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Structural development studies of anti-hepatitis C virus agents with a phenanthridinone skeleton

Masahiko Nakamura [a], Atsushi Aoyama [a], Mohammed T. A. Salim [b], Mika Okamoto [b], Masanori Baba [b,*], Hiroyuki Miyachi [c], Yuichi Hashimoto [a], Hiroshi Aoyama [a,*]

[a] Institute of Molecular & Cellular Biosciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan
[b] Division of Antiviral Chemotherapy, Center for Chronic Viral Diseases, Graduate School of Medical and Dental Sciences, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8544, Japan
[c] Division of Pharmaceutical Sciences, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 1-1-1 Tsushima-Naka, Kita-ku, Okayama 700-8530, Japan

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ABSTRACT

A phenanthridinone skeleton was derived from our previous researches on thalidomide and retinoids as a multi-template for generation of anti-viral lead compounds. Structural development studies focusing on anti-hepatitis C virus activity afforded 5-butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenanthridin-6(5*H*)-one (**10**) and 5-butylbenzo[*b*]phenanthridin-6(5*H*)-one (**39**), which showed EC_{50} values of approximately 3.7 and 3.2 μM, respectively.

1. Introduction

The efficient identification of small-molecular scaffolds for the development of biologically active compounds is very important in chemical genetics and medicinal chemistry. As one approach, we have been utilizing the multi-template hypothesis,[1–5] based on the idea that the number of protein fold structure types that comprise all the domains occurring in natural proteins is quite limited, in spite of the huge number of natural proteins.[6–8] A given fold structure might be characteristic of many natural proteins, and therefore, ignoring physical/chemical interactions, one might expect that a template/scaffold structure which is spatially complementary to one fold structure might serve as a multi-template for structural development of ligands that would interact specifically with many different natural proteins. As candidate multi-template structures, we have focused particularly on thalidomide (**1**) and retinoids, including synthetic retinoids Am80 (**2**) and Am580 (**3**) (Fig. 1).[1,9–13] All of these compounds **1**–**3** elicit a wide range of biological activities, and thalidomide (**1**) is well-established to be multi-target drug.[1,9–13] In fact, we recently applied thalidomide (**1**) and/or Am80 (**2**)/Am580 (**3**) as multi-templates to develop anti-viral agents and/or anti-proliferative agents for virus-infected cells, that is, anti-bovine viral diarrhea virus (anti-BVDV) agents, including SK3M4M5M (**4**) derived from thalidomide (**1**)[14,15] and adult T-cell leukemia (ATL) cell-selective proliferation inhibitors, including TMN($COCH_3$) (**5**) and TMN(OH)($COCH_3$) (**6**) derived from Am80 (**2**)/Am580 (**3**) (Fig. 1).[4] We next aimed to develop anti-hepatitis C virus (HCV) agents.

HCV infection is thought to be a major cause of human hepatitis,[16,17] and it is estimated that at least 170 million people worldwide are chronically infected with this virus.[18] Most infections become persistent and about 60% of cases progress to chronic liver disease, which in turn can lead to development of cirrhosis, hepatocellular carcinoma, and liver failure.[19,20] Currently, no vaccine is available against HCV infection, and the standard treatment for chronic hepatitis C consists of pegylated interferon (IFN)-α in combination with the nucleoside analog ribavirin (1-β-D-ribofuranosyl-1,2,4-triaxole-3-carboxamide). However, the virus cannot be eliminated from approximately half of infected patients treated with these agents.[21] In addition, the side effects of these agents are sometimes serious and unacceptable to patients. Therefore, alternative agents for the treatment and prevention of HCV infection are urgently needed.

As mentioned above, we have been succeeded in the development of potent anti-BVDV agents, including SK3M4M5M (**4**), which

* Corresponding authors. Tel.: +81 3 5841 7848; fax: +81 5841 8495 (H.A.); tel.: +81 99 275 5930; fax: +81 99 275 5932 (M.B.).
E-mail addresses: m-baba@vanilla.ocn.ne.jp (M. Baba), aoyama@iam.u-tokyo.ac.jp (H. Aoyama).

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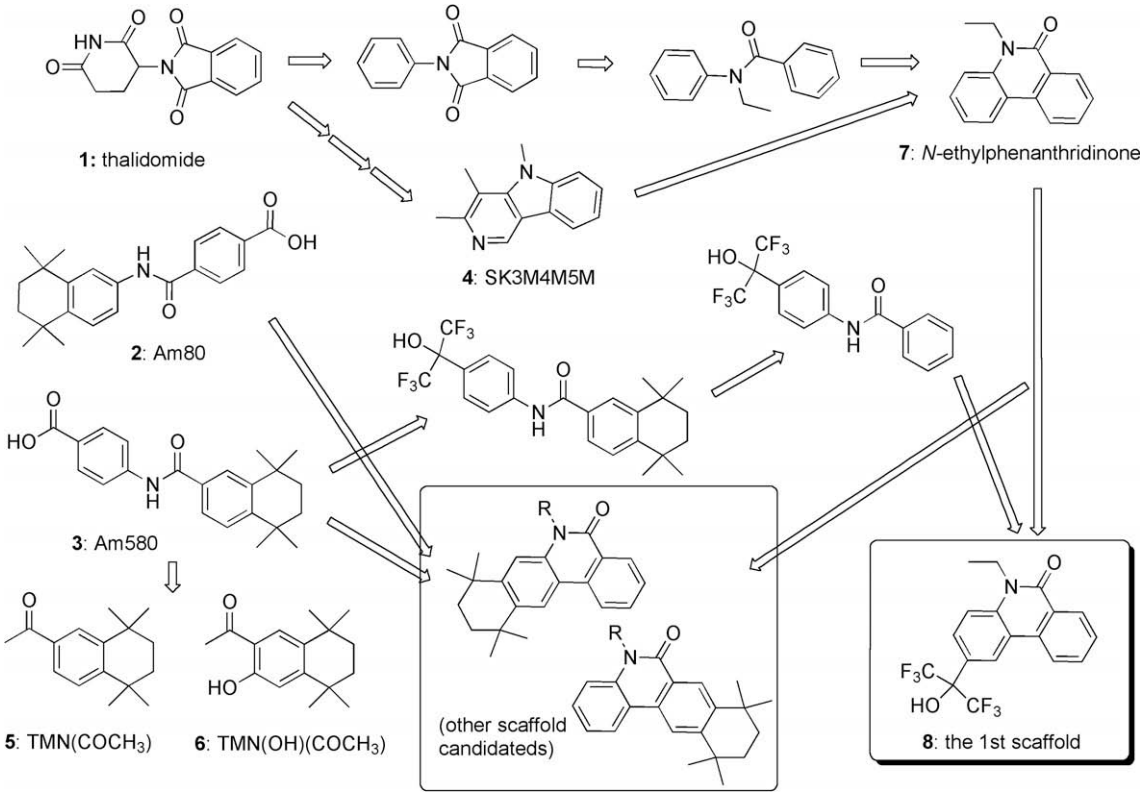


Figure 1. Armchair structural development of thalidomide (**1**) and retinoids (**2**, **3**) to the first scaffold **8**.

have an EC_{50} value of 3.5 nM.[15] Although BVDV belongs to the *Flaviviridae* family, as HCV does,[22] and is thought to be a surrogate model for HCV,[23–25] SK3M4M5M (**4**) showed only a very weak activity against HCV. Therefore, we tried to develop another scaffold for the development of anti-HCV agents, as shown in Figure 1.

2. Results and discussion

2.1. Armchair structural development leading to phenanthridinone derivatives

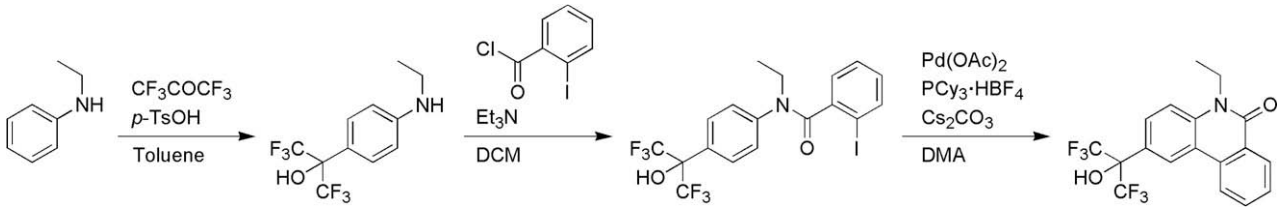
First, we applied armchair structural development to phenylphthalimide, which was itself developed from thalidomide (**1**). The phenylphthalimide skeleton is a superior multi-template, and we have developed various biologically active phenylphthalimide derivatives, including tumor necrosis factor-α production regulators, tubulin polymerization inhibitors, dipeptidylpeptidase type IV inhibitors, liver X receptor antagonists, α-glucosidase inhibitors, and so on.[1,9–12] The armchair ring opening of phenylphthalimide gave *N*-ethylbenzanilide, whose recyclization should afford *N*-ethylphenanthridinone (**7**) (Fig. 1). On the other hand, synthetic retinoids Am80 (**2**) and Am580 (**3**) both possess a benzoic acid moiety. Because our previous studies on anti-BVDV agents suggested that carboxyl acid derivatives are not suitable as lead structures, we considered that a bioisosteric functional group, the 1,1,1,3,3,3-hexafluoropropan-2-ol-substituted phenyl moiety, might be introduced into *N*-ethylphenanthridinone (**7**) to give the first scaffold structure, 5-ethyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl) phenanthridin-6(5*H*)-one (**8**) (Fig. 1). In addition, both Am80 (**2**) and Am580 (**3**) possess a teterahydrotetramethylnaphthalene moiety, which has been established to be a useful core structure to develop ATL cell-selective proliferation inhibitors, including TMN($COCH_3$) (**5**) and TMN(OH)($COCH_3$) (**6**).[4] We therefore considered that docking of teterahydrotetramethylnaphthalene moiety and *N*-ethylphenanthridinone (**7**) would afford other scaffold candidate structures, as shown in Figure 1.

