8), a member of the heat shock protein 70 (Hsp70) family, could be a new target to inhibit HCV. Hsc70 is a cytoplasmic adenosine triphosphate (ATP)-binding protein with 646 amino acids.[11–13] A study by Parent et al. demonstrated that host Hsc70 was a part of the HCV particle and that it modulated HCV infectivity, as well as lipid droplet-dependent virus release, after HCV entered host cells.[14] Additionally, we have reported that down-regulation of this protein in HCV-infected host cells can reduce the level of Hsc70 packaging into HCV particles, and therefore inhibit HCV replication in the next infection cycle.[15]

(+)-Lycoricidine (**1**, Fig. 1), a benzylphenethylamine alkaloid mainly isolated from the Amaryllidaceae family, has attracted considerable interest due to its promising cytotoxic activities.[16–21] It is interesting, however, that (+)-lycoricidine and other lycoris alkaloids also exhibit broad-spectrum capabilities against a series of RNA viruses,[22] which suggests that these alkaloids do not directly affect the virus but act on the host cell.

Thus, (+)-lycoricidine and two other lycoris alkaloids, **6** and **7** (Fig. 1), were initially evaluated for both the inhibition of HCV and the expression of host Hsc70 in Huh-7.5 cells. Under treatment with compound **1,** at the concentration of 0.1 μg/mL, Hsc70 expression in host cells was reduced by 28%. Simultaneously, the replication of HCV decreased dramatically to 6%. The correlation between Hsc70 expression and HCV replication was clear from western blot analysis (Fig. 2). Furthermore, **1** also exhibited an inhibitory activity on Hsc70 mRNA expression with 91.4% inhibition at the concentration of 10 μg/mL and 83.1% inhibition at the concentration of 100 μg/mL. According to the results above, it could be speculated that **1** might inhibit HCV replication by suppressing Hsc70 expression.

(+)-Lycoricidine (**1**) exhibited a strong inhibitory activity against HCV expression, but it exhibited obvious cytotoxicity, as

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http://dx.doi.org/10.1016/j.bmcl.2013.02.089

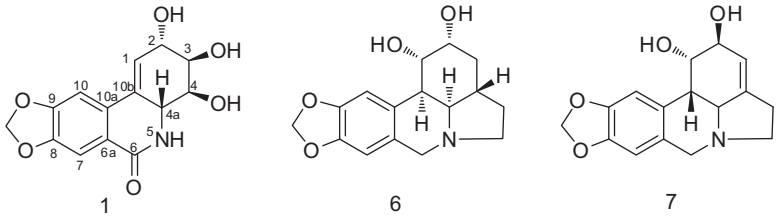
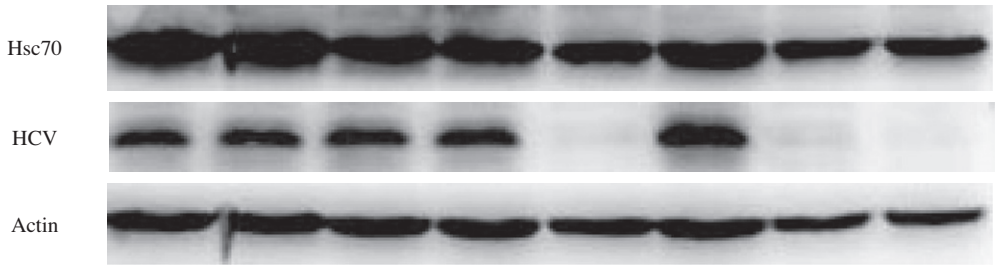


Figure 1. Structures of compounds **1** ((+)-lycoricidine), **6** and **7**.



Comp.	Control	**6** (0.1)	**6** (1.0)	**6** (10)	**6** (100)	**7** (0.1)	**7** (1.0)	**1** (0.1)
Hsc70/actin	1.00	1.01	0.68	0.60	0.67	0.78	0.79	0.72
HCV/actin	1.00	1.20	1.16	1.22	0.10	1.86	0.20	0.06
								(μg /mL)

Figure 2. Intracellular Hsc70 protein concentration decreased dose-dependently in Huh-7.5 cells either untreated or treated with **1** (0.1 μg/mL), **6** (0.1, 1.0, 10, 100 μg/mL) and **7** (0.1, 1.0 μg/mL) for 24 h.

well. Therefore, further exploration of this compound's active sites was necessary and valuable. As shown in Fig. 3, the structure modifications of **1** were initiated with the transformation of substituents at the 2-, 3- and 4-positions, as well as the double bond between the 1- and 10b-positions. Compounds **2**–**5** were designed and synthesised as four key intermediates (Fig. 3) and then modified these four compounds to afford other 16 derivatives (Fig. 4). All these 20 compounds were evaluated for their anti-HCV activities (Table 1).

