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POLYMETHYLATED γ-CARBOLINES WITH POTENT ANTI-BOVINE VIRAL DIARRHEA VIRUS (BVDV) ACTIVITY

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Abstract – Several anti-BVDV agents with a polymethylated γ-carboline skeleton were synthesized, and their anti-BVDV activity was evaluated. The most potent antiviral agent, SK3M4M5M (**20**), was synthesized by Pd-catalyzed Buchwald-Hartwig amination reaction followed by annulation reaction as key steps. The structure-activity relationship was analyzed.

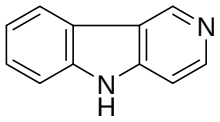
INTRODUCTION

Human hepatitis C virus (HCV：a member of the *Flaviviridae* family) infection is thought to be a major cause of human hepatitis globally.[1,2] The WHO estimates that approximately 170 million people are infected by this virus and the infection persists in more then 80% of the infected population.[3] Moreover, about 60% of cases progress to chronic liver disease, which in turn, can lead to development of cirrhosis, hepatocellular carcinoma, and liver failure.[4,5] Currently, the most effective treatment of chronic hepatitis C is the use of pegylated interferon-α in combination with the nucleoside analogue ribavirin. However, the virus cannot be eliminated from approximately 50-60% of the infected patients treated with these agents.[6] In addition, this therapy is associated with serious side-effects.[7,8] Therefore, alternative agents for the treatment and prevention of HCV infection are urgently needed.

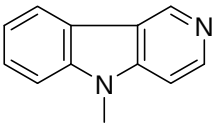
Bovine viral diarrhea virus (BVDV) is a member of *Pestivirus*, which also belongs to the *Flaviviridae* family.[9] Since the anti-HCV agent rivabirin showed activity against BVDV replication *in vitro*, BVDV is

This paper is dedicated to Professor Emeritus Keiichiro Fukumoto on the occasion of his 75th birthday.

thought to be a good model for human HCV (HCV does not replicate efficiently in cell cultures or animals).[10-12] Recently, we found that 6-5-6 fused heteroaromatic compounds such as γ-carboline (**1**) and its mono-methylated analogs, including SK5M (**2**), exhibit rather potent anti-BVDV activity in antiviral screening using Madin–Darby bovine kidney (MDBK) cells infected with BVDV (Figure 1).[13] Preliminary structure-activity relationship studies indicated that the introduced methyl group is quite critical for the activity, depending on its position.[13]



γ-carboline (**1**; SK00)
EC_{50}: 2.0 μM, CC_{50}: 44 μM
SI = 22



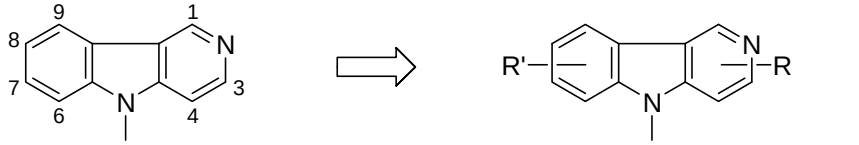
SK5M (**2**)
EC_{50}: 0.26 μM, CC_{50}: 29 μM
SI = 111

Figure 1. Structures of γ-carboline (**1**; SK00) and SK5M (**2**). EC_{50}: anti-BVDV activity, CC_{50}: cytotoxicity toward the host cells, SI: the selectivity index ($SI = CC_{50}/EC_{50}$).

In this paper, we disclose syntheses of di- and tri-methylated γ-carboline analogs and the results of evaluation of their anti-viral activity against BVDV.

RESULTS AND DISCUSSION

Our previous report indicates that the introduction of a methyl group at the 1, 4, or 5 position of γ-carboline (**1**) efficiently enhanced the anti-BVDV activity of γ-carboline (**1**).[13] Among the mono-methylated γ-carboline analogs, 5-methyl-γ-carboline (**2**, SK5M) exhibits the most potent anti-BVDV activity.[13] On the basis of this result, we expected that additional methylation of SK5M (**2**) at various positions might lead to the development of more potent antiviral agents.



SK5M (**2**)

R = Me, R' = H or R = H, R' = Me

Figure 2. Structural development of dimethylated γ-carbolines based on SK5M (**2**).