Compound **8** was prepared as shown in Scheme 1.[26,27] Briefly, *N*-ethylaniline was treated with hexafluoroacetone to give the 1,1,1,3,3,3-hexafluoro-2-hydroxypropyl derivative, which was coupled with 2-iodobenzoyl chloride. The resulting anilide was cyclized to give compound **8**. The anti-HCV activity of compound **8** was determined in the established HCV RNA replicon cells.[28] Briefly, NNC #2 cells carrying full-genomic HCV RNA replicons were cultured in the presence of various concentrations of the test compounds for 3 days. The cells were examined for HCV RNA and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) RNA levels by real-time reverse transcription (RT)-PCR. The anti-HCV activity and cytotoxicity of test compounds were expressed as 50% effective concentration (EC_{50}) and 50% cytotoxic concentration (CC_{50}), defined in terms of decrease of HCV RNA and GAPDH RNA levels to 50% of the respective control levels. As shown in Figure 2, compound **8** showed apparent anti-HCV activity (EC_{50} and CC_{50}: 14.1 and >40 μM, respectively), suggesting that compound **8** could be a lead compound for structural development of anti-HCV agents.

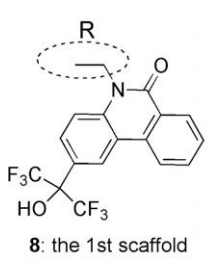
2.2. Effects of N-substituents

Since compound **8** showed anti-HCV activity, we examined the effects of *N*-substituents. Several N-alkylated derivatives of **8** (**9**–**14**) were prepared as shown in Scheme 2, and their anti-HCV activity was measured as described above (Figs. 2 and 3).

As shown in Figures 2 and 3, introduction of three fluorine atoms at the terminal methyl group (**9**) did not improve the activity. However, introduction of a longer-chain alkyl group, *n*-butyl (**10**), *n*-hexyl (**11**), or *n*-nonyl (**12**), resulted in enhancement of anti-HCV activity, though at the same time, the cytotoxicity was increased. The anti-HCV activity of these compounds decreased



Scheme 1.



8: the 1st scaffold

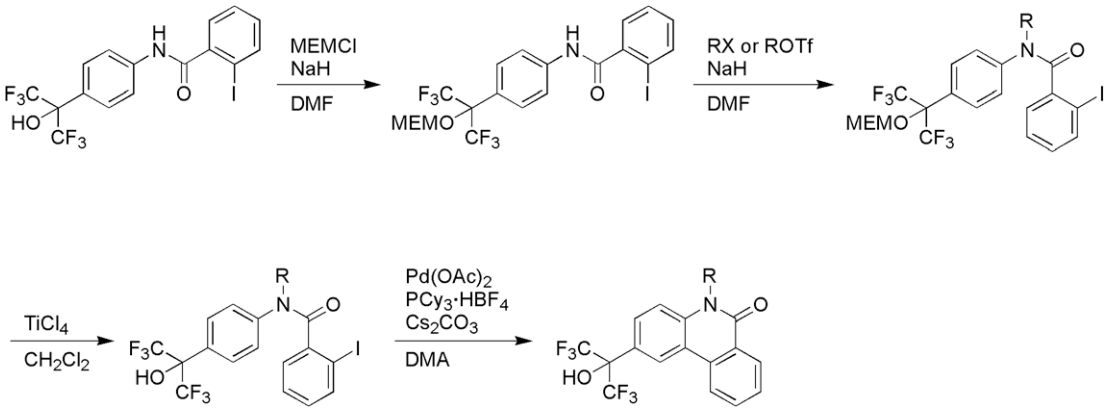
R	EC_{50} (μM)	CC_{50} (μM)
8: CH_3CH_2-	14.1	>40
9: CF_3CH_2-	24.8	30.3
10: $CH_3(CH_2)_3$-	3.7	12.5
11: $CH_3(CH_2)_5$-	4.8	12.2
12: $CH_3(CH_2)_8$-	8.3	10.2
13: $PhCH_2$-	7.7	18.2
14: c-$C_6H_{11}CH_2$-	6.7	14.4

Figure 2. Effects of N-substituents on anti-HCV activity. EC_{50}: 50% effective concentration, based on the decrease of the amount of HCV RNA. CC_{50}: 50% cytotoxic concentration, based on the decrease of the amount of GAPDH RNA.

in the order of: **10** > **11** > **12**. On the other hand, their cytotoxicity tended to decrease in the reverse order, though the differences were small. The benzyl analog (**13**) and the cyclohexylmethyl analog (**14**) showed moderate anti-HCV activity (EC_{50}: 7.7 and 6.7 μM, respectively) with weaker cytotoxicity (CC_{50}: 18.2 and 14.4 μM, respectively). Cytotoxicity was not affected by the length of the introduced alkyl group (CC_{50}: 10.2–12.5 μM). Among this series, compound **10** showed the most potent anti-HCV activity (EC_{50}: 3.7 μM). Therefore, we selected the *N*-butylphenanthridinone skeleton (corresponding to compound **10**) as a scaffold structure for further structural development studies.

2.3. Regioisomers of methyl-substituted *N*-butylphenanthridinone

Based on our previous structure–activity relationship studies of anti-BVDV γ-carboline analogs, which indicated that regio-selective methyl-substitution dramatically influenced the anti-viral activity,[14,15] the effect of methyl-substitution was investigated. For this purpose, we synthesized all the regioisomers of methyl-



Scheme 2.

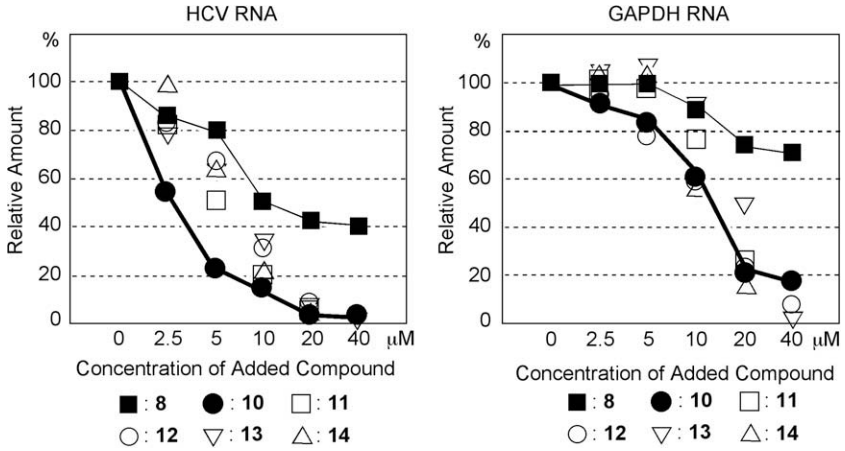


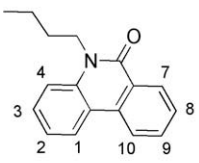
Figure 3. Dose-dependency curves of anti-HCV and cytotoxic activity elicited by compounds **8** and **10**–**14**.

substituted *N*-butylphenanthridinone (Fig. 4) by the method shown in Scheme 3.

As shown in Figure 4, *N*-butylphenanthridinone itself (an extracted structure from compound **10**) was completely inactive. However, methyl-substitution at position 2, that is, the position at which the 1,1,1,3,3,3-hexafluoropropan-2-ol group was attached in compound **10**, resulted in the appearance of anti-HCV activity (EC_{50}: 42.0 μM). All the other regioisomers, except the 8-methyl analog (**21**), that is, compounds **16**, **18**–**20**, **22**, and **23**, were inactive, and the anti-HCV activity elicited by **21** was very weak. Therefore, position 2 seems to be the best position at which to introduce a substituent.

2.4. Effect of 2-subsituents on anti-HCV activity

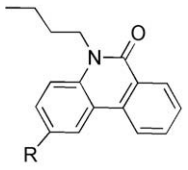
The results described above prompted us to examine the effect of 2-substitutents, and we prepared various derivatives (**24**–**33**) as shown in Figure 5. Although some derivatives, including compounds **28**–**33**, showed improved anti-HCV activity compared to the 2-methyl analog **17**, their activity was weaker than that of **10**. As already mentioned, the unsubstituted analog **15** was inactive. Its fluoro analog **24** was also inactive, suggesting that a mono-atomic substituent is inappropriate to improve the activity. Though the 2-methyl analog **17** showed some activity (EC_{50}: 35.8 μM), its trifluoromethyl analog **26** was inactive. On the contrary, its hydroxyl analog **25** showed activity comparable to that of the 2-methyl analog **17**. The 2-ethyl analog **27** also showed activity comparable to that of **17**/**25**.



