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Bioorganic & Medicinal Chemistry 16 (2008) 3780–3790
Bioorganic & Medicinal Chemistry

γ-Carboline derivatives with anti-bovine viral diarrhea virus (BVDV) activity

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Received 7 January 2008; revised 25 January 2008; accepted 27 January 2008
Available online 31 January 2008

Abstract—Based on anti-viral screening of our heteroaromatics derived from thalidomide, the γ-carboline skeleton has been identified as a superior scaffold structure for compounds with potent anti-bovine viral diarrhea virus (BVDV) activity. Structural development studies led to a potent anti-viral agent, SK5M (5-methyl-γ-carboline), with the EC_{50} of 0.26 μM.

1. Introduction

Viruses belonging to the *Flaviviridae* family cause clinically significant diseases in humans and animals. This virus family includes three genera, that is, *Pestiviruses* [including bovine viral diarrhea virus (BVDV)], *Flaviviruses* [including yellow fever (YFV), dengue, and West Nile viruses], and *Hepaciviruses* [including hepatitis C virus (HCV)].[1]

HCV infection is thought to be a major cause of human hepatitis globally.[2,3] The World Health Organization (WHO) estimates that at least 170 million people worldwide are chronically infected with this virus.[4] 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.[5,6] With the exception of YFV, no vaccine exists against the members of *Flaviviridae*. Currently, 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 the infected patients treated with these agents.[7] In addition, the side effects of these agents are sometimes serious. Therefore, alternative agents for the treatment and prevention of HCV infection are urgently needed.

We have been engaged in structural development studies of thalidomide (**1**: *N*-α-phthalimidoglutarimide),[8–11] which was launched as a sedative/hypnotic agent, but subsequently withdrawn because of its teratogenicity.[12] Nevertheless it has since been found to be effective against various diseases, including multiple myeloma (MM), AIDS, and Hansen's disease.[8–12] Our structural development studies based on anti-viral activity-related bioassay systems/molecular targets, including tumor necrosis factor (TNF)-α production inhibition, α-glucosidase inhibition, etc.,[8–11] led us develop compounds with a 6-5-6 fused heteroaromatic ring system (Fig. 1). Based on the structural development scheme shown in Figure 1, we screened commercially available 6-5-6 fused heteroaromatic compounds, that is, fluorene (**2**), carbazole (**3**) and dibenzofuran (**4**), for anti-viral activity (Fig. 2).

Because BVDV is thought to be a good model for human HCV (HCV does not replicate efficiently in cell cultures or animals),[13–15] we performed the anti-viral screening of typical 6-5-6 fused heteroaromatic compounds using Madin–Darby bovine kidney (MDBK) cells infected with BVDV (Nose strain), as described previously.[15,16] We found that all three compounds (**2**–**4**) examined possess anti-BVDV activity without apparent cytotoxicity, though they are not so potent, having

Keywords: Anti-BVDV agents; γ-Carboline; Thalidomide.
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doi:10.1016/j.bmc.2008.01.052

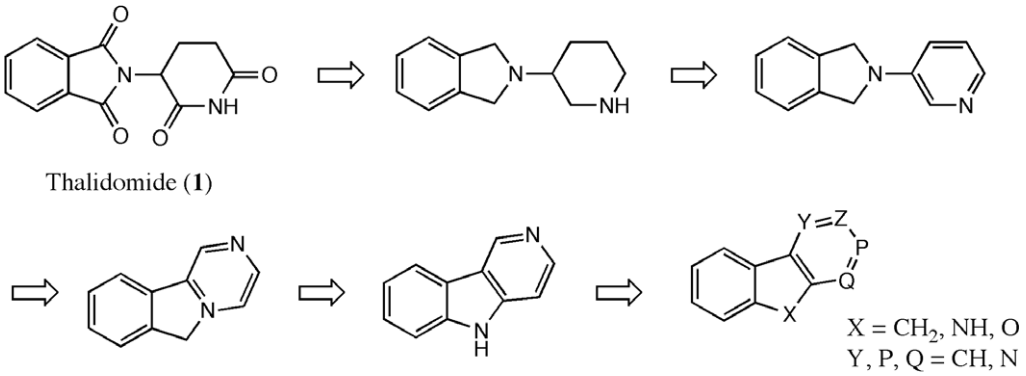


Figure 1. Armchair structural development of thalidomide (**1**) to a 6-5-6 fused heteroaromatic ring system.

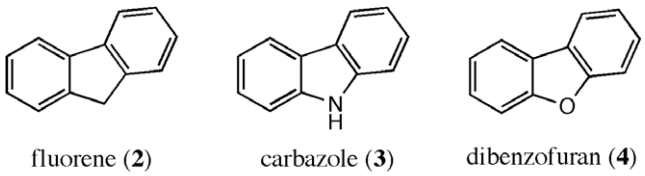


Figure 2. Anti-viral activity and cytotoxicity of commercially available 6-5-6 fused heteroaromatic compounds. EC_{50}: 50% effective concentration, based on the reduction of BVDV replication-induced cell destruction. CC_{50}: 50% cytotoxic concentration, based on the reduction of viable cell number.

EC_{50} values of 58–71 μM (Fig. 2). Among these compounds, we focused on carbazole (**3**) because of its potential for easy derivatization, and we searched for derivatives with potent anti-BVDV activity. Thalidomide (**1**) itself did not show the activity.

In this article, we describe the identification of the γ-carboline skeleton as a scaffold for potent anti-BVDV agents, its structure–activity relationship, and the creation of a potent anti-BVDV agent, SK5M (5-methyl-γ-carboline).

2. Carboline isomers

Based on our preliminary investigation mentioned above, we examined the anti-BVDV activity of carboline isomers (Fig. 3). All of the carboline isomers were prepared, basically as described by other researchers. The synthetic routes to α- (**5**), γ- (**7**), and δ-carboline (**8**) are summarized in Scheme 1.[17] Briefly, Pd-catalyzed cross-coupling reaction of iodobenzenes and aminopyridines with $Pd_2(dba)_3$ and DPPF, followed by *ortho*-coupling reaction using $Pd(OAc)_2$ gave the three carboline isomers, **5**, **7**, and **8**.

The anti-BVDV activity of the prepared carboline isomers is illustrated in Figure 3. As shown in the figure, the introduction of a nitrogen atom into one of the phenyl rings of carbazole (**3**) had a dramatic effect. Introduction of a nitrogen atom at the α (α-carboline: **5**) or *δ* position (δ-carboline: **8**) caused decrease or disappearance of anti-BVDV activity, with no apparent effect on cytotoxicity. On the other hand, introduction of a nitrogen atom at the β (β-carboline: **6**) or γ position (γ-carboline: **7**) greatly enhanced the anti-BVDV activity, affording EC_{50} values of 8.8 and 2.0 μM, respectively. Though the cytotoxicity was also enhanced by the derivatization, the CC_{50} values of β-carboline (**6**) and γ-carboline (**7**) are 8.8 and 20 times larger than the corresponding EC_{50} values, respectively, which seems permissible for lead compounds that are to be structurally developed. Since γ-carboline (**7**) possessed more potent anti-BVDV activity, it was selected for further structural development.

3. γ-Carbolines

Next, we examined the effect of methylation of γ-carboline (**7**) on its anti-BVDV activity and cytotoxicity. The methylated analogs were synthesized as shown in