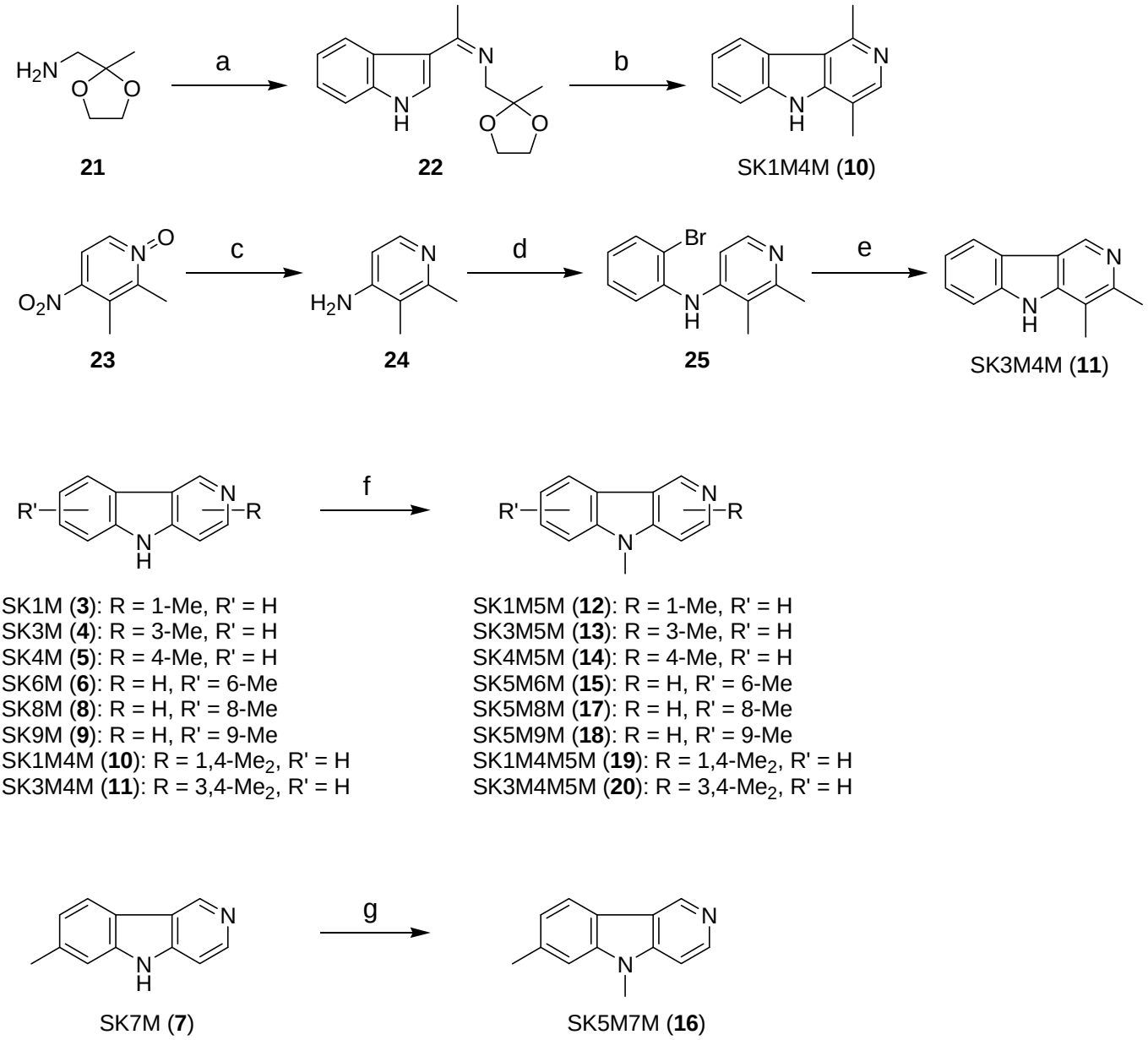
We planned to synthesize dimethylated γ-carbolines **12-18**, and trimethylated γ-carbolines **19**, **20** from **3-11** by *N*-methylation using dimethyl carbonate as a methylating agent in the final step. Monomethylated

γ-carbolines **3**-**9** were prepared according to the method described in our previous report, and 1,4-dimethyl-γ-carboline (**10**, SK1M4M) was synthesized from 1-aminopropan-2-one ethylene acetal (**21**) by a similar procedure to that used for the synthesis of **5** (Scheme 1).[13] Although synthesis of 3,4-dimethyl-γ-carboline (**11**, SK3M4M) was expected to be feasible via a similar procedure, the corresponding starting material was difficult to obtain. Thus, we attempted to prepare **11** from commercially available 2,3-dimethyl-4-nitropyridine *N*-oxide (**23**) by employing Pd-catalyzed amination and subsequent annulation reaction as key steps. The nitro group and *N*-oxide of **23** were reduced by iron powder under an acidic condition, followed by Pd-catalyzed Buchwald-Hartwig amination reaction with 1-bromo-2-iodobenzene to give intermediate **25**.[14] Finally, the annulation reaction of **25** with $Pd(OAc)_2$ afforded 3,4-dimethyl-γ-carboline, SK3M4M (**11**).[14]

N-Methylation of γ-carbolines **3**-**11** was performed as summarized in Scheme 1. As expected, *N*-methylation of **3**-**6**, **8**-**11** proceeded smoothly to give **12**-**15**, **17**-**20** in moderate yields. However, compound **16** could not be obtained from **7** under similar conditions. Therefore we tried to synthesize **16** by Mitsunobu reaction using **7**, TMAD, P(*n*-Bu)$_3$, and MeOH. Although the yield was not high, **16** was obtained.