%-Inhibition of HCV gene and host cell gene (GAPDH) expression at 10 μM

	HCV	GAPDH
15: none	inactive	inactive
16: 1-CH_3	inactive	85.2
17: 2-CH_3	42.0	98.5
18: 3-CH_3	inactive	70.7
19: 4-CH_3	inactive	inactive
20: 7-CH_3	inactive	inactive
21: 8-CH_3	87.0	88.8
22: 9-CH_3	inactive	inactive
23: 10-CH_3	inactive	94.9

Figure 4. Anti-HCV activity of methyl-substituted regioisomers of *N*-butylphenanthridinone, **16**–**23**.



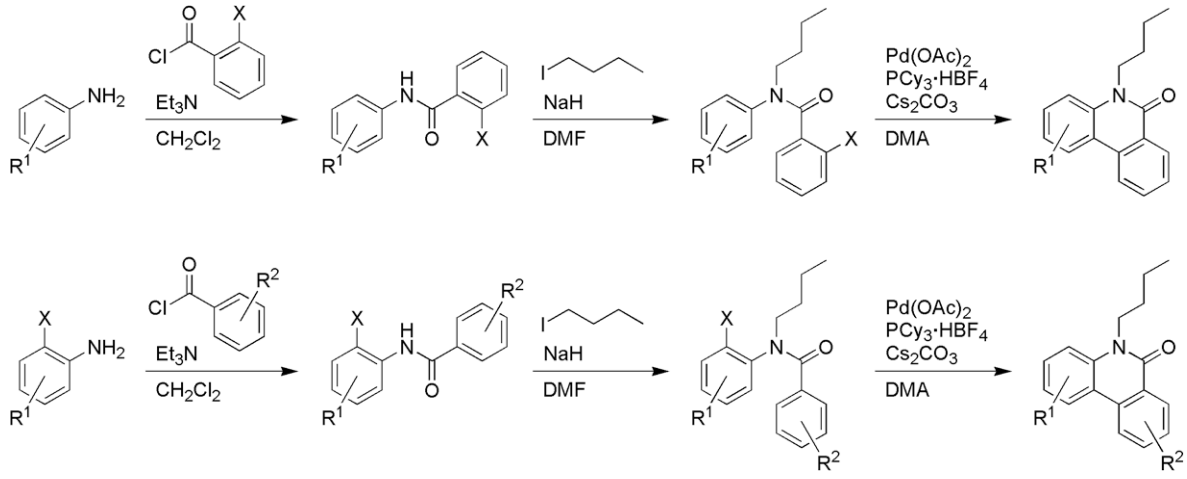
R	EC_{50} (μM)	CC_{50} (μM)
10: $(CF_3)_2(OH)C$-	3.7	12.5
15: H	>58.3	58.3
17: CH_3	35.8	38.4
24: F	>50	>50
25: OH	37.9	43.7
26: CF_3	>50	>50
27: CH_3CH_2	34.7	40.5
28: $(CH_3)_2CH$-	16.2	29.7
29: CH_2OH	21.5	>50
30: $CH_3CH(OH)$-	21.8	51.4
31: $(CH_3)_3CH$	10.6	24.9
32: CH_3CO-	18.7	27.3
33: $Ph(CH_2)_2$-	21.0	24.1

Figure 5. Anti-HCV activity of 2-substituted *N*-butylphenanthridinone, **15**, **17** and **24**–**33**.

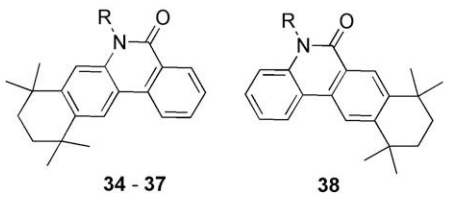
Compounds **28**–**33** showed moderate anti-HCV activity (EC_{50}: 10.6–21.8 μM). Among the 2-alkylated derivatives, the activity decreased in the order of *t*-butyl (**31**) > *i*-propyl (**28**) > ethyl (**27**) > methyl (**17**), suggesting that the hydrophobicity of the 2-substituent contributes to the activity, at least in part. On the other hand, introduction of a hydroxyl group into the ethyl (**30**) or methyl (**29**) group of **27** and **17**, respectively, seemed to slightly enhance the activity, that is, **27** (EC_{50}: 34.7 μM) versus **30** (EC_{50}: 21.8 μM) and **17** (EC_{50}: 35.8 μM) versus **29** (EC_{50}: 21.5 μM). The 2-acetyl derivative **32** showed slightly more potent activity than **30**.

2.5. Tetrahydrotetramethylnaphthalene-related analogs and benzophenanthridinone analogs

Although the structure–activity relationships of 2-substituted *N*-butylphenanthridinone were clearly interpretable, hydrophobicity around the 2-position also seemed to contribute to the activity. This and the armchair structural development shown in Figure 1 prompted us to prepare compounds **34**–**38** (Fig. 6). We also prepared benzophenanthridinone derivatives **39**–**44** (Fig. 7). Among the tetrahydrotetramethylnaphthalene-related analogs **34**–**38**, only the *N*-butyl and *N*-benzyl derivatives (**35** and **36**, respectively) were moderately active, as shown in Figure 6. The regioisomer of **35**, that is, compound **38**, was inactive.

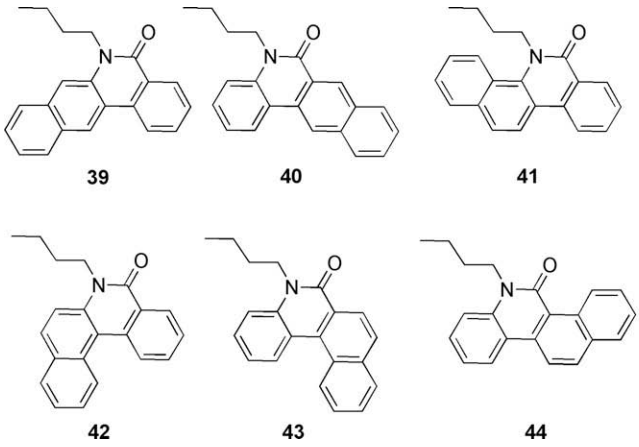


Scheme 3.



R	EC_{50} (μM)	CC_{50} (μM)
34: H	inactive	inactive
35: $CH_3(CH_2)_3$-	18.7	27.3
36: $PhCH_2$-	21.2	31.8
37: c-$C_6H_{11}CH_2$-	inactive	inactive
38: $CH_3(CH_2)_3$-	inactive	inactive

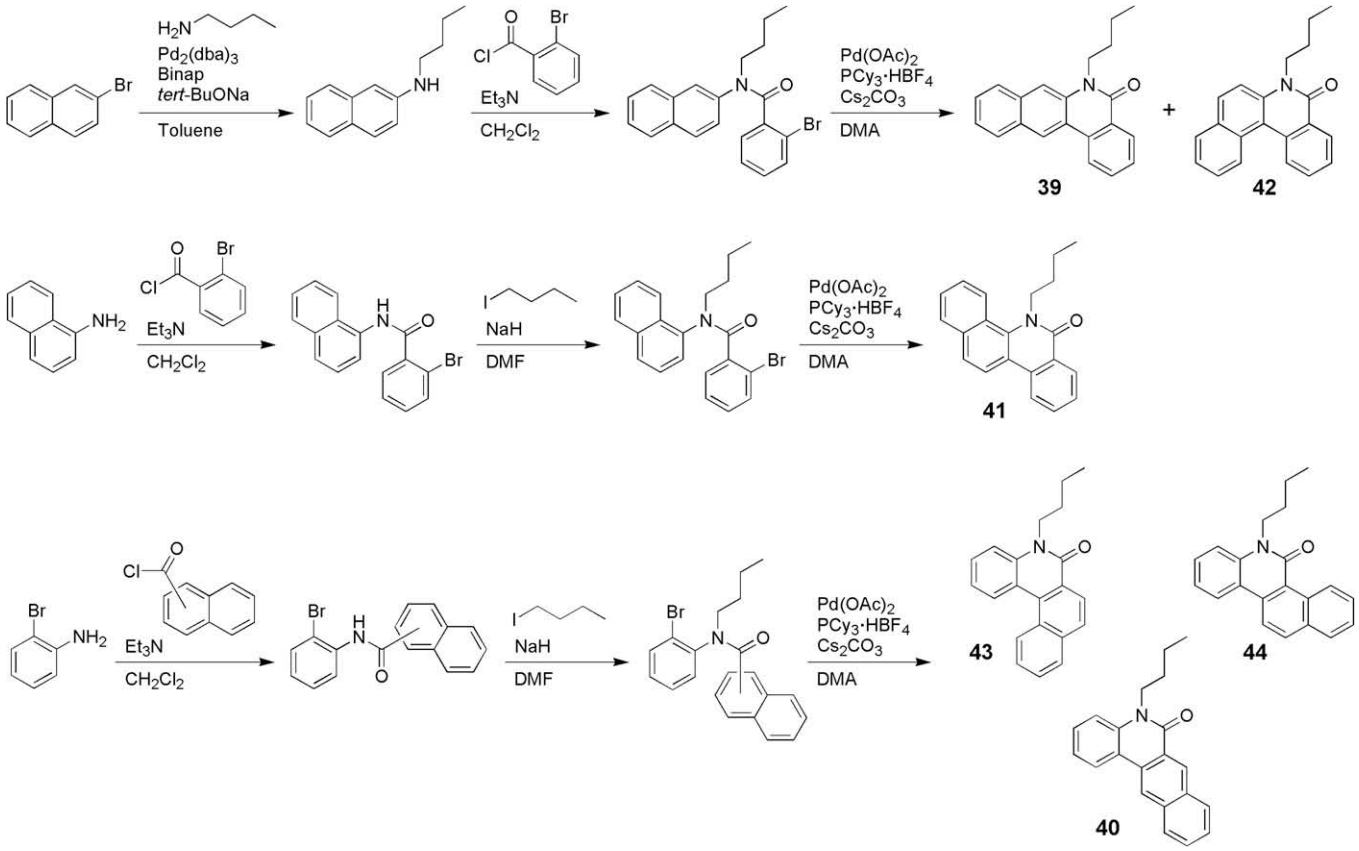
Figure 6. Anti-HCV activity of tetrahydrotetramethylnaphthalene-related analogs, **34**–**38**.