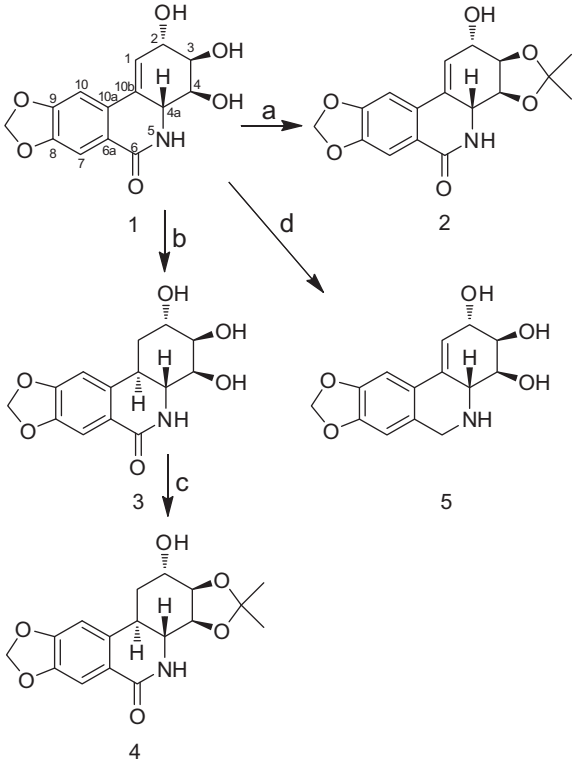


Figure 3. Synthesis of the intermediates **2**–**5**. Reagents and conditions: (a) 2,2-dimethoxypropane, *p*-TSA, DMF, 12 h, rt, 85%; (b) 10% Pb–C, H_2, Cl_2CH_2/CH_3OH, 24 h, rt, 82%; (c) 2,2-dimethoxypropane, *p*-TSA, DMF, 20 h, rt, 90%. (d) $LiAlH_4$, THF, −10 °C, 4 h, 45%.

The SAR analysis first concentrated on the double bond between the 1- and 10b-positions, which contributes to a large conjugated system within **1**. Hydrogenation of this double bond would notably affect the electronic cloud distribution within the compound and would have an impact on the bioactivities. The comparison of **3** with **1** indicated that the compound without the double bond exhibited higher CC_{50} and SI values. Specifically, **3** had a similar EC_{50} value (0.68 nmol/mL) to that of **1,** but its CC_{50} value (23 nmol/mL) was significantly (threefold) greater than that of **1**. Similar results can also be observed in the comparisons of **2** with **4**, **2c**–**d** with **4a**–**b** and **3a**–**c** with **1b**–**d**, respectively. Therefore, it can be deduced that the double bond between the 1- and 10b-positions is crucial to the anti-HCV activity: reduction of this bond might reduce the cytotoxicity of the compounds and improve their SI values.

Ether linkage of propylidene was achieved on the hydroxyl groups at the 3- and 4-positions of **1**. Such conversion may influence the formation of hydrogen bonds between the compound and its target protein. These results showed that the CC_{50} of **2** was much higher than that of **1**, that **4** exhibited a lower cytotoxicity than **3** and that both **2** and **4** lost their inhibitory activities to a certain extent. These results suggested that the ether linkage at the 3-, 4-position affected both the cytotoxicity and the anti-HCV activity but was most pronounced in its effects by increasing the CC_{50} for the title compound.