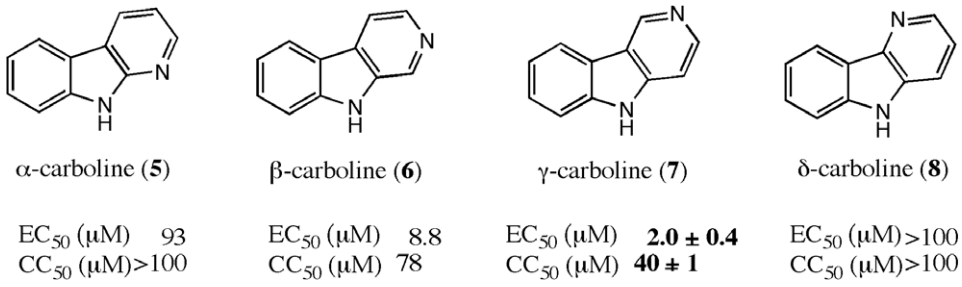
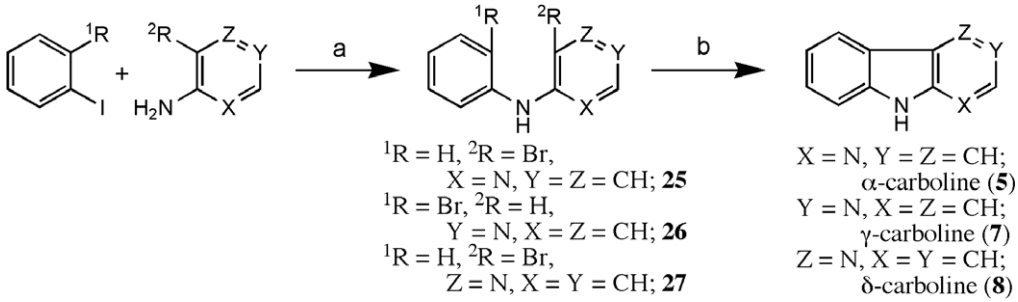
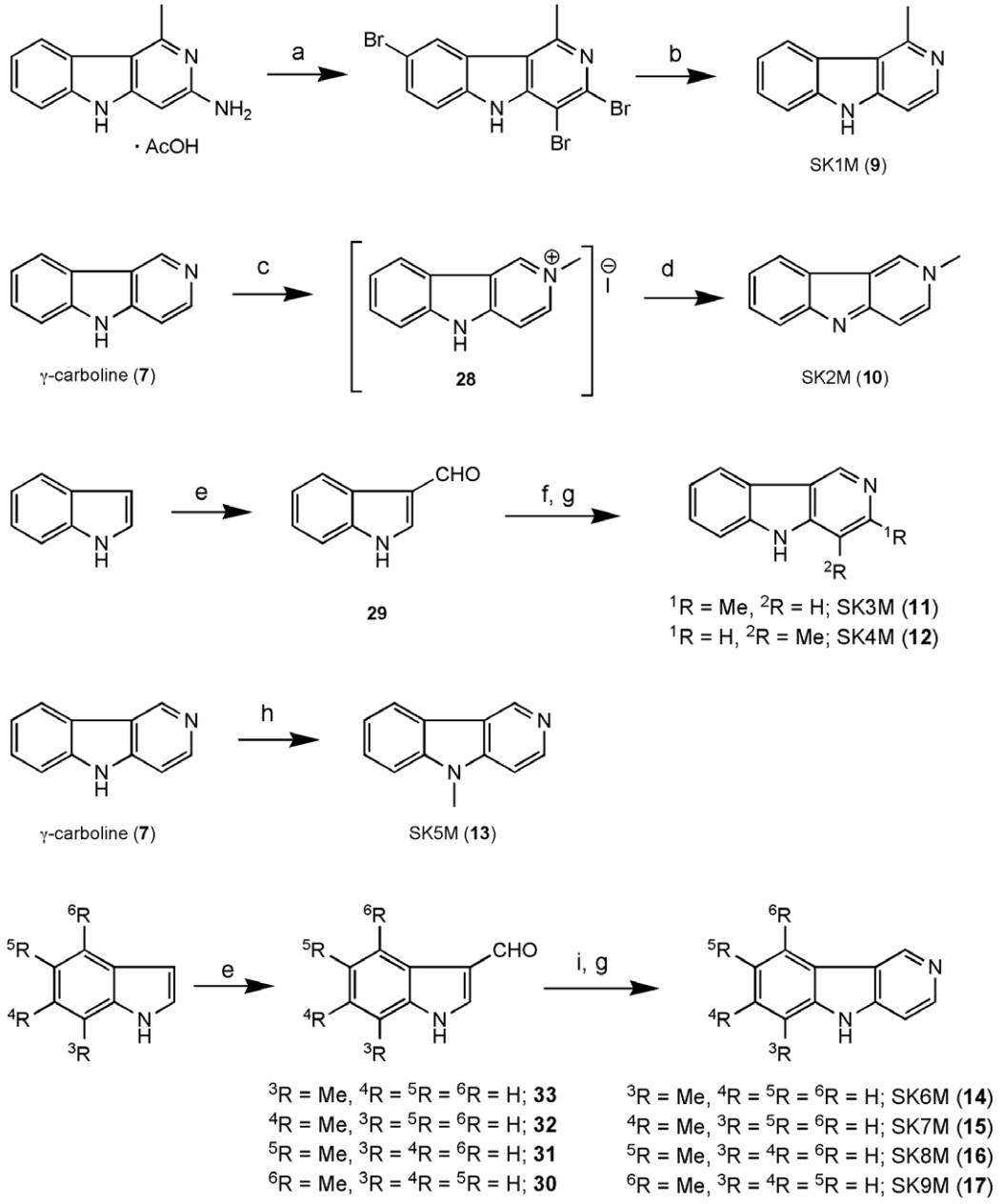


Figure 3. Anti-viral activity and cytotoxicity of carboline isomers (**5**–**8**). EC_{50}: 50% effective concentration, based on the reduction of BVDV replication-induced cell destruction. CC_{50}: 50% cytotoxic concentration, based on the reduction of viable cell number.

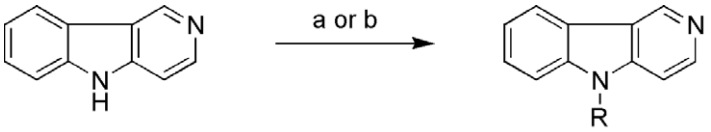


Scheme 1. Reagents and conditions: (a) $Pd_2(dba)_3$, DPPF, *t*-BuONa, toluene, 100–115 °C; (b) $Pd(OAc)_2$, Na_2CO_3, DMF, 165 °C.

Schemes 2 and 3. 1-Methyl-γ-carboline (**9**, SK1M) and 2-methyl-γ-carboline (**10**, SK2M) were synthesized from Trp-P-2·AcOH[18] and γ-carboline (**7**), respectively. Methylated γ-carboline analogs **11**–**17** were synthesized from the 3-formylindole analogs **29**–**33** by means of acid-supported cyclization via the imine derivatives. As



Scheme 2. Reagents and conditions: (a) $NaNO_2$, KBr, aq HBr, 0–80 °C; (b) H_2, 10% Pd/C, MeOH, rt; (c) MeI, 2-PrOH, 100 °C; (d) KOH, hot H_2O, rt; (e) NCS, PPh_3, DMF, THF, H_2O, reflux; (f) R-NH_2, benzene, 110 °C; (g) H_3PO_4, 165 °C; (h) DMC, K_2CO_3, DMF, 160 °C; (i) $(MeO)_2CHCH_2NH_2$, benzene, 110 °C.



R = Et; SK5E (**18**)
R = *n*-C_3H_7; SK5nP (**19**)
R = *iso*-C_3H_7; SK5iP (**20**)
R = *n*-C_4H_9; SK5Bu (**21**)
R = *n*-C_6H_{13}; SK5H (**22**)
R = Bn; SK5Bn (**23**)
R = $CH_2CH_2NEt_2$; SK5DAE (**24**)

Scheme 3. Reagents and conditions: (a) RI, NaH, DMF, THF, rt; (b) ROH, TMAD, P(*n*-Bu)$_3$, THF, toluene, 50 °C.

shown in Scheme 2, bromination of Trp-P-2·AcOH with $NaNO_2$ and KBr under acidic conditions, followed by debromination with Pd/C under an H_2 atmosphere, afforded SK1M (**9**). The methylpyridinium iodide intermediate **28** was prepared from γ-carboline and MeI, and then treatment of **28** with KOH aqueous solution gave SK2M (**10**). The formylation of indole and its methyl analogs with NCS, PPh_3, and DMF by Sugimoto's method[19] gave the 3-formylindole derivatives **29**–**33**, followed by condensation with acetalamine and cyclization with H_3PO_4 to afford SK3M (**11**), SK4M (**12**), SK6M (**14**), SK7M (**15**), SK8M (**16**), and SK9M (**17**), respectively. *N*-Methylation of γ-carboline at the 5-position was carried out using dimethyl carbonate with K_2CO_3 to afford SK5M (**13**).