	EC_{50} (μM)	CC_{50} (μM)
35	18.7	27.3
39	3.2	6.3
40	11.0	>40
41	19.5	24.3
42	29.1	44.5
43	>31.4	31.4
44	29.9	>40

Figure 7. Anti-HCV activity of benzophenathridinone derivatives, **39**–**44**.

Benzophenanthridinone derivatives **39**–**44** were prepared as shown in Scheme 4. All the benzophenanthridinone derivatives, except **43**, showed potent or moderate anti-HCV activity. The activity decreased in the order of: benzo[*b*] (**39**) > benzo[*j*] (**40**) > benzo[*c*] (**41**) > benzo[*a*] (**42**) > benzo[*i*] (**44**) >> benzo[*k*] (**43**) as shown in Figures 7 and 8. Although compound **39** showed the most



Scheme 4.

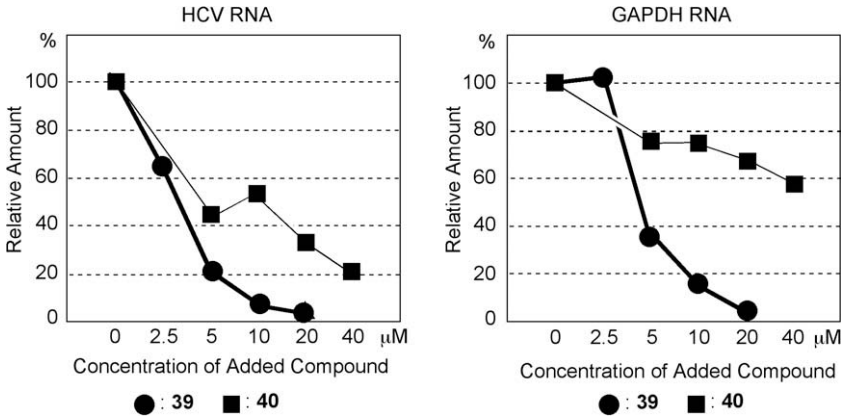


Figure 8. Dose-dependency curves of anti-HCV and cytotoxic activity elicited by compounds **39** and **40**.

potent anti-HCV activity (EC_{50}: 3.2 μM) among the compounds in this paper, its selectivity index (SI = CC_{50}/EC_{50}) was low (SI: 1.97). In terms of SI, compound **40** (EC_{50}: 11.0 μM) seems to be the best (SI: >3.64).

3. Conclusion

Based on a phenanthridinone skeleton derived by armchair structural development of thalidomide and retinoids, we developed candidate anti-HCV agents, **10** (EC_{50}: 3.7 μM,), **39** (EC_{50}: 3.2 μM,), and **40** (EC_{50}: 11 μM, CC_{50}: >40 μM). Further structural development may yield highly potent and selective drug candidates.

4. Experimental

4.1. General comments

Melting points were determined by using a Yanagimoto hot-stage melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a JEOL JNM-GX500 (500 MHz) spectrometer. Chemical shifts are expressed in δ (ppm) values with tetramethylsilane (TMS) as an internal reference. The following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet, dd = double doublet, dt = double triplet, br = broad. Mass spectra (MS) and high-resolution mass spectra (HRMS) were recorded on a JEOL JMS-DX303 spectrometer. Elemental analysis was performed with a Yanagimoto MT-6 elemental analyser.

4.2. 5-Ethyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-phenanthridin-6(5*H*)-one (8)

To a solution of 5 mmol of *N*-ethylaniline and 25 mL of toluene were added hexafluoroacetone trihydrate (1.2 equiv) and *p*-TsOH (0.1 equiv). The mixture was stirred for 22 h at 120 °C and the solvent was evaporated in vacuo. The residue was purified by silica gel column chromatography (eluent; *n*-hexane/ethyl acetate) to afford 2-(4-ethylaminophenyl)-1,1,1,3,3,3-hexafluoropropan-2-ol. The obtained compound was dissolved in dichloromethane (0.1 mmol/mL), and then triethylamine (1:40 v/v) and 2-iodobenzoyl chloride (1.2 equiv) were added. The mixture was stirred for 15 h at room temperature and the solvent was evaporated in vacuo. The residue was purified by silica gel column chromatography (eluent; *n*-hexane/ethyl acetate) to afford benzanilide derivatives. To a solution of the obtained benzanilide derivative, cesium carbonate (2 equiv) and *N*,*N*-dimethylacetamide were added palladium (II) acetate (10 mol %) and tricyclohexylphosphine tetrafluoroborate (0.15 equiv), and the mixture was heated to 130 °C. The catalyst was filtered off and washed several times with ethyl acetate. The combined organic layers were washed with water and brine successively, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (eluent; *n*-hexane/ethyl acetate) to afford 5-ethyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenanthridin-6(5*H*)-one (**8**) as a white solid. Mp 208.0–209.0 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.68 (d, J = 1.5 Hz, 1H), 8.56 (dd, J = 7.9, 1.5 Hz, 1H), 8.31 (d, J = 7.9 Hz, 1H), 7.85 (d, J = 9.4 Hz, 1H), 7.80 (td, J = 7.9, 1.3 Hz, 1H), 7.63 (t, J = 7.7 Hz, 1H), 7.50 (d, J = 9.0 Hz, 1H), 4.48 (q, J = 7.3 Hz, 2H), 3.71 (s, 1H), 1.44 (t, J = 7.3 Hz, 3H). HRMS (FAB) calcd for $C_{18}H_{14}F_6NO_2$ 390.0929; found: 390.0918 $(M+H)^+$.

4.3. 2-(1,1,1,3,3,3-Hexafluoro-2-hydroxypropan-2-yl)-5-(2,2,2-trifluoroethyl)phenanthridin-6(5*H*)-one (9)

The title compound was prepared by a method similar to that described for the synthesis of **8**, using aniline as a starting material, with slight modifications. 2-(4-Aminophenyl)-1,1,1,3,3,3-hexafluoropropan-2-ol (prepared from aniline and 1,1,1,3,3,3-hexafluoroacetone)[29] was coupled with 2-iodobenzoyl chloride, followed by protection of the hydroxyl group with 2-methoxylethoxymethyl chloride. It was then N-alkylated by the use of 2,2,2-trifluoroethyl triflate. The 2-methoxyethoxymethyl group of the obtained benzanilide derivative was removed by treatment with titanium tetrachloride, and the deprotected benzanilide derivative was cyclized by the method used for the synthesis of **8**. White solid. Mp 76.1–77.8 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.70 (d, J = 1.1 Hz, 1H), 8.56 (dd, J = 8.1, 1.1 Hz, 1H), 8.33 (d, J = 8.1 Hz, 1H), 7.87 (dd, J = 7.7, 1.1 Hz, 1H), 7.85 (dd, J = 7.7, 1.1 Hz, 1H), 7.66 (td, J = 8.1 Hz, 1H), 7.51 (d, J = 9.0 Hz, 1H), 5.13 (s, 2H), 3.78 (s, 1H). HRMS (FAB) calcd for $C_{18}H_{10}F_9NO_2$ 444.0646; found: 444.0653 $(M+H)^+$.

4.4. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-phenanthridin-6(5*H*)-one (10)

The title compound was prepared by a method similar to that described for the synthesis of **9** using 1-iodobutane instead of 2,2,2-trifluoroethyl triflate. White solid. Mp 166.0–166.6 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.55 (d, J = 7.9 Hz, 1H), 8.30 (d, J = 7.9 Hz, 1H), 7.84 (d, J = 7.9 Hz, 1H), 7.79 (dd, J = 7.9, 7.3 Hz, 1H), 7.62 (dd, J = 7.9, 7.3 Hz, 1H), 7.47 (d, J = 7.9 Hz, 1H), 4.39 (t, J = 7.9 Hz, 2H), 3.81 (s, 1H), 1.83–1.76 (m, 2H), 1.56–1.50 (m, 2H),

1.03 (t, J = 7.3 Hz, 3H). HRMS (FAB) calcd for $C_{20}H_{18}F_6NO_2$ 418.1242; found: 418.1262 $(M+H)^+$.