When the hydroxyl groups of the 3- and 4-positions were replaced by propylidene, the substitution at the 2-position influenced the bioactivities of the compounds. Comparing **2** with its

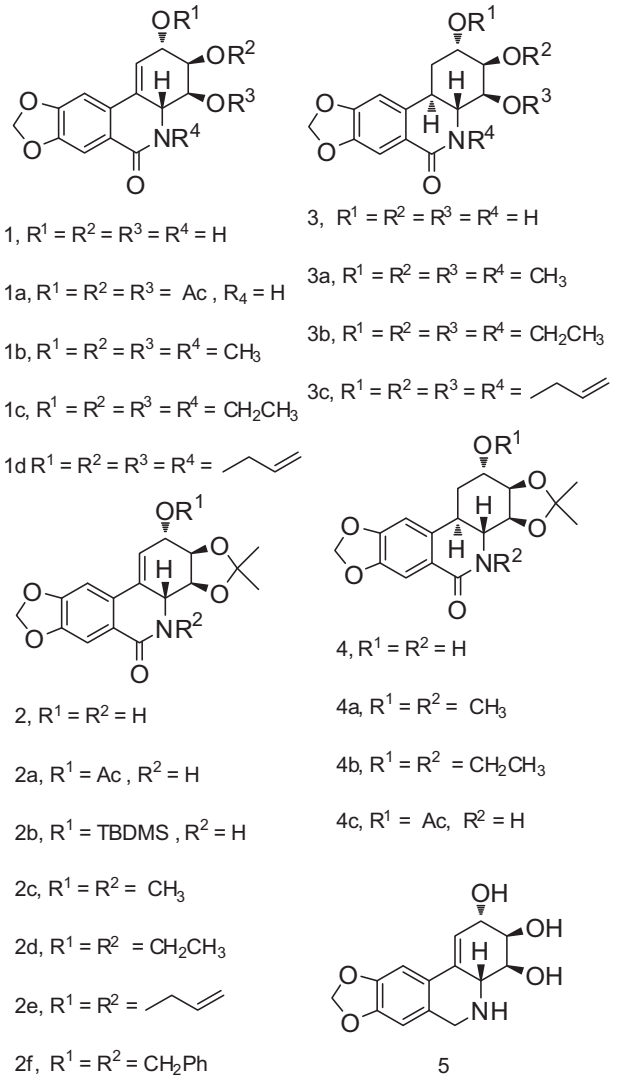


Figure 4. Structures of (+)-lycoricidine derivatives.

Table 1
Inhibition of HCV (+)-lycoricidine derivatives

Compd.	HCV		
	CC_{50} (nmol/mL)	EC_{50} (nmol/mL)	SI
1	7.2 ± 0.48	0.55 ± 0.07	12.72
1a	33 ± 3.36	>0.98	<33.8
1b	180 ± 9.34	22 ± 1.79	8.2
1c	252 ± 5.59	>83	<3.01
1d	120 ± 9.98	74 ± 7.91	1.62
2	170 ± 10.16	11 ± 0.85	9.09
2a	>300	63 ± 3.51	>4.76
2b	160 ± 10.49	75 ± 5.41	2.13
2c	280 ± 12.36	93 ± 6.88	3.01
2d	180 ± 10.95	15 ± 1.05	12.00
2e	250 ± 6.43	40 ± 4.82	6.25
2f	200 ± 9.94	65 ± 4.77	3.08
3	23 ± 1.46	0.68 ± 0.07	33.82
3a	>300	54 ± 4.34	>5.56
3b	>300	43 ± 2.30	>6.98
3c	38 ± 1.89	12 ± 0.64	3.17
4	280 ± 9.28	>11	< 25.46
4a	250 ± 14.55	92 ± 3.85	2.17
4b	260 ± 13.74	85 ± 3.85	3.06
4c	270 ± 15.27	89 ± 4.04	3.03
5	>360	33 ± 4.47	>10.91
Intron A	> 6000 (U/mL)	0.250 (U/mL)	>24000

derivatives (**2a**, **2b**), in which the 2-hydroxyl was acetylated (**2a**) or silylated (**2b**), revealed that the EC_{50} values increased significantly from 11 nmol/mL to 63 nmol/mL (**2a**) and 75 nmol/mL (**2b**), respectively. Coincidentally, compound **4c**, in which the 2-position was also acetylated, exhibited a much weaker anti-HCV activity than that of **4**. In this sense, a hydroxyl group at the 2-position may play an important role in inhibiting HCV replication.