The anti-BVDV activity and cytotoxicity of the prepared methylated γ-carboline derivatives (**9**–**17**) are shown in Figure 4. The effect of methylation on the activity seems to be dependent on the position at which

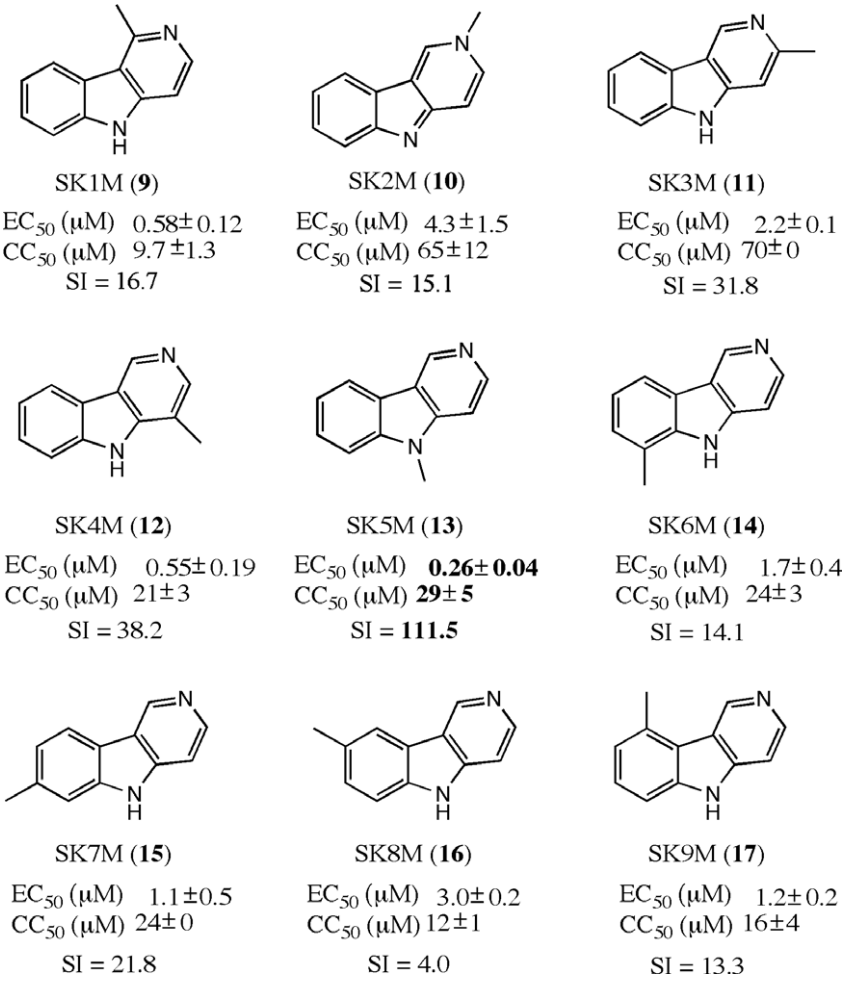


Figure 4. Anti-viral activity, cytotoxicity, and selectivity index of methylated γ-carboline derivatives (**9**–**17**). EC_{50}: 50% effective concentration, based on the reduction of BVDV replication-induced cell destruction. CC_{50}: 50% cytotoxic concentration, based on the reduction of viable cell number. SI: CC_{50}/EC_{50}.

the methyl substituent is introduced. Introduction of a methyl substituent at positions 2, 3, and 8 decreased the anti-BVDV activity, whereas introduction at other sites, that is, positions 1, 4, 5, 6, 7, and 9, increased the activity. Broadly speaking, methylation at sites that cause the resulting structure to become longer (positions 2, 3, and 8: that is, the β and γ positions) seems to decrease the activity, with the exception of SK7M (**15**), while methylation at sites that cause the resulting structure to become wider (positions 1, 4, 5, 6, and 9: that is, the nitrogen atom at position 1, and the α and δ positions) seems to enhance the activity. The 5-methylated analog, SK5M (**13**), showed the most potent anti-BVDV activity among this series of the compounds, with an EC_{50} value of 0.26 μM.

As for cytotoxicity, the introduction of a methyl group at positions 2 and 3 decreased the cytotoxicity, while introduction at other positions increased it. Thus, the methyl substituent effects on anti-BVDV activity and cytotoxicity showed only a limited correlation, and as a result, the values of the selectivity index (SI value = CC_{50}/EC_{50}) varied from 4.0 to 111.5. From the standpoints of both potent anti-BVDV activity and high selectivity index, SK5M (**13**) seems to be the best lead compound, having EC_{50} and SI values of 0.26 μM and 111.5, respectively. The dose–response curves of the anti-BVDV activity and the cytotoxicity elicited by SK5M (**13**) are shown in Figure 5.

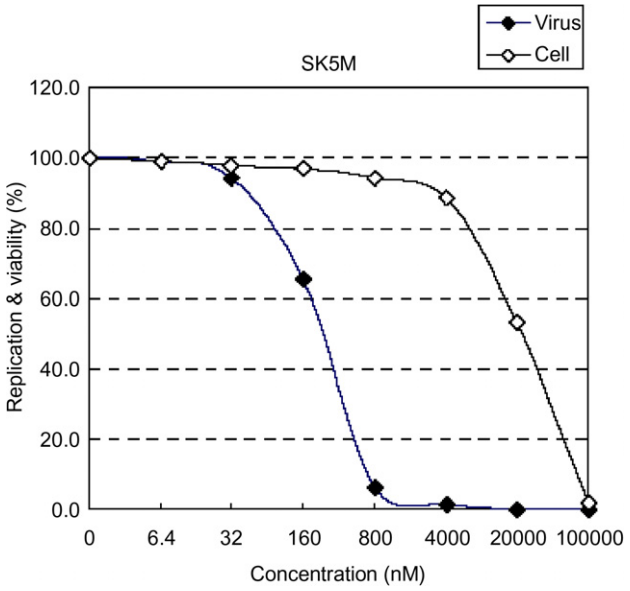
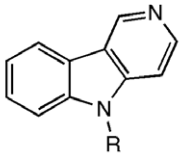


Figure 5. Anti-viral activity and cytotoxicity elicited by SK5M (**13**).

Because the nitrogen atom at the 5 position appeared to be the most appropriate position at which to introduce a substituent (vide supra), other substituents were introduced at this position (compounds **18**–**24**, Fig. 6). Synthesis of these *N*-alkylated derivatives of γ-carboline **18**–**24** is summarized in Scheme 3. Compounds **18**–**22** were prepared by general *N*-alkylation using NaH and the corresponding alkyl iodide. Compounds **23** and **24** were synthesized by using the corresponding alkyl alcohol with TMAD and P(*n*-Bu)$_3$.

As shown in Figure 6, introduction of an ethyl group at the nitrogen atom at the 5-position (SK5E: **18**) did not affect the anti-BVDV activity of γ-carboline (**7**), but enhanced the cytotoxicity. Substitution at the same position with a larger group decreased the anti-BVDV activity compared with that of γ-carboline (**7**). The result suggests that introduction of a bulky group at the nitrogen atom at position 5 is unfavorable for the activity of the derivatives. The substituent effect seems to be larger for cytotoxicity than for anti-BVDV activity, because the anti-BVDV activities of compounds **19**–**24** are similar, with EC_{50} values of 8.2–11 μM [except SK5H (**22**) whose EC_{50} value is larger than its CC_{50} value], whereas CC_{50} of these compounds range from 7.4 to 30 μM. In the case of derivatives with a normal alkyl chain pendant, a clear structure–activity relationship for cytotoxicity was observed, that is, cytotoxicity increased in the order of: SK5M (methyl: **13**) > SK5E (ethyl: **18**) > SK5nP (*n*-propyl: **19**) > SK5Bu (*n*-butyl: **21**) > SK5H (*n*-hexyl: **22**). The potency order of anti-BVDV activity of these compounds is just the reverse,



compound	R	EC_{50} (μM)	CC_{50} (μM)
SK5E (**18**)	CH_2CH_3	2.0	29
SK5nP (**19**)	$CH_2CH_2CH_3$	8.2	26
SK5iP (**20**)	$CH(CH_3)_2$	11	30
SK5Bu (**21**)	$CH_2(CH_2)_2CH_3$	9.2	14
SK5H (**22**)	$CH_2(CH_2)_4CH_3$	>7.4	7.4
SK5Bn (**23**)	CH_2Ph	8.7	11
SK5DAE (**24**)	$CH_2CH_2N(CH_2CH_3)_2$	8.9	10

Figure 6. Anti-viral activity and cytotoxicity of *N*-substituted γ-carboline derivatives (**18**–**24**). EC_{50}: 50% effective concentration, based on the reduction of BVDV replication-induced cell destruction. CC_{50}: 50% cytotoxic concentration, based on the reduction of viable cell number.

except for SK5H (**22**), whose EC_{50} value could not be determined (vide supra). Thus, SK5M (**13**) was considered to be the best lead compound, having the most potent anti-BVDV activity and the largest SI value among the compounds prepared. Since SK5M could suppress viral RNA synthesis at 6 h after virus infection in a dose-dependent fashion (data not shown), it is assumed that the compound inhibits an early step of the replication cycle of BVDV.