4.5. 2-(1,1,1,3,3,3-Hexafluoro-2-hydroxypropan-2-yl)-5-hexylphenanthridin-6(5*H*)-one (11)

The title compound was prepared by a method similar to that described for the synthesis of compound **9**, using 1-iodohexane instead of 2,2,2-trifluoroethyl triflate. White solid. Mp 132.0–132.2 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (d, J = 1.8 Hz, 1H), 8.54 (d, J = 7.9, 1.8 Hz, 1H), 8.29 (d, J = 7.9 Hz, 1H), 7.84 (d, J = 8.5 Hz, 1H), 7.78 (dd, J = 8.5, 7.3 Hz, 1H), 7.62 (dd, J = 8.5, 7.3 Hz, 1H), 7.46 (d, J = 8.5 Hz, 1H), 4.37 (t, J = 7.9 Hz, 2H), 3.91 (s, 1H), 1.84–1.76 (m, 2H), 1.53–1.47 (m, 2H), 1.42–1.32 (m, 4H), 0.91 (t, J = 7.3 Hz, 3H). HRMS (FAB) calcd for $C_{22}H_{22}F_6NO_2$ 446.1555; found: 446.1516 $(M+H)^+$.

4.6. 2-(1,1,1,3,3,3-Hexafluoro-2-hydroxypropan-2-yl)-5-nonylphenanthridin-6(5*H*)-one (12)

The title compound was prepared by a method similar to that described for the synthesis of compound **9**, using 1-bromononane instead of 2,2,2-trifluoroethyl triflate. White solid. Mp 98.1–99.0 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (d, J = 1.8 Hz, 1H), 8.54 (d, J = 7.9, 1.8 Hz, 1H), 8.29 (d, J = 7.9 Hz, 1H), 7.84 (d, J = 8.5 Hz, 1H), 7.79 (dd, J = 7.9, 7.3 Hz, 1H), 7.62 (dd, J = 7.9, 7.3 Hz, 1H), 7.46 (d, J = 8.5 Hz, 1H), 4.37 (t, J = 7.9 Hz, 2H), 3.87 (s, 1H), 1.84–1.76 (m, 2H), 1.53–1.46 (m, 2H), 1.43–1.36 (m, 2H), 1.33–1.24 (m, 8H), 0.88 (t, J = 7.3 Hz, 3H). HRMS (FAB) calcd for $C_{25}H_{28}F_6NO_2$ 488.2024; found: 488.1981 $(M+H)^+$.

4.7. 5-Benzyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-phenanthridin-6(5*H*)-one (13)

The title compound was prepared by a method similar to that described for the synthesis of compound **9**, using benzyl bromide instead of 2,2,2-trifluoroethyl triflate. White solid. Mp 80.0–80.9 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (d, J = 1.3 Hz, 1H), 8.63 (d, J = 8.1, 1.3 Hz, 1H), 8.34 (d, J = 8.1 Hz, 1H), 7.84 (td, J = 7.2, 1.3 Hz, 1H), 7.68 (q, J = 8.3 Hz, 2H), 7.38 (d, J = 9.4 Hz, 1H), 7.34–7.23 (m, 5H), 5.66 (s, 2H), 3.63 (s, 1H). HRMS (FAB) calcd for $C_{23}H_{15}F_6NO_2$ 452.1085; found: 452.1057 $(M+H)^+$. Anal. Calcd for $C_{23}H_{15}F_6NO_2 \cdot 1/3\ H_2O$: C, 60.40; H, 3.45; N, 3.06. Found: C, 60.46; H, 3.47; N, 3.08.

4.8. 5-(Cyclohexylmethyl)-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenanthridin-6(5*H*)-one (14)

The title compound was prepared by a method similar to that described for the synthesis of compound **9**, using cyclohexylmethyl bromide instead of 2,2,2-trifluoroethyl triflate. White solid. Mp 200.9–201.7 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (d, J = 1.8 Hz, 1H), 8.55 (d, J = 8.5, 1.8 Hz, 1H), 8.30 (d, J = 8.5 Hz, 1H), 7.83 (d, J = 8.5 Hz, 1H), 7.79 (dd, J = 8.5, 7.3 Hz, 1H), 7.62 (dd, J = 8.5, 7.3 Hz, 1H), 7.46 (d, J = 8.5 Hz, 1H), 4.30 (br s, 2H), 3.75 (s, 1H), 1.98–1.88 (m, 1H), 1.76–1.64 (m, 5H), 1.24–1.17 (m, 5H). HRMS (FAB) calcd for $C_{23}H_{22}F_6NO_2$ 458.1555; found: 458.1569 $(M+H)^+$.

4.9. 5-Butylphenanthridin-6(5*H*)-one (15)

The title compound was prepared by a method similar to that described for the synthesis of **9**, using aniline as a starting material, with slight modifications, that is, 1-iodobutane was used in place of 2,2,2-trifluoroethyl triflate. Colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.54 (dd, 1H, J = 7.9, 1.3 Hz), 8.29 (dd, 1H, J = 8.6, 1.3 Hz), 8.27 (d, 1H, J = 8.6 Hz), 7.74 (ddd, 1H, J = 8.0, 7.4, 1.2 Hz), 7.57 (t, 1H, J = 7.4, Hz), 7.53 (ddd, 1H, J = 8.5, 7.4, 1.2 Hz), 7.40 (d, 1H, J = 8.5 Hz), 7.30 (dd, 1H, J = 8.0, 7.4 Hz), 4.38 (t, 2H, J = 7.9, Hz), 1.78 (td, 2H, J = 7.9, 7.3 Hz), 1.52 (sextet, 2H, J = 7.3 Hz), 1.00 (t, 3H, J = 7.3, Hz). HRMS (FAB) calcd for $C_{17}H_{17}NO$ 252.1388; found: 252.1349 $(M+H)^+$.

4.10. 5-Butyl-1-methylphenanthridin-6(5*H*)-one (16)

The title compound was prepared by a method similar to that described for the synthesis of **15**, using 2-iodo-3-methylaniline as a starting material. Benzoyl chloride was used instead of 2-iodobenzoyl chloride. Pale brown oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.64 (d, 1H, J = 8.0 Hz), 8.44 (d, 1H, J = 8.0 Hz), 7.73 (t, 1H, J = 8.0 Hz), 7.59 (t, 1H, J = 8.0 Hz), 7.42 (t, 1H, J = 8.0 Hz), 7.33 (d, 1H, J = 8.0 Hz), 7.17 (d, 1H, J = 8.0 Hz), 4.40 (t, 2H, J = 7.6 Hz), 2.96 (s, 3H), 184–1.77 (m, 2H), 158–1.48 (m, 2H), 1.02 (t, 3H, J = 7.6 Hz). HRMS (FAB) calcd for $C_{18}H_{19}NO$ 266.1545; found: 266.1568 $(M+H)^+$.

4.11. 5-Butyl-2-methylphenanthridin-6(5*H*)-one (17)

The title compound was prepared by a method similar to that described for the synthesis of **15**, using 4-methylaniline as a starting material. 2-Bromobenzoyl chloride was used instead of 2-iodobenzoyl chloride. Colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.55 (dd, 1H, J = 8.0, 1.2 Hz), 8.28 (d, 1H, J = 8.0 Hz), 8.10 (s, 1H), 7.74 (td, 1H, J = 8.0, 1.2 Hz), 7.57 (m, 1H), 7.37–7.30 (d, 1H, J = 7.6 Hz), 4.38 (t, 2H, J = 7.6 Hz), 2.49 (s, 3H), 1.82–1.75 (m, 3H), 1.56–1.48 (m, 2H), 1.01 (t, 3H, J = 7.3 Hz). HRMS (FAB) calcd for $C_{18}H_{19}NO$ 266.1545; found: 266.1584 $(M+H)^+$.

4.12. 5-Butyl-3-methylphenanthridin-6(5*H*)-one (18)

The title compound was prepared by a method similar to that described for the synthesis of **16**, using 2-bromo-5-methylaniline as a starting material. Pale brown oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (d, 1H, J = 8.0 Hz), 8.24 (d, 1H, J = 8.0 Hz), 8.80 (d, 1H, J = 8.0 Hz), 7.73 (t, 1H, J = 8.0 Hz), 7.55 (t, 1H, J = 8.0 Hz), 7.20 (s, 1H), 7.13 (d, 1H, J = 8.0 Hz), 4.39 (t, 2H, J = 7.5 Hz), 2.52 (s, 3H), 1.83–1.76 (m, 2H), 1.57–1.49 (m, 2H), 1.03 (t, 3H, J = 7.5 Hz). HRMS (FAB) calcd for $C_{18}H_{19}NO$ 266.1545; found: 266.1512 $(M+H)^+$.

4.13. 5-Butyl-4-methylphenanthridin-6(5*H*)-one (19)

The title compound was prepared by a method similar to that described for the synthesis of **17**, using 2-methylaniline as a starting material. Colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.50 (dd, 1H, J = 8.0, 1.2 Hz), 8.22 (d, 1H, J = 8.0 Hz), 8.13 (d, 1H, J = 8.0 Hz), 7.20 (td, 1H, J = 7.6, 1.4 Hz), 7.55 (t, 1H, J = 8.0 Hz), 7.30 (d, 1H, J = 7.6 Hz), 7.21 (t, 1H, J = 7.6 Hz), 4.49 (t, 2H, J = 7.6 Hz), 2.66 (s, 3H), 1.66–1.60 (m, 2H), 1.20–1.19 (m, 2H), 0.86 (t, 3H, J = 7.3 Hz). HRMS (FAB) calcd for $C_{18}H_{19}NO$ 266.1545; found: 266.1557 $(M+H)^+$.