The hydroxyl groups at the 2-, 3-, and 4-positions, as well as the amide group at the 5-position, each could form hydrogen bonds with target proteins. Acylation or etherification of these groups would significantly affect the cytotoxicity of the compounds. The respective comparisons of 1 with **1a**–**d** and 3 with **3a**–**c,** indicated that **1** and **3** exhibited much higher cytotoxicities. Additionally, the analogue modifications decreased the ability to inhibit the replication of HCV to varying degrees, which suggested that these modifications influence not only the cytotoxicity but also the anti-HCV activity. It was notable that compound **2d** had a good inhibitory

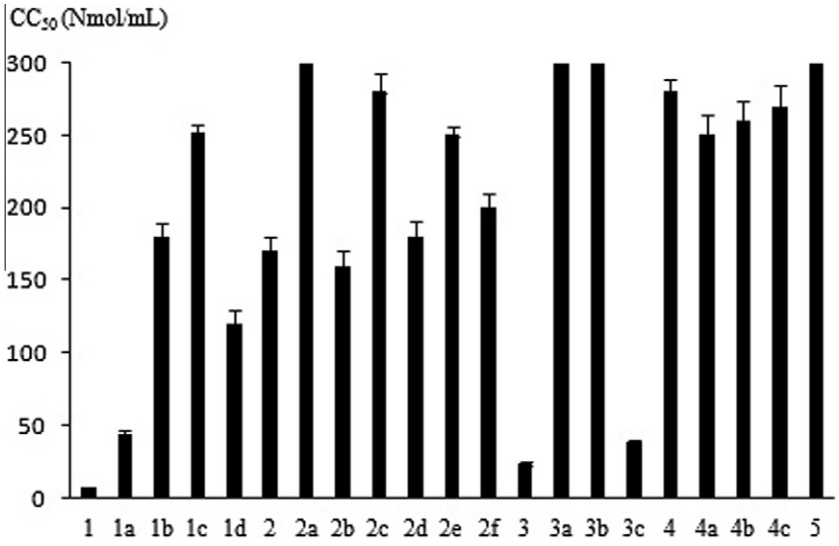


Figure 5. The cytotoxicity of (+)-lycoricidine derivatives. The CC_{50} values of **2a**, **3a**, **3b** and **5** were greater than 300.

activity, with an EC_{50} value of 15 nmol/mL and an SI value of 12.0; thus, we deduced that the ethyl substituents at the 2- and 5-positions with propylidene at the 3-, 4-position might together improve anti-HCV activity of compounds.

The electron-withdrawing carbonyl group of the 6-position was crucial to the bioactivities of (+)-lycoricidine, as it can connect to peptide chains through H-bonds and can contribute to the conjugated system of **1**. Furthermore, the reduction derivative **5** also showed a higher CC_{50} value, which suggested that the amide group might be another functional group that can affect the cytotoxicity of (+)-lycoricidine.

This study was the first evaluation of the anti-HCV activity of (+)-lycoricidine (**1**) and its derivatives. The results indicated that compound **1** inhibited the replication of HCV in HCV-infected Huh-7.5 cells by suppressing host Hsc70 expression, which was different from the mechanism of nucleosides. Based on this finding, 20 (+)-lycoricidine derivatives were further synthesised to evaluate their anti-HCV activities. The SAR analysis revealed that the conjugated system of **1** was crucial to its bioactivities. Reduction of the double bond between the 1- and 10b-positions, or of the carbonyl group at the 6-position, may reduce the cytotoxicity and improve the SI of the compound. Additionally, acyl or ether substitutions at the 2-, 3-, 4- and 5-positions were introduced to prevent hydrogen bonding with the groups' respective host proteins and were shown to be efficacious, as demonstrated by the increased CC_{50} value of the derivatives (Fig. 5). However, these modifications also reduced the inhibitory activity of the derivatives. Furthermore, it should be noted that compound **2d** exhibited relatively good anti-HCV activity, which suggested that certain ether substituents at 2-, 3-, 4- and 5-positions may improve the SI values of compounds.

Acknowledgments

This research was supported financially by the National Natural Science Foundation of China (30830114 and 31270404) and the Ministry of Science and Technology (2009CB522300).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.02.089.

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