4. Growth inhibition of HL-60 cells by γ-carbolines

To explore the mechanism of cytotoxicity of γ-carbolines, we examined the effect of γ-carboline (**7**) (low cytotoxicity) and SK1M (**9**) (high cytotoxicity) on apoptosis involving the caspase cascade. First, we investigated the cell growth-inhibitory activity of γ-carboline (**7**) and SK1M (**9**) toward HL-60 cells. Both compounds inhibited HL-60 cell growth, with IC_{50} values of 11.4 μM for γ-carboline (**7**) and 15.3 μM for SK1M (**9**). Next, we examined the effect of Z-VAD fmk, a caspase-family inhibitor, on the HL-60 cell growth-inhibitory activity elicited by γ-carboline (**7**) and SK1M (**9**). As shown in Figure 7, the cell growth inhibition elicited by γ-carboline (**7**) was completely reversed by Z-VAD fmk (closed column), although the growth inhibition elicited by SK1M (**9**) was only partially reversed by addition of Z-VAD fmk (closed column). These results suggest that the cytotoxicity elicited by γ-carboline (**7**) and SK1M (**9**) is at least partly due to the induction of apoptosis via the caspase cascade.

5. Conclusion

Based on a 6-5-6 fused heteroaromatic system derived from thalidomide, we developed a potent lead compound for anti-BVDV agents, SK5M (**13**), which has an EC_{50} value of 0.26 μM and an SI value of 111.5. Further structural development should yield highly potent and selective drug candidates.

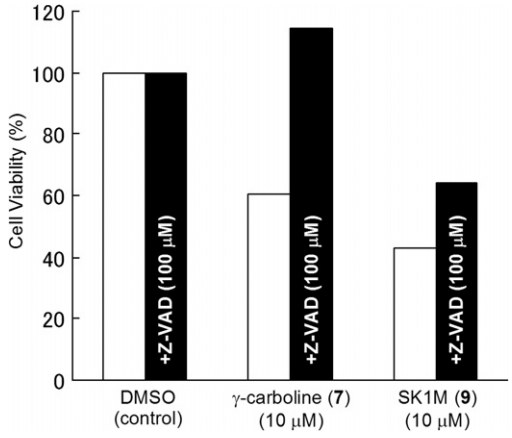


Figure 7. Growth inhibition of HL-60 cells by 10 μM γ-carboline (**7**) and SK1M (**9**) in the presence (filled column) or absence (open column) of Z-VAD fmk (100 μM).

6. Experimental

6.1. 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.

6.2. Materials

Fluorene (**2**) was purchased from Sigma–Aldrich, Inc. Carbazole (**3**) and dibenzofuran (**4**) were purchased from Kanto Chemical Co., Inc. β-Carboline (**6**) was purchased from Tokyo Chemical Industry Co., Ltd. All other compounds were of the best available commercial grade.

6.2.1. 2-Anilino-3-bromopyridine (25)[17]. A mixture of 2-amino-3-bromopyridine (204 mg, 1.18 mmol), iodobenzene (0.160 mL, 1.43 mmol), *t*-BuONa (162 mg, 1.68 mmol), tris(dibenzylideneacetone)-dipalladium (54.7 mg, 0.060 mmol), and 1,1′-bis(diphenylphosphino)ferrocene (66.7 mg, 0.120 mmol) in dry toluene (15 mL) was stirred for 14 h at 100 °C. After cooling to room temperature, the mixture was diluted with Et_2O, filtered through a Celite pad, and washed with Et_2O. The filtrate was evaporated, and the residue was purified by silica-gel column chromatography using *n*-hexane/AcOEt (20:1) as the eluent to give 204 mg (69.3%) of the title compound as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.16 (br, 1H), 7.75 (d, J = 7.3 Hz, 1H), 7.61 (d, J = 7.9 Hz, 2H), 7.36 (t, J = 7.9 Hz, 2H), 7.09–7.05 (m, 2H), 6.64 (br, 1H). MS (FAB, M^+) *m*/*z* 249.

6.2.2. α-Carboline (5)[17]. A mixture of 2-anilino-3-bromopyridine (109 mg, 0.437 mmol), $Pd(OAc)_2$ (10.5 mg, 0.047 mmol), and Na_2CO_3 (67.8 mg, 0.640 mmol) in dry DMF (5 mL) was refluxed at 165 °C for 67 h. After cooling to room temperature, the mixture was diluted with AcOEt and filtered through a Celite pad. The filtrate was washed successively with water and brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica-gel column chromatography using *n*-hexane/AcOEt (10:1) as the eluent to give 13.5 mg (18.4%) of the title compound as a yellow powder. Mp 210–211 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.91 (s, 1H), 8.56 (d, J = 7.7 Hz, 1H), 8.43 (d, J = 4.7 Hz, 1H), 8.18 (d, J = 8.1 Hz, 1H), 7.52 (d, J = 8.1 Hz, 1H), 7.47 (t, J = 8.1 Hz, 1H), 7.26–7.23 (m, 2H). HRMS (FAB) calcd for $C_{11}H_9N_2$ 169.0766; found: 169.0816 $(M+H)^+$.

6.2.3. 4-(2′-Bromoanilino)pyridine (26)[17]. A mixture of 4-aminopyridine (944 mg, 10.0 mmol), 1-bromo-2-iodo-

benzene (1.35 mL, 10.5 mmol), *t*-BuONa (1.14 g, 11.9 mmol), tris(dibenzylideneacetone)-dipalladium (137 mg, 0.150 mmol), and 1,1′-bis(diphenylphosphino)ferrocene (200 mg, 0.361 mmol) in dry toluene (30 mL) was stirred for 18 h at 115 °C. After cooling to room temperature, the mixture was diluted with Et_2O and filtered through a Celite pad. The filtrate was evaporated, and purified by silica-gel column chromatography using CH_2Cl_2/MeOH (29:1 to 9:1) as the eluent to give 2.50 g (99.9%) of the title compound as a gray solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.34 (dd, J = 2.0, 6.5 Hz, 2H), 7.60 (dd, J = 1.5, 8.0 Hz, 1H), 7.44 (dd, J = 1.5, 8.0 Hz, 1H), 7.29 (ddd, J = 1.5, 8.0, 8.0 Hz, 1H), 6.96 (ddd, J = 1.5, 8.0, 8.0 Hz, 1H), 6.87 (dd, J = 2.0, 6.5 Hz, 1H), 6.18 (s, 1H). MS (FAB, $[M+H]^+$) m/z 249, 251.

6.2.4. γ-Carboline (7)[17]. A mixture of 4-(2′-bromoanilino) pyridine (2.50 g, 10.0 mmol), $Pd(OAc)_2$ (112 mg, 0.500 mmol), and Na_2CO_3 (1.48 g, 14.0 mmol) in DMF (20 mL) was stirred for 20 h at 165 °C. After cooling to room temperature, the mixture was diluted with AcOEt and washed successively with water. The organic phase was extracted with 2 N HCl aq. and the aqueous phase was neutralized with NaOH pellets. The resulting brown precipitate was removed by filtration and additional NaOH pellets were added to the pale-yellow filtrate until it became basic. The resulting precipitate was collected in a funnel by suction, washed with water, and dried in vacuo. The crude product was purified by recrystallization from toluene to give 1.21 g (72.0%) of the title compound as a pale-yellow powder. Mp 230–231 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.70 (s, 1H), 9.32 (s, 1H), 8.41 (dd, J = 1.0, 5.5 Hz, 1H), 8.22 (d, J = 8.0 Hz, 1H), 7.55 (d, J = 8.0 Hz, 1H), 7.46 (dd, J = 8.0, 8.0 Hz, 1H), 7.45 (d, J = 5.5 Hz, 1H), 7.25 (dd, J = 8.0, 8.0 Hz, 1H). HRMS (FAB) calcd for $C_{11}H_9N_2$ 169.0766; found: 169.0785 $(M+H)^+$.