4.14. 5-Butyl-7-methylphenanthridin-6(5*H*)-one (20)

The title compound was prepared by a method similar to that described for the synthesis of **16**, using 2-bromoaniline as a starting material. 2-Methylbenzoyl chloride was used instead of benzoyl chloride. White amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (d, 1H, J = 7.5 Hz), 8.17 (d, 1H, J = 7.5 Hz), 7.58 (t, 1H, J = 7.5 Hz), 7.51 (t, 1H, J = 7.5 Hz), 7.35 (t, 1H, J = 7.5 Hz), 7.26 (t, 1H, J = 7.5 Hz), 4.31 (t, 2H, J = 8.0 Hz), 2.98 (s, 3H), 1.81–1.74 (m, 2H), 1.58–1.49 (m, 2H), 1.02 (t, 3H, J = 7.3 Hz). HRMS (FAB) calcd for $C_{18}H_{19}NO$ 266.1545; found: 266.1573 $(M+H)^+$.

4.15. 5-Butyl-8-methylphenanthridin-6(5*H*)-one (21)

The title compound was prepared by a method similar to that described for the synthesis of **20**. 3-Methylbenzoyl chloride was used instead of 2-methylbenzoyl chloride. White amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.36 (s, 1H), 8.28 (d, 1H, J = 8.0 Hz), 8.18 (d, 1H, J = 8.0 Hz), 7.58 (dd, 1H, J = 8.0, 1.8 Hz), 7.52 (t, 1H, J = 8.0 Hz), 7.41 (d, 1H, J = 8.0 Hz), 7.30 (t, 1H, J = 8.0 Hz), 4.40 (t, 2H, J = 8.0 Hz), 2.52 (s, 3H), 1.83–1.76 (m, 2H), 1.56–1.50 (m, 2H), 1.02 (t, 3H, J = 7.3 Hz). HRMS (FAB) calcd for $C_{18}H_{19}NO$ 266.1545; found: 266.1588 $(M+H)^+$.

4.16. 5-Butyl-9-methylphenanthridin-6(5*H*)-one (22)

The title compound was prepared by a method similar to that described for the synthesis of **20**. 4-Methylbenzoyl chloride was used instead of 2-methylbenzoyl chloride. White solid. Mp 70.0–73.0 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.44 (d, 1H, J = 7.5 Hz), 8.30 (d, 1H, J = 7.5 Hz), 8.07 (s, 1H7.5), 7.53 (td, 1H, J = 7.5 Hz), 7.40 (d, 2H, J = 7.5 Hz), 7.30 (dd, 1H, J = 7.5 Hz), 4.39 (t, 2H, J = 8.0 Hz), 2.57 (s, 3H), 1.81.75 (m, 2H), 1.51.49 (m, 2H), 1.02 (t, 3H, J = 7.3 Hz). HRMS (FAB) calcd for $C_{18}H_{19}NO$ 266.1545; found: 266.1527 $(M+H)^+$.

4.17. 5-Butyl-10-methylphenanthridin-6(5*H*)-one (23)

The title compound was prepared by a method similar to that described for the synthesis of **20**. Colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (d, 1H, J = 8.0 Hz), 8.70 (dd, 1H, J = 8.0, 1.2 Hz), 7.61 (d, 1H, J = 8.0 Hz), 7.56–7.52 (m, 1H), 7.49 (d, 1H, J = 8.0 Hz), 7.46 (dd, 1H, J = 8.0, 1.2 Hz), 7.32–7.27 (m, 1H), 4.40 (t, 2H, J = 7.3 Hz), 2.97 (s, 3H), 1.85–1.78 (m, 2H), 1.50–1.50 (m, 2H), 1.02 (t, 3H, J = 7.3 Hz). HRMS (FAB) calcd for $C_{18}H_{19}NO$ 266.1545; found: 266.1553 $(M+H)^+$.

4.18. 5-Butyl-2-fluorophenanthridin-6(5*H*)-one (24)

The title compound was prepared by a method similar to that described for the synthesis of **17**, using 4-fluoroaniline as a starting material. White solid. FAB-MS m/z 270 $(M+H)^+$. Mp 114.0–117.5 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.56 (dd, 1H, J = 8.0, 1.5 Hz), 8.17 (d, 1H, J = 8.0 Hz), 7.90 (dd, 1H, J = 9.7, 3.0 Hz), 7.77 (t, 1H, J = 8.0 Hz), 7.63 (t, 1H, J = 8.0 Hz), 7.37 (dd, 1H, J = 9.0, 4.5 Hz), 7.29–7.24 (m, 1H), 4.38 (t, 2H, J = 8.0 Hz), 1.82–1.75 (m, 2H), 1.57–1.48 (m, 2H), 1.02 (t, 3H, J = 7.3 Hz). Anal. Calcd for $C_{17}H_{16}NFO$: C, 75.82; H, 5.99; N, 5.20. Found: C, 76.22; H, 6.24; N, 5.24.

4.19. 5-Butyl-2-trifluoromethylphenanthridin-6(5*H*)-one (26)

The title compound was prepared by a method similar to that described for the synthesis of **17**, using 4-trifluoromethylaniline as a starting material. White solid. FAB-MS m/z 320 $(M+H)^+$. Mp 111.0–112.0 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.56 (d, 1H, J = 8.0 Hz), 8.53 (s, 1H), 8.30 (d, 1H, J = 8.0 Hz), 7.82 (t, 1H, J = 8.0 Hz), 7.77 (dd, 1H, J = 8.0, 1.8 Hz), 7.65 (t, 1H, J = 8.0 Hz), 7.00 (d, 1H, J = 8.0 Hz), 4.41 (t, 2H, J = 7.9 Hz), 1.83–1.75 (m, 2H), 1.58–1.49 (m, 2H), 1.03 (t, 3H, J = 7.3 Hz). Anal. Calcd for $C_{18}H_{16}NF_3O$: C, 67.70; H, 5.05; N, 4.39. Found: C, 67.91; H, 5.33; N, 4.38.

4.20. 5-Butyl-2-ethylphenanthridin-6(5*H*)-one (27)

The title compound was prepared by a method similar to described that for the synthesis of **17**, using 4-ethylaniline as a starting material. Pale brown oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.55 (dd, 1H, J = 8.0, 1.5 Hz), 8.30 (d, 1H, J = 8.0 Hz), 8.12 (d, 1H, J = 1.5 Hz), 7.75 (td, 1H, J = 8.0, 1.5 Hz), 7.58 (t, 1H, J = 8.0 Hz), 7.39 (dd, 1H, J = 8.0, 1.5 Hz), 7.34 (d, 1H, J = 8.0 Hz), 4.39 (t, 2H, J = 8.0 Hz), 2.79 (q, 2H, J = 8.0 Hz), 1.83–1.76 (m, 2H), 1.57–1.49 (m, 2H), 1.34 (t, 3H, J = 7.6 Hz), 1.02 (t, 3H, J = 7.3 Hz). HRMS (FAB) calcd for $C_{19}H_{21}NO$ 280.1701; found: 280.1696 $(M+H)^+$.

4.21. 5-Butyl-2-isopropylphenanthridin-6(5*H*)-one (28)

The title compound was prepared by a method similar to that described for the synthesis of **15**, using 4-isopropylaniline as a starting material. Colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.54 (dd, 1H, J = 8.0, 1.2 Hz), 8.30 (d, 1H, J = 8.0 Hz), 8.12 (d, 1H, J = 1.9 Hz), 7.74 (ddd, 1H, J = 7.9, 7.4, 1.2 Hz), 7.56 (dd, 1H, J = 8.0, 7.4 Hz), 7.41 (dd, 1H, J = 8.6, 1.9 Hz), 7.34 (d, 1H, J = 8.6 Hz), 4.37 (t, 2H, J = 8.0 Hz), 3.05 (septet, 1H, J = 7.4 Hz), 1.77 (quintet, 2H, J = 8.0 Hz), 1.51 (sextet, 2H, J = 7.4 Hz), 1.34 (d, 6H, J = 7.4 Hz), 1.00 (t, 3H, J = 7.4 Hz). HRMS (FAB) calcd for $C_{20}H_{23}NO$ 294.1858; found: 294.1851 $(M+H)^+$.

4.22. 5-Butyl-2-*tert*-butylphenanthridin-6(5*H*)-one (31)

The title compound was prepared by a method similar to that described for the synthesis of **17** using 4-*tert*-burylaniline as a starting material. Pale brown oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.56 (dd, 1H, J = 8.0, 1.5 Hz), 8.32 (d, 1H, J = 8.0 Hz), 8.31 (d, 1H, J = 1.5 Hz), 7.78–7.74 (m, 1H), 7.60 (dd, 1H, J = 8.0, 1.5 Hz), 7.57 (d, 1H, J = 8.0 Hz), 7.37 (d, 1H, J = 8.0 Hz), 4.39 (t, 2H, J = 7.6 Hz), 1.83–1.76 (m, 2H), 1.56–1.50 (m, 2H), 1.44 (s, 9H), 1.02 (t, 3H, J = 7.3 Hz). HRMS (FAB) calcd for $C_{21}H_{25}NO$ 308.2014; found: 308.2008 $(M+H)^+$.

4.23. 5-Butyl-2-hydroxyphenanthridin-6(5*H*)-one (25)

The title compound was prepared by a method similar to that described for the synthesis of **8**, using 4-*tert*-butyldimethylsilyloxyaniline (prepared from *p*-nitrophenol) as a starting material, with slight modifications. 4-*tert*-Butyldimethylsilyloxyaniline was acylated with butyryl chloride in the presence of triethylamine in dichloromethane, and then hydrogenated with lithium aluminum hydride in tetrahydrofuran. Obtained *N*-butylaniline was coupled with 2-iodobenzoyl chloride, and then cyclized in the presence of palladium (II) acetate, tricyclohexylphosphine tetrafluoroborate and potassium carbonate in *N*,*N*-dimethylacetamide. White solid. Mp 187.0–192.0 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.55 (d, 1H, J = 7.9 Hz), 8.10 (d, 1H, J = 7.9 Hz), 7.78 (d, 1H, J = 2.4 Hz), 7.67 (t, 1H, J = 7.3 Hz), 7.55 (t, 1H, J = 7.3 Hz), 7.28 (d, 1H, J = 9.2 Hz), 7.14 (dd, 1H, J = 9.2, 2.4 Hz), 6.68 (br s, 1H), 4.37 (t, 2H, J = 7.3 Hz), 1.77 (quintet, 2H, J = 7.3 Hz), 1.49 (sextet, 2H, J = 7.3 Hz), 0.97 (t, 3H, J = 7.3 Hz). HRMS (FAB) calcd for $C_{17}H_{17}NO_2$ 268.1338; found: 268.1344 $(M+H)^+$.

4.24. 5-Butyl-2-hydroxymethylphenanthridin-6(5*H*)-one (29)

White solid. FAB-MS m/z 282 $(M+H)^+$. Mp 150.0–153.0 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 18.54 (d, 1H, J = 8.0 Hz), 8.28–8.26 (m, 2H), 7.75 (t, 1H, J = 8.0 Hz), 7.59 (t, 1H, J = 8.0, 1.8 Hz), 7.52 (dd, 1H, J = 8.0, 1.8 Hz), 7.36 (d, 1H, J = 8.0 Hz), 4.83 (d, 2H, J = 6.0 Hz), 4.37 (t, 2H, J = 7.5 Hz), 1.99–1.94 (m, 1H), 1.81–1.74 (m, 2H), 1.55–1.48 (m, 2H), 1.01 (t, 3H, J = 7.5 Hz). Anal. Calcd for $C_{18}H_{19}NO_2$: C, 76.84; H, 6.81; N, 4.98. Found: C, 76.94; H, 6.71; N, 5.01.

4.25. 5-Butyl-2-(1′-hydroxyethyl)phenanthridin-6(5*H*)-one (30)

The title compound was prepared by a method similar to that described for the synthesis of **25**, using 1-(4-aminophenyl)ethanol as a starting material. Pale yellow solid. Mp 95.0–99.0 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.51 (dd, 1H, J = 7.9, 1.2 Hz), 8.23 (d, 1H, J = 8.5 Hz), 8.22 (d, 1H, J = 1.8 Hz), 7.72 (td, 1H, J = 7.3, 1.2 Hz),

7.56 (dd, 1H, *J* = 7.9, 7.3 Hz), 7.49 (dd, 1H, *J* = 8.5, 1.8 Hz), 7.29 (d, 1H, *J* = 8.5 Hz), 5.03 (q, 1H, *J* = 6.7 Hz), 4.33 (t, 2H, *J* = 7.9 Hz), 1.74 (quintet, 2H, *J* = 7.9 Hz), 1.57 (d, 3H, *J* = 6.7 Hz), 1.49 (sextet, 2H, *J* = 7.3 Hz), 0.99 (t, 3H, *J* = 7.3 Hz). HRMS (FAB) calcd for $C_{19}H_{21}NO_2$ 296.1651; found: 296.1661 $(M+H)^+$.

4.26. 5-Butyl-2-acetylphenanthridin-6(5*H*)-one (32)

The title compound was prepared by the same method as described for the synthesis of **30**. The title compound was generated by partial oxidization of the alcoholic hydroxy group at the final cyclization step. White solid. Mp 114.0–118.0 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.92 (d, 1H, *J* = 1.8 Hz), 8.53 (d, 1H, *J* = 7.3 Hz), 8.37 (d, 1H, *J* = 7.9 Hz), 8.10 (dd, 1H, *J* = 9.2, 1.8 Hz), 7.79 (t, 1H, *J* = 7.3 Hz), 7.61 (dd, 1H, *J* = 7.9, 7.3 Hz), 7.44 (d, 1H, *J* = 9.2 Hz), 4.39 (t, 2H, *J* = 7.9 Hz), 2.69 (s, 3H), 1.77 (quintet, 2H, *J* = 7.9 Hz), 1.52 (sextet, 2H, *J* = 7.3 Hz), 1.01 (t, 3H, *J* = 7.3 Hz). HRMS (FAB) calcd for $C_{19}H_{19}NO_2$ 294.1494; found: 294.1501 $(M+H)^+$.

4.27. 5-Butyl-2-phenethylphenanthridin-6(5*H*)-one (33)

Compound **29** was oxidized with manganese oxide in dichloromethane to give an aldehyde derivative, which was coupled by Wittig reaction to give a styryl derivative. This product was hydrogenated in the presence of palladium–carbon under a hydrogen atmosphere to give the title compound. Colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.55 (dd, 1H, *J* = 8.0, 1.2 Hz), 8.22 (d, 1H, *J* = 8.0 Hz), 8.05 (s, 1H), 7.74 (ddd, 1H, *J* = 8.0, 6.5, 1.2 Hz), 7.58 (t, 1H, *J* = 8.0 Hz), 7.37–7.29 (m, 4H), 7.24–7.21 (m, 3H), 4.38 (t, 2H, *J* = 7.9 Hz), 3.09–2.99 (m, 4H), 1.82–1.76 (m, 2H), 1.55–1.49 (m, 2H), 1.02 (t, 3H, *J* = 7.3 Hz). HRMS (FAB) calcd for $C_{25}H_{26}NO$ 356.2014; found: 356.1995 $(M+H)^+$.

4.28. 6-Butyl-8,9,10,11-tetrahydro-8,8,11,11-tetramethylbenzo-[2,3-*b*]phenanthridin-5(6*H*)-one (35)

The title compound was prepared by a method similar to that described for the synthesis of **17**, using 2-amino-5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalene as a starting material. Colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.52 (d, 1H, *J* = 8.0 Hz), 8.26 (d, 1H, *J* = 8.0 Hz), 8.21 (s, 1H), 7.73 (t, 1H, *J* = 8.0 Hz), 7.54 (t, 1H, *J* = 8.0 Hz), 7.31 (s, 1H), 4.39 (t, 2H, *J* = 7.3 Hz), 1.84–1.75 (m, 6H), 1.59–1.50 (m, 2H), 1.40 (s, 6H), 1.38 (s, 6H), 1.04 (t, 3H, *J* = 7.3 Hz). HRMS (FAB) calcd for $C_{25}H_{31}NO$ 362.2484; found: 362.2473 $(M+H)^+$.

4.29. 6-Benzyl-8,9,10,11-tetrahydro-8,8,11,11-tetramethylbenzo-[2,3-*b*]phenanthridin-5(6*H*)-one (36)

The title compound was prepared by a method similar to that described for the synthesis of **35**. N-Alkylation was performed by using benzyl bromide. White amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.60 (dd, 2H, *J* = 8.0, 1.5 Hz), 8.29 (d, 1H, *J* = 8.0 Hz), 8.18 (s, 1H), 7.79–7.75 (m, 1H), 7.58 (t, 1H, *J* = 8.0 Hz), 7.37–7.28 (m, 4H), 7.23 (s, 1H), 7.22 (t, 1H, *J* = 8.0 Hz), 5.65 (s, 2H), 1.69 (d, 4H, *J* = 1.2 Hz), 1.36 (s, 6H), 1.14 (s, 6H). HRMS (FAB) calcd for $C_{28}H_{29}NO$ 396.2327; found: 396.2296 $(M+H)^+$.

4.30. 6-Cyclohexylmethyl-8,9,10,11-tetrahydro-8,8,11,11-tetramethylbenzo[2,3-*b*]phenanthridin-5(6*H*)-one (37)

The title compound was prepared by a method similar to that described for the synthesis of **35**. N-Alkylation was performed by using cyclohexylmethyl bromide. White solid. Mp 148.0–153.0 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.52 (dd, 2H, *J* = 8.0, 1.2 Hz), 8.27 (d, 1H, *J* = 8.0 Hz), 8.21 (s, 1H), 7.73 (td, 1H, *J* = 8.0, 1.2 Hz), 7.54 (td, 1H, *J* = 8.0, 1.2 Hz), 7.29 (s, 1H), 4.30 (br s, 2H), 1.94–1.89 (m, 1H), 1.78 (s, 4H), 1.76 (t, 4H, *J* = 13.00 Hz), 1.41 (s, 6H), 1.38 (s, 6H), 1.29–1.15 (m, 6H). HRMS (FAB) calcd for $C_{28}H_{35}NO$ 402.2797; found: 402.2791 $(M+H)^+$.