6.2.5. 3-Anilino-2-bromopyridine (27)[17]. A mixture of 3-amino-2-bromopyridine (205 mg, 1.19 mmol), iodobenzene (0.160 mL, 1.43 mmol), *t*-BuONa (161 mg, 1.67 mmol), tris(dibenzylideneacetone)-dipalladium (57.3 mg, 0.0626 mmol), and 1,1′-bis(diphenylphosphino)ferrocene (68.9 mg, 0.124 mmol) in dry toluene (15 mL) was stirred at 100 °C for 14 h. After cooling to room temperature, the mixture was diluted with Et_2O and filtered through a Celite pad. The filtrate was evaporated, and the residue was purified by silica-gel column chromatography using *n*-hexane/AcOEt (20:1) as the eluent to give 109 mg (36.6%) of the title compound as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (dd, J = 4.7 1.7 Hz, 1H), 7.44 (dd, J = 8.1, 1.7 Hz, 1H), 7.36 (dt, J = 7.3, 0.86 Hz, 2H), 7.16 (dd, J = 7.3, 0.86 Hz, 2H), 7.14–7.08 (m, 2H), 6.16 (s, 1H). MS (FAB, M^+) m/z 249.

6.2.6. δ-Carboline (8)[17]. A mixture of 3-anilino-2-bromopyridine (109 mg, 0.436 mmol), $Pd(OAc)_2$ (12.3 mg, 0.055 mmol), and Na_2CO_3 (66.5 mg, 0.627 mmol) in dry DMF (5 mL) was refluxed at 165 °C for 67 h. After cooling to room temperature, the mixture was diluted with AcOEt and filtered through a Celite pad. The filtrate was washed successively with water and brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica-gel column chromatography using *n*-hexane/AcOEt (10:1) as the eluent to give 30.2 mg (41.2%) of the title compound as a yellow powder. Mp 189–190 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.42 (s, 1H), 8.44 (d, J = 4.3 Hz, 1H), 8.17 (d, J = 7.7 Hz, 1H), 7.86 (d, J = 8.1 Hz, 1H), 7.55 (d, J = 7.7 Hz, 1H), 7.49 (t, J = 7.7 Hz, 1H), 7.38 (dd, J = 8.1, 4.3 Hz, 1H), 7.23 (t, J = 7.7 Hz, 1H). HRMS (FAB) calcd for $C_{11}H_9N_2$ 169.0766; found: 169.0794 $(M+H)^+$.

6.2.7. 1-Methyl-γ-carboline (9, SK1M). A solution of Trp-P-2·AcOH[18] (103 mg, 0.400 mmol) in a mixture of concd HBr aq/H_2O = 4:1 (5.0 mL) was added to a solution of $NaNO_2$ (55.2 mg, 0.800 mmol) in H_2O (0.5 mL) at 0 °C and the mixture was stirred for 5 min at the same temperature. To this was added a solution of KBr (191 mg, 1.60 mmol) in H_2O (1.0 mL) and the whole was stirred at 80 °C for 3 h. After cooling to room temperature, the reaction was quenched by adding 10 N NaOH aq. and saturated aqueous $NaHCO_3$ solution until the solution became basic. The aqueous solution was then extracted with AcOEt and the organic phase was washed with water, dried over $MgSO_4$, and evaporated. The residue was purified by silica-gel column chromatography using *n*-hexane/AcOEt (6:1) as the eluent to give 19.1 mg (11.4%) of tribrominated 1-methyl-γ-carboline as a pale-yellow powder. This material was used in the next step without further examination. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.28 (s, 1H), 8.25 (d, J = 1.5 Hz, 1H), 7.69 (dd, J = 1.5, 9.0 Hz, 1H), 7.59 (d, J = 9.0 Hz, 1H), 2.88 (s, 3H). MS (FAB, $[M+H]^+$) m/z 419, 422.

A solution of the tribrominated 1-methyl-γ-carboline (19.1 mg, 0.046 mmol) in a mixture of MeOH (1.5 mL) and AcOEt (1.0 mL) was added to Et_3N (25.0 μL, 0.179 mmol) and 10% Pd/C (0.8 mg), and the mixture was stirred at room temperature under an H_2 atmosphere. After 18 h, the reaction mixture was filtered through a Celite pad and the filtrate was evaporated. To the residue was added saturated aqueous $NaHCO_3$ solution and the aqueous phase was extracted with AcOEt. The organic phase was dried over $MgSO_4$ and evaporated. The residue was purified by silica-gel column chromatography using acetone/MeOH (20:1) as the eluent to give 7.7 mg (92.6%) of the title compound as a white powder. Mp 229–230 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.72 (s, 1H), 8.27 (d, J = 5.5 Hz, 1H), 8.13(d, J = 8.5 Hz, 1H), 7.56 (d, J = 8.5 Hz, 1H), 7.46 (t, J = 7.5 Hz, 1H), 7.32 (d, J = 5.5 Hz, 1H), 7.28 (t, J = 7.5 Hz, 1H), 2.92 (s, 3H). HRMS (FAB) calcd for $C_{12}H_{11}N_2$ 183.0922; found: 183.0953 $(M+H)^+$.

6.2.8. 2-Methyl-γ-carbolinium iodide (28)[19]. A mixture of γ-carboline (**7**) (173 mg, 1.03 mmol) and iodomethane (0.100 mL, 1.61 mmol) in 2-propanol (8 mL) was refluxed at 100 °C for 3 h. Then the excess iodomethane and 2-propanol were removed under reduced pressure, and the residue was recrystallized from EtOH to give 268 mg (83.7%) of the title compound as a white pow-

der. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.61 (s, 1H), 8.55 (d, J = 7.3 Hz, 1H), 8.34 (d, J = 7.9 Hz, 1H), 7.92 (d, J = 7.3 Hz, 1H), 7.77–7.71 (m, 2H), 7.53 (dd, J = 7.9, 8.1 Hz, 1H), 4.43 (s, 3H). MS (FAB, $[M+H]^+$) m/z 183.

6.2.9. 2-Methyl-γ-carboline (10, SK2M)[19]. A solution of 2-methyl-γ-carbolinium iodide (163 mg, 0.526 mmol) in hot water (5 mL) was added to 50% aqueous KOH solution (2.6 mL). The resulting light yellow precipitate was separated by filtration, washed with cold water, and dried in vacuo to give 39.0 mg (52.6%) of the title compound as a light yellow powder. Mp 172–173 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.67 (s, 1H), 8.02 (d, J = 7.7 Hz, 1H), 7.87(d, J = 7.9 Hz, 1H), 7.66–7.62 (m, 2H), 7.57 (dd, J = 7.3, 8.1 Hz, 1H), 7.29 (dd, J = 7.3, 7.9 Hz, 1H), 4.24 (s, 3H). HRMS (FAB) calcd for $C_{12}H_{11}N_2$ 183.0922; found: 183.0967 $(M+H)^+$.

6.2.10. 3-Formylindole (29): typical procedure for the formylation of indoles. *N*-Chlorosuccinimide (1.07 g, 8.01 mmol) was added little by little to a solution of PPh_3 (2.09 g, 7.98 mmol) in dry THF (80 mL) with stirring for 30 min at room temperature. *N*,*N*-Dimethylformamide (1.24 mL, 16.0 mmol) was added to the suspension, and the mixture was refluxed for 1 h. Then to this was added 1*H*-indole (313 mg, 2.67 mmol), and the mixture was refluxed for 1 h. The volatile solvent was removed, and to the remaining solution was added H_2O (30 mL). The mixture was refluxed for an additional 1 h. The reaction was quenched by adding NaOH aqueous solution and the mixture was extracted with AcOEt. The organic phase was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by silica-gel column chromatography using *n*-hexane/AcOEt (1:1) as the eluent to give 3-formylindole (292 mg, 75.3%) as a yellow powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.1 (s, 1H), 8.66 (br, 1H), 8.33 (dd, J = 3.0, 6.0 Hz, 1H), 7.85 (d, J = 3.0 Hz, 1H), 7.47–7.43 (m, 1H), 7.36–7.31 (m, 2H). MS (FAB, $[M+H]^+$) m/z 146.

6.2.11. 3-Formyl-4-methylindole (30). According to the typical procedure, 3-formyl-4-methylindole (**30**) was obtained from 4-methyl-1*H*-indole as a yellow powder, which was used in the next step without purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.2 (s, 1H), 8.73 (br, 1H), 7.95 (d, J = 3.4 Hz, 1H), 7.28 (d, J = 8.1 Hz, 1H), 7.21 (dd, J = 7.1, 8.1 Hz, 1H), 7.09 (d, J = 7.1 Hz, 1H), 2.86 (s, 3H). MS (FAB, $[M+H]^+$) m/z 160.

6.2.12. 3-Formyl-5-methylindole (31). According to the typical procedure, 3-formyl-5-methylindole (**31**) was obtained from 5-methyl-1*H*-indole as a yellow powder, which was used in the next step without purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.0 (s, 1H), 8.73 (br, 1H), 8.13 (s, 1H), 7.81 (d, J = 3.0 Hz, 1H), 7.33 (d, J = 8.6 Hz, 1H), 7.15 (d, J = 8.6 Hz, 1H), 2.49 (s, 3H). MS (FAB, $[M+H]^+$) m/z 160.

6.2.13. 3-Formyl-6-methylindole (32). According to the typical procedure, 3-formyl-6-methylindole (**32**) was obtained from 6-methyl-1*H*-indole as a pale red powder, which was used in the next step without purification. ^{1}H NMR (500 MHz, $CDCl_3$) d 10.0 (s, 1H), 8.59 (br, 1H), 8.18 (d, J = 8.3 Hz, 1H), 7.78 (d, J = 3.0 Hz, 1H), 7.23 (s, 1H), 7.16 (d, J = 8.3 Hz, 1H), 2.48 (s, 3H). MS (FAB, $[M+H]^+$) m/z 160.

6.2.14. 3-Formyl-7-methylindole (33). According to the typical procedure, 3-formyl-7-methylindole (**33**) was obtained from 7-methyl-1*H*-indole as a yellow powder, which was used in the next step without purification. ^{1}H NMR (500 MHz, DMSO-d_6) d 10.1 (s, 1H), 9.25 (br, 1H), 8.15 (d, J = 7.9 Hz, 1H), 7.81 (d, J = 3.1 Hz, 1H), 7.23 (dd, J = 7.9, 7.3 Hz, 1H), 7.12 (d, J = 7.3 Hz, 1H), 2.53 (s, 3H). MS (FAB, $[M+H]^+$) m/z 160.

6.2.15. Benzyl 1-(*N*-methoxy-*N*-methylcarbamoyl)ethylcarbamate (34). A mixture of *N*-CBZ-L-alanine (230 mg, 1.03 mmol), *N*,*O*-dimethylhydroxylamine hydrochloride (204 mg, 2.09 mmol), 1-hydroxybenzotriazole monohydrate (169 mg, 1.25 mmol), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (246 mg, 1.59 mmol), and diisopropylethylamine (0.220 mL, 1.26 mmol) in dry CH_2Cl_2 (10 mL) was stirred for 30 min at room temperature. The reaction was quenched by adding water and the organic phase was washed with water, dried over $MgSO_4$, and evaporated. The residue was purified by silica-gel column chromatography using *n*-hexane/AcOEt (1:1) as the eluent to give 239 mg (87.0%) of the title compound as a white powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37–7.28 (m, 5H), 5.53 (br s, 1H), 5.10 (q, J = 12.4 Hz, 2H), 4.76–4.73 (m, 1H), 3.77 (s, 3H), 3.21 (s, 3H), 1.35 (d, J = 6.8 Hz, 3H). MS (FAB, $[M+H]^+$) m/z 267.

6.2.16. Benzyl 1-formylethylcarbamate (35). A mixture of **34** (239 mg, 0.896 mmol) and lithium aluminum hydride (38.7 mg, 1.02 mmol) in dry THF (5 mL) was stirred for 15 min at 0 °C. The reaction was quenched by adding $Na_2SO_4{\cdot}10H_2O$ and $MgSO_4$. The resulting precipitate was removed by filtration. The filtrate was evaporated and the residue was purified by silica-gel column chromatography using *n*-hexane/AcOEt (3:1) as the eluent to give 77.3 mg (41.6%) of the title compound as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.64 (s, 1H), 7.27–7.22 (m, 5H), 5.35 (br s, 1H), 5.02 (s, 2H), 4.24–4.16 (m, 1H), 1.27 (d, J = 7.7 Hz, 3H). MS (FAB, $[M+H]^+$) m/z 208.

6.2.17. Benzyl 1-(1,3-dioxolan-2-yl)ethylcarbamate (36). A mixture of **35** (73.7 mg, 0.356 mmol), pyridinium *p*-toluenesulfonate (4.3 mg, 17.1 μmol), and ethylene glycol (64 μL, 1.15 mmol) in dry benzene (6 mL) was refluxed for 4 h at 110 °C. The reaction was quenched by adding water, and the organic phase was washed with water, saturated aqueous $NaHCO_3$ solution, and brine, dried over $MgSO_4$, and evaporated. The residue was purified by Chromatorex® column chromatography using*n*-hexane/AcOEt (4:1) as the eluent to give 56.9 mg (63.6%) of the title compound as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36–7.30 (m, 5H), 5.11 (s, 2H), 4.85 (s, 1H), 4.02–3.86 (m, 4H), 1.16 (d, J = 7.3 Hz, 3H). MS (FAB, $[M+H]^+$) m/z 252.

6.2.18. 3-Methyl-γ-carboline (11, SK3M). A mixture of **36** (397 mg, 1.58 mmol) and 20% $Pd(OH)_2$/C (404 mg)

in AcOEt (10 mL) was stirred under an H_2 atmosphere for 15 h at room temperature. The reaction mixture was filtered through a Celite pad and the filtrate was evaporated. To this was added a solution of **29** (188 mg, 1.30 mmol) in dry benzene (10 mL) and the mixture was refluxed for 5 h at 110 °C. After the mixture had cooled to room temperature, the benzene was removed under reduced pressure. Then 90% phosphoric acid, which had been stirred for 2 h at 165 °C, was added to the residue and stirring was continued for another 30 min at 165 °C. To the reaction mixture was added ice and the resulting precipitate was filtered through a Celite pad. The filtrate was basified with dil NH_3 and extracted with CH_2Cl_2. The organic phase was extracted with dil. HCl, and the HCl layer was basified with dil. NaOH aqueous solution. The resulting precipitate was collected in a funnel and purified by PLC using chloroform/EtOH (10:1) as the eluent to give 4.3 mg (3.1%, three steps) of the title compound as a pale brown powder. Mp 225–226 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.4 (s, 1H), 8.75 (s, 1H), 8.17 (d, J = 7.7 Hz, 1H), 7.93 (s, 1H), 7.54 (d, J = 8.1 Hz, 1H), 7.50 (dd, J = 6.8, 8.1 Hz, 1H), 7.19 (dd, J = 6.8, 7.7 Hz, 1H), 2.60 (s, 3H). HRMS (FAB) calcd for $C_{12}H_{11}N_2$ 183.0922; found: 183.0952 $(M+H)^+$.

6.2.19. Benzyl 2-hydroxypropylcarbamate (37). To a mixture of 1-amino-2-propanol (0.780 mL, 9.97 mmol), 4-(dimethylamino)pyridine (250 mg, 2.05 mmol), and triethylamine (6.0 mL, 42.9 mmol) in dry DMSO (10 mL) was added benzyl chloroformate (6.42 mL, 45.0 mmol) little by little, and the mixture was stirred for 5 h at 50 °C. The reaction was quenched by adding saturated aqueous $NaHCO_3$ solution, and the whole was extracted with AcOEt. The organic phase was washed with dil. HCl and brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica-gel column chromatography using *n*-hexane/AcOEt (5:1) as the eluent to give 1.70 g (81.5%) of the title compound as a pale-yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28–7.19 (m, 5H), 5.06 (br, 1H), 5.02 (s, 2H), 3.86–3.81 (m, 1H), 3.28–3.23 (m, 1H), 3.00–2.94 (m, 1H), 1.09 (d, J = 6.4 Hz, 3H). MS (FAB, $[M+H]^+$) *m*/*z* 210.

6.2.20. Benzyl 2-oxopropylcarbamate (38). A mixture of **37** (1.70 g, 8.15 mmol), K_2CO_3 (11.4 g, 8.21 mmol), *N*-chlorosuccinimide (1.21 g, 9.06 mmol), *N*-*tert*-butylbenzenesulfenamide (74.0 μL, 0.470 mmol), and molecular sieves 4 Å (8.15 g) in dry CH_2Cl_2 was stirred for 2 h at 0 °C. The resulting precipitate was removed by filtration through a Celite pad, and the filtrate was washed with saturated aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica-gel column chromatography using *n*-hexane/AcOEt (3:1) as the eluent to give 761 mg (45.0%) of the title compound as a white powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28–7.19 (m, 5H), 5.37 (br, 1H), 5.02 (s, 2H), 4.01 (d, J = 5.1 Hz, 2H), 2.09 (s, 3H). MS (FAB, $[M+H]^+$) *m*/*z* 208.