4.31. 5-Butyl-8,9,10,11-tetrahydro-8,8,11,11-tetramethylbenzo-[2,3-*j*]phenanthridin-6(5*H*)-one (38)

The title compound was prepared by a method similar to that described for the synthesis of **20**. 5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthoyl chloride (prepared from 5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphthalene-2-carboxylic acid)[4] was used instead of 2-methylbenzoyl chloride. White solid. FAB-MS *m/z* 362 $(M+H)^+$. Mp 103.0–109.0 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.51 (s, 1H), 8.29 (d, 1H, *J* = 7.9 Hz), 8.21 (s, 1H), 7.50 (t, 1H, *J* = 7.9 Hz), 7.38 (d, 1H, *J* = 7.9 Hz), 7.29 (t, 1H, *J* = 7.3 Hz), 4.38 (t, 2H, *J* = 7.6 Hz), 1.81–1.74 (m, 6H), 1.56–1.50 (m, 2H), 1.42 (s, 6H), 1.40 (s, 6H), 1.02 (t, 3H, *J* = 7.3 Hz). Anal. Calcd for $C_{25}H_{31}NO{\cdot}1/7\ H_2O$: C, 82.47; H, 8.66; N, 3.85. Found: C, 82.64; H, 8.76; N, 3.71.

4.32. 8,9,10,11-Tetrahydro-8,8,11,11-tetramethylbenzo[2,3-*b*]-phenanthridin-5(6*H*)-one (34)

The title compound was prepared by a method similar to that described for the synthesis of **35**, using 4-methoxybenzyl bromide instead of 1-iodobutane, followed by deprotection of the 4-methoxybenzyl group with trifluoroacetic acid. White solid. Mp 280 °C (decomp). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.27 (s, 1H), 8.51 (dd, 1H, *J* = 7.8, 1.3 Hz), 8.28 (t, 1H, *J* = 7.8 Hz), 8.14 (s, 1H), 7.78 (td, 1H, *J* = 7.8, 1.3 Hz), 7.57 (t, 1H, *J* = 7.8 Hz), 7.11 (s, 1H), 1.76 (s, 4H), 1.39 (s, 6H), 1.38 (s, 6H). HRMS (FAB) calcd for $C_{21}H_{23}NO$ 306.1858; found: 306.1817 $(M+H)^+$.

4.33. 6-Butylbenzo[*b*]phenanthridin-5(6*H*)-one (39)

The title compound was prepared by a method similar to that described for the synthesis of **8**, using 2-butylaminonaphthalene as a starting material. 2-Butylaminonaphthalene was prepared from 2-bromonaphthalene by coupling reaction with *n*-butylamine in the presence of tris(dibenzylidenacetone)dipalladium, sodium *tert*-butoxide and n-butylamine. 2-Bromobenzoyl chloride was used instead of 2-iodobenzoyl chloride. White solid. Mp 101.0–104.0 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.90 (s, 1H), 8.57 (dd, 1H, *J* = 8.0, 1.8 Hz), 8.46 (d, 1H, *J* = 8.0 Hz), 7.98 (d, 1H, *J* = 8.0 Hz), 7.92 (d, 1H, *J* = 8.0 Hz), 7.82–7.78 (m, 1H), 7.73 (s, 1H), 7.62 (t, 1H, *J* = 7.3 Hz), 7.55 (t, 1H, *J* = 7.3 Hz), 7.48 (t, 1H, *J* = 7.3 Hz), 4.50 (t, 3H, *J* = 7.9 Hz), 1.92–1.85 (m, 2H), 1.63–1.52 (m, 2H), 1.06 (t, 3H, *J* = 7.3 Hz). HRMS (FAB) calcd for $C_{21}H_{19}NO$ 302.1545; found: 302.1555 $(M+H)^+$.

4.34. 5-Butylbenzo[*j*]phenanthridin-6(5*H*)-one (40)

The title compound was prepared by a method similar to that described for the synthesis of **20**. 2-Naphthoyl chloride was used instead of 2-methylbenzoyl chloride. White solid. Mp 111.0–115.0 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.14 (s, 1H), 8.74 (s, 1H), 8.48 (dd, 1H, *J* = 8.0, 1.5 Hz), 8.09 (d, 1H, *J* = 8.0 Hz), 8.04 (d, 1H, *J* = 8.0 Hz), 7.63 (t, 1H, *J* = 8.0 Hz), 7.57 (d, 1H, *J* = 8.0 Hz), 7.54 (dd, 1H, *J* = 8.0, 1.5 Hz), 7.42 (d, 1H, *J* = 8.0 Hz), 7.35 (t, 1H, *J* = 8.0 Hz), 4.43 (t, 2H, *J* = 7.6 Hz), 1.87–1.80 (m, 2H), 1.58–1.52 (m, 2H). HRMS (FAB) calcd for $C_{21}H_{19}NO$ 302.1545; found: 302.1591 $(M+H)^+$.

4.35. 5-Butylbenzo[*c*]phenanthridin-6(5*H*)-one (41)

The title compound was prepared by a method similar to that described for the synthesis of **17**, using 1-aminonaphthlene as a

starting material. White amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.55 (dd, 1H, J = 8.0, 1.2 Hz), 8.29–8.22 (m, 3H), 7.92–7.89 (m, 1H), 7.81–7.77 (m, 1H), 7.73 (d, 1H, J = 8.0 Hz), 7.60 (t, 3H, J = 8.0 Hz), 7.56–7.50 (m, 2H), 4.59 (t, 2H, J = 7.3 Hz), 1.93–1.86 (m, 2H), 1.26–1.18 (m, 2H), 0.83 (t, 3H, J = 7.3 Hz). HRMS (FAB) calcd for $C_{21}H_{19}NO$ 302.1545; found: 302.1574 $(M+H)^+$.

4.36. 6-Butylbenzo[*a*]phenanthridin-5(6*H*)-one (42)

The title compound was prepared by the same method as described for the synthesis of **39**. The title compound was fractionated by means of HPLC. Colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.80 (d, 1H, J = 8.5 Hz), 8.68 (d, 1H, J = 8.5 Hz), 8.64 (dd, 1H, J = 8.5, 1.5 Hz), 7.95 (t, 2H, J = 8.5 Hz), 7.79 (td, 1H, J = 8.5, 1.5 Hz), 7.65–7.60 (m, 3H), 7.52 (t, 1H, J = 8.5 Hz), 4.50 (t, 2H, J = 7.5 Hz), 1.88–1.80 (m, 2H), 1.58–1.53 (m, 2H), 1.03 (t, 3H, J = 7.3 Hz). HRMS (FAB) calcd for $C_{21}H_{19}NO$ 302.1545; found: 302.1534 $(M+H)^+$.

4.37. 5-Butylbenzo[*k*]phenanthridin-6(5*H*)-one (43)

The title compound was prepared by the same method as described for the synthesis of **40**. The title compound was fractionated by means of HPLC. White solid. Mp 54.0–60.0 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.88 (d, 1H, J = 7.3 Hz), 8.66 (d, 1H, J = 8.5 Hz), 8.51 (d, 1H, J = 8.5 Hz), 8.01 (dd, 2H, J = 7.3, 3.0 Hz), 7.95 (d, 1H, J = 8.5 Hz), 7.70–7.63 (m, 3H), 7.59 (t, 1H, J = 7.3 Hz), 7.53 (d, 2H, J = 8.5 Hz), 7.36 (t, 2H, J = 8.5 Hz), 4.45 (t, 2H, J = 7.9 Hz), 1.90–1.83 (m, 3H), 1.57–1.51 (m, 2H), 1.03 (t, 3H, J = 7.5 Hz). HRMS (FAB) calcd for $C_{21}H_{19}NO$ 302.1545; found: 302.1567 $(M+H)^+$.

4.38. 6-Butylbenzo[*i*]phenanthridin-5(6*H*)-one (44)

The title compound was prepared by a method similar to that described for the synthesis of **40**. 1-Naphthoyl chloride was used instead of 2-naphthoyl chloride. White amorpous solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.30 (d, 1H, J = 9.0 Hz), 8.44 (dd, 1H, J = 8.0, 1.2 Hz), 8.39 (d, 1H, J = 9.0 Hz), 8n.17 (d, 1H, J = 9.0 Hz), 7.94 (dd, 1H, J = 8.0, 1.2 Hz), 7.77–7.73 (m, 1H), 7.65–7.60 (m, 2H), 7.49 (d, 1H, J = 8.0 Hz), 7.36 (t, 1H, J = 8.0 Hz), 4.50 (t, 2H, J = 7.6 Hz), 1.90–1.83 (m, 2H), 1.63–1.53 (m, 2H), 1.06 (t, 3H, J = 7.3 Hz). HRMS (FAB) calcd for $C_{21}H_{19}NO$ 302.1545; found: 302.1540 $(M+H)^+$.

4.39. Bioassay

NNC #2 cells carrying full-genomic HCV RNA replicons were maintained in high-glucose Dulbecco's modified Eagle's medium (DMEM) in the presence of 10% (v/v) fetal bovine serum (FBS) and 1 mg/mL G418 (Sigma). Cells at 70% confluence were collected after treatment with trypsin and resuspended in the same medium (5×10^4 cells/mL). One hundred microliters of the cell suspension was transferred to each well of a 96-well plate and cultured at 37 °C for 24 h. Then the medium was removed, and 200 μL of DMEM supplemented with 10% FBS containing various concentrations of test compound was added to each well. After incubation for 3 days, the cells were treated with lysis buffer of a TaqMan Gene Expression Cell-to-CT™ Kit. Expression levels of HCV RNA and GAPDH RNA were measured with the kit, according to the manufacturer's instructions. The RNA levels were quantified by real-time RT-PCR using an ABI 7500 Real-Time PCR System (Applied Biosystems). The anti-HCV activity and cytotoxicity of test compounds were expressed as EC_{50} and CC_{50} determined from the decrease of HCV RNA and GAPDH RNA levels, respectively, as described above.

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