6.2.21. Benzyl (2-methyl-1,3-dioxolan-2-yl)methylcarbamate (39). A mixture of **38** (761 mg, 3.67 mmol), pyridinium *p*-toluenesulfonate (38.5 mg, 0.153 mmol), and ethylene glycol (0.660 mL, 11.8 mmol) in dry benzene (10 mL) was refluxed for 4 h at 110 °C. The reaction was quenched by adding water, and the organic phase was washed with water, saturated aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$, and evaporated. The residue was purified by Chromatorex® column chromatography using *n*-hexane/AcOEt (3:1) as the eluent to give 791 mg (85.8%) of the title compound as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27–7.20 (m, 5H), 5.02 (s, 1H), 4.89 (br, 1H), 3.89–3.82 (m, 4H), 3.26 (d, J = 6.0 Hz, 1H), 1.24 (s, 3H). MS (FAB, $[M+H]^+$) *m*/*z* 252.

6.2.22. 4-Methyl-γ-carboline (12, SK4M). A mixture of **39** (791 mg, 3.15 mmol) and 20% $Pd(OH)_2$/C (811 mg) in AcOEt (20 mL) was stirred under an H_2 atmosphere for 5 h at room temperature. The reaction mixture was filtered through a Celite pad and the filtrate was evaporated. To this was added a solution of **29** (458 mg, 3.91 mmol) in dry benzene (10 mL) and the mixture was refluxed for 5 h at 110 °C. After the mixture had cooled to room temperature, the benzene was removed under reduced pressure. Then 90% phosphoric acid, which had been treated for 2 h at 165 °C, was added to the residue and stirring was continued for another 30 min at 165 °C. To the reaction mixture was added ice, and the resulting precipitate was removed by filtration through a Celite pad. The filtrate was basified with dil. NH_3 and extracted with CH_2Cl_2. The organic phase was extracted with dil. HCl and the HCl layer was basified with dil NaOH aqueous solution. The resulting precipitate was collected in a funnel and purified by silica-gel column chromatography using chloroform/EtOH (10:1) as the eluent to give 32.6 mg (5.7%, three steps) of the title compound as a pale brown powder. Mp 197–198 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.7 (s, 1H), 9.17 (s, 1H), 8.24 (s, 1H), 8.19 (d, J = 7.5 Hz, 1H), 7.56 (d, J = 8.1 Hz, 1H), 7.46 (dd, J = 7.1, 8.1 Hz, 1H), 7.25 (dd, J = 7.1, 7.5 Hz, 1H), 2.51 (s, 3H). HRMS (FAB) calcd for $C_{12}H_{11}N_2$ 183.0922; found: 183.0875 $(M+H)^+$.

6.2.23. 5-Methyl-γ-carboline (13, SK5M). A mixture of γ-carboline (**7**) (83.0 mg, 0.493 mmol), K_2CO_3 (32.3 mg, 0.234 mmol) and dimethyl carbonate (0.850 mL, 1.01 mmol) in dry DMF (5 mL) was refluxed for 7 h at 160 °C. After cooling to room temperature, the reaction mixture was poured into water and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica-gel column chromatography using chloroform/EtOH (15:1) as the eluent to give 54.3 mg (60.4%) of the title compound as a pale-yellow powder. Mp 84–85 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.34 (s, 1H), 8.48 (d, J = 5.6 Hz, 1H), 8.25 (d, J = 8.1 Hz, 1H), 7.67 (d, J = 8.1 Hz, 1H), 7.61 (d, J = 5.6 Hz, 1H), 7.55 (dd, J = 7.9, 8.1 Hz, 1H), 7.31 (dd, J = 7.9, 8.1 Hz, 1H), 3.89 (s, 3H). HRMS (FAB) calcd for $C_{12}H_{11}N_2$ 183.0922; found: 183.0898 $(M+H)^+$.

6.2.24. 6-Methyl-γ-carboline (14, SK6M): typical procedure for the synthesis of methyl analogs of γ-carboline at the 6–9 positions. A mixture of **33** and aminoacetalde-

hyde dimethylacetal (0.550 mL, 4.84 mmol) in dry benzene (10 mL) was refluxed for 5 h at 110 °C. After the solution had cooled to room temperature, the benzene was evaporated. Then 90% phosphoric acid (10 mL), which had been treated for 2 h at 165 °C, was added to the residue and stirring was continued for another 30 min at 165 °C. To the reaction mixture was added ice, and the resulting precipitate was removed by filtration through a Celite pad. The filtrate was basified with dil. NH_3 and extracted with CH_2Cl_2. The organic phase was extracted with dil. HCl and the HCl layer was basified with dil NaOH aqueous solution. The resulting precipitate was collected and purified by silica-gel column chromatography using chloroform/EtOH (5:1) as the eluent to give 5.7 mg (0.8%, three steps) of SK6M as a brown powder. Mp 219–220 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.32 (s, 1H), 8.52 (d, J = 5.6 Hz, 1H), 8.46 (br, 1H), 8.00 (d, J = 7.7 Hz, 1H), 7.40 (d, J = 5.6 Hz, 1H), 7.32–7.23 (m, 2H), 2.60 (s, 3H). HRMS (FAB) calcd for $C_{12}H_{11}N_2$ 183.0922; found: 183.0909 $(M+H)^+$.

6.2.25. 7-Methyl-γ-carboline (15, SK7M). According to the typical procedure, 106 mg (30.1%, two steps) of 7-methyl-γ-carboline (**15**) was obtained from **32** (307 mg, 1.93 mmol) as a pale-yellow powder. Mp 200–201 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.6 (s, 1H), 9.24 (s, 1H), 8.36 (d, J = 5.8 Hz, 1H), 8.07 (d, J = 7.9 Hz, 1H), 7.40 (d, J = 5.8 Hz, 1H), 7.34 (s, 1H), 7.07 (d, J = 7.9 Hz, 1H), 2.48 (s, 3H). HRMS (FAB) calcd for $C_{12}H_{11}N_2$ 183.0922; found: 183.0925 $(M+H)^+$.

6.2.26. 8-Methyl-γ-carboline (16, SK8M). According to the typical procedure, 15.6 mg (2.1%, three steps) of 8-methyl-γ-carboline (**16**) was obtained from **31** as a brown powder. Mp 207–208 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.6 (s, 1H), 9.27 (br, 1H), 8.37 (br, 1H), 8.00 (s, 1H), 7.43 (d, J = 8.1 Hz, 1H), 7.41 (br, 1H), 7.28 (d, J = 8.1 Hz, 1H), 2.47 (s, 3H). HRMS (FAB) calcd for $C_{12}H_{11}N_2$ 183.0922; found: 183.0935 $(M+H)^+$.

6.2.27. 9-Methyl-γ-carboline (17, SK9M). According to the typical procedure, 83.1 mg (53.6%, two steps) of 9-methyl-γ-carboline (**17**) was obtained from **30** (135 mg, 0.851 mmol) as a white powder. Mp 180–181 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.7 (s, 1H), 9.28 (s, 1H), 8.41 (d, J = 5.6 Hz, 1H), 7.46 (d, J = 5.6 Hz, 1H), 7.40–7.34 (m, 2H), 7.06 (d, J = 6.8 Hz, 1H), 2.81 (s, 3H). HRMS (FAB) calcd for $C_{12}H_{11}N_2$ 183.0922; found: 183.0919 $(M+H)^+$.

6.2.28. 5-Ethyl-γ-carboline (18, SK5E): typical procedure for *N*-alkylation of γ-carboline. A mixture of γ-carboline (**7**) (101 mg, 0.603 mmol), NaH (42.2 mg, 0.967 mmol), and iodoethane (0.14 mL, 1.75 mmol) in dry THF (5 mL) and dry DMF (5 mL) was stirred for 30 min at room temperature. The volatile materials were removed under reduced pressure and the residue was poured into water. The mixture was extracted with AcOEt. The organic phase was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica-gel column chromatography using chloroform as the eluent to give 79.6 mg (67.3%) of the title compound as a yellow powder. Mp 64–65 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.34 (s, 1H), 8.47 (d, J = 5.8 Hz, 1H), 8.25 (d, J = 7.5 Hz, 1H), 7.69 (d, J = 8.1 Hz, 1H), 7.62 (d, J = 5.8 Hz, 1H), 7.53 (dd, J = 7.3, 8.1 Hz, 1H), 7.30 (dd, J = 7.3, 7.5 Hz, 1H), 4.46 (q, J = 7.3 Hz, 2H), 1.32 (t, J = 7.3 Hz, 3H). HRMS (FAB) calcd for $C_{13}H_{13}N_2$ 197.1079; found: 197.1116 $(M+H)^+$.

6.2.29. 5-*n*-Propyl-γ-carboline (19, SK5nP). According to the typical procedure using 1-iodopropane (0.16 mL, 1.60 mmol), 98.8 mg (86.7%) of 5-*n*-propyl-γ-carboline (**19**) was obtained as a pale brown powder. Mp 65–66 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.34 (s, 1H), 8.46 (d, J = 5.6 Hz, 1H), 8.25 (d, J = 7.7 Hz, 1H), 7.70 (d, J = 8.3 Hz, 1H), 7.63 (d, J = 5.6 Hz, 1H), 7.52 (dd, J = 7.3, 8.3 Hz, 1H), 7.30 (dd, J = 7.3, 7.7 Hz, 1H), 4.38 (t, J = 7.3 Hz, 2H), 1.80 (dt, J = 7.3, 7.3 Hz, 2H), 0.85 (t, J = 7.3 Hz, 3H). HRMS (FAB) calcd for $C_{14}H_{15}N_2$ 211.1235; found: 211.1203 $(M+H)^+$.

6.2.30. 5-*i*-Propyl-γ-carboline (20, SK5iP). According to the typical procedure using 2-iodopropane (0.16 mL, 1.60 mmol), 64.9 mg (55.7%) of 5-*i*-propyl-γ-carboline (**20**) was obtained as a white powder. Mp 100–101 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.34 (s, 1H), 8.43 (d, J = 5.8 Hz, 1H), 8.26 (d, J = 7.7 Hz, 1H), 7.78 (d, J = 8.1 Hz, 1H), 7.69 (d, J = 5.8 Hz, 1H), 7.50 (dd, J = 8.1, 8.2 Hz, 1H), 7.28 (dd, J = 7.7, 8.2 Hz, 1H), 5.16–5.10 (m, 1H), 1.62 (d, J = 6.8 Hz, 6H). HRMS (FAB) calcd for $C_{14}H_{15}N_2$ 211.1235; found: 211.1217 $(M+H)^+$.

6.2.31. 5-*n*-Butyl-γ-carboline (21, SK5Bu). According to the typical procedure using 1-iodobutane (0.16 mL, 1.60 mmol), 80.8 mg (65.6%) of 5-*n*-butyl-γ-carboline (**21**) was obtained as a pale brown powder. Mp 58–59 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.34 (s, 1H), 8.46 (d, J = 5.6 Hz, 1H), 8.25 (d, J = 7.9 Hz, 1H), 7.68 (d, J = 8.1 Hz, 1H), 7.61 (d, J = 5.6 Hz, 1H), 7.52 (dd, J = 7.1, 8.1 Hz, 1H), 7.29 (dd, J = 7.1, 7.9 Hz, 1H), 4.40 (t, J = 7.3 Hz, 2H), 1.77–1.71 (m, 2H), 1.30–1.23 (m, 2H), 0.85 (t, J = 7.3 Hz, 3H). HRMS (FAB) calcd for $C_{15}H_{17}N_2$ 225.1392; found: 225.1366 $(M+H)^+$.

6.2.32. 5-*n*-Hexyl-γ-carboline (22, SK5H). According to the typical procedure using 1-iodohexane (0.23 mL, 1.56 mmol), 22.4 mg (16.9%) of 5-*n*-hexyl-γ-carboline (**22**) was obtained as a brown oil. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.33 (s, 1H), 8.46 (d, J = 5.6 Hz, 1H), 8.25 (d, J = 7.7 Hz, 1H), 7.68 (d, J = 8.3 Hz, 1H), 7.61 (d, J = 5.6 Hz, 1H), 7.53 (d, J = 7.3, 8.3 Hz, 1H), 7.30 (d, J = 7.3, 7.7 Hz, 1H), 4.40 (t, J = 7.3 Hz, 2H), 1.26–1.18 (m, 8H), 0.79 (t, J = 7.3 Hz, 3H). HRMS (FAB) calcd for $C_{17}H_{21}N_2$ 253.1705; found: 253.1735 $(M+H)^+$.

6.2.33. 5-Benzyl-γ-carboline (23, SK5Bn). A mixture of γ-carboline (**7**) (86.0 mg, 0.511 mmol), P(*n*-Bu)$_3$ (0.250 mL, 1.03 mmol), and benzyl alcohol (0.105 mL, 1.01 mmol) in dry THF (3 mL) and dry toluene (3 mL) was added to *N*,*N*,*N*′,*N*′-tetramethylazodicarboxamide (177 mg, 1.03 mmol) at 0 °C. The mixture was stirred for 15 h at 50 °C, then poured into water, and the whole was extracted with AcOEt. The organic phase was

washed with water and brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica-gel column chromatography using chloroform/EtOH (50:1) as the eluent to give 127.5 mg (96.6%) of the title compound as a yellow powder. Mp 109–110 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.37 (s, 1H), 8.47 (d, J = 5.8 Hz, 1H), 8.28 (d, J = 7.7 Hz, 1H), 7.69–7.67 (m, 2H), 7.50 (dd, J = 6.8, 8.1 Hz, 1H), 7.31 (dd, J = 7.7, 8.1 Hz, 1H), 7.29–7.17 (m, 5H), 5.69 (s, 2H). HRMS (FAB) calcd for $C_{18}H_{15}N_2$ 259.1235; found: 259.1270 $(M+H)^+$.

6.2.34. 5-(*N*,*N*-Diethylaminoethyl)-γ-carboline (24, SK5DAE). A mixture of γ-carboline (**7**) (104 mg, 0.615 mmol), P(*n*-Bu)$_3$ (0.300 mL, 1.24 mmol), and *N*,*N*-diethylethanolamine (0.16 mL, 1.20 mmol) in dry THF (5 mL) and dry toluene (5 mL) was added to *N*,*N*,*N*′,*N*′-tetramethylazodicarboxamide (214 mg, 1.24 mmol) at 0 °C. The mixture was stirred for 15 h at 50 °C, then poured into water, and the whole was extracted with AcOEt. The organic phase was washed with water and brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica-gel column chromatography using chloroform/EtOH (30:1) as the eluent to give 46.8 mg (28.5%) of the title compound as a yellow oil. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.32 (s, 1H), 8.45 (d, J = 5.8 Hz, 1H), 8.24 (d, J = 7.5 Hz, 1H), 7.66 (d, J = 7.7 Hz, 1H), 7.58 (d, J = 5.8 Hz, 1H), 7.52 (dd, J = 7.3, 7.7 Hz, 1H), 7.28 (dd, J = 7.3, 7.5 Hz, 1H), 4.43 (t, J = 6.4 Hz, 2H), 2.73 (t, J = 6.4 Hz, 2H), 2.44 (q, J = 7.3 Hz, 4H), 0.74 (t, J = 7.3 Hz, 6H). HRMS (FAB) calcd for $C_{17}H_{22}N_3$ 268.1814; found: 268.1783 $(M+H)^+$.

6.3. Bioassays[15,16]

MDBK cells (1×10^4 cells/well) were infected with BVDV (Nose strain) at a multiplicity of infection of 0.01 and incubated at 37 °C in the presence of various concentrations of test compounds. After 3 days, cell viability of virus-infected and mock-infected cells were determined by the MTT and LDH methods, respectively.

HL-60 cells were cultured in RPMI-1640 containing 10% heat-inactivated fetal bovine serum (FBS; Gibco BRL) at 37 °C under a 5% CO_2 atmosphere. HL-60 cells (1×10^5/mL) were seeded on a 96-well plate and incubated with γ-carboline (**7**) (10 μM final concentration, DMSO final concentration 0.5%, v/v) or SK1M (**9**) (10 μM final concentration, DMSO final concentration 0.5%, v/v) in RPMI-1640 containing 10% FBS with or without Z-VAD fmk (100 μM final concentration, DMSO final concentration 0.1%, v/v) at 37 °C under a 5% CO_2 atmosphere. After 62 h in culture, the viability of the cells was evaluated by cell counting under a microscope. The viability was estimated by comparison with that of DMSO (final concentration 0.6%, v/v)-treated cells.

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