The anti-BVDV activity of the prepared compounds was evaluated by the method described in the previous report.[13] The EC_{50} values for anti-BVDV activity, the CC_{50} values for cytotoxicity toward the host cells, and the values of the selectivity index (CC_{50}/EC_{50}: SI) of the polymethylated γ-carbolines **10**-**20** are summarized in Table 1. Among the dimethylated compounds, SK3M4M (**11**), SK3M5M (**13**) and SK4M5M (**14**) exhibited anti-BVDV activity one order of magnitude higher than that of the monomethyl compound SK5M (**2**): the EC_{50} values of **11**, **13** and **14** were 0.072, 0.062 and 0.043 μM, respectively. Other dimethylated compounds showed similar potency of anti-BVDV activity to **2**, but their cytotoxicity seemed to be increased except in the case of SK5M7M (**16**). The trimethylated compound SK1M4M5M (**19**) exhibited favorable anti-viral activity, but also showed the highest cytotoxicity. Interestingly, SK3M4M5M (**20**),[15] in which one methyl group of **19** is switched from the1-position to the 3-position, showed the most potent anti-BVDV activity with the EC_{50} value of 3.5 nM. Moreover, the SI value of SK3M4M5M (**20,** SI = 2057) was about 20-fold higher than that of the lead compound SK5M (**2**, SI = 111), indicating great improvement of the selectivity. These results suggest that the best position of SK5M (**2**) for methyl group substitution, in terms of enhancement of anti-BVDV activity, is the 3 or 4 position. In addition, methylation at the 1, 6, 8, or 9 position seems to enhance the cytotoxicity. The relationship between anti-BVDV activity and introduction position of methyl group is unclear. However, enhancement of the basicity of pyridine moiety in association with electron-donating character of methyl group(s) might be one of the factor(s) of the improved anti-BVDV activity, because introduction of methyl group(s) on the pyridine ring caused enhancement of the

anti-BVDV activity whereas those on the indole ring are not effective.

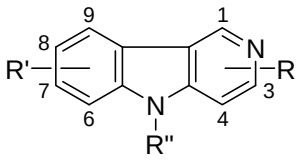


Scheme 1. Synthetic route to di- and tri-methylated γ-carbolines **10**-**20**.
Reagents and comments. a) 3-Acetylindole, benzene; b) 90% H_3PO_4, 4% (2 steps); c) Fe, AcOH, 76%; d) 1-bromo-2-iodobenzene, $Pd_2(dba)_3$, DPPF, NaO^tBu, toluene, 99%; e) $Pd(OAc)_2$, Na_2CO_3, DMF, 20%; f) DMC, K_2CO_3, DMF, 8-73%; g) MeOH, TMAD, P(*n*-Bu)$_3$, THF, toluene, 11%.

The carboline skeleton generally has the ability to intercalate into DNA, so we next evaluated the interaction of polymethylated γ-carbolines with double-stranded DNA. As expected, all of the polymethylated γ-carbolines showed affinity for double-stranded DNA, with association constants of the order of 10^4 M^{-1}. However, no clear correlation between the anti-BVDV activity or cytotoxicity and the

DNA affinity was observed (*data not shown*). This result suggests that the anti-BVDV activity and cytotoxicity of polymethylated γ-carbolines are not attributable to interaction with DNA.

Table 1. Anti-viral activity, cytotoxicity, and selectivity index of polymethylated γ-carbolines (**10**-**20**).



	R	R'	R"	EC_{50} (μM)	CC_{50} (μM)	SI[a]
SK5M (**2**)	H	H	Me	0.26	29	111
SK1M4M (**10**)	1,4-Me_2	H	H	0.12	5.2	43
SK3M4M (**11**)	3,4-Me_2	H	H	0.072	25	347
SK1M5M (**12**)	1-Me	H	Me	0.20	5.2	26
SK3M5M (**13**)	3-Me	H	Me	0.062	21	339
SK4M5M (**14**)	4-Me	H	Me	0.043	9.6	223
SK5M6M (**15**)	H	6-Me	Me	0.30	11	37
SK5M7M (**16**)	H	7-Me	Me	0.30	40	133
SK5M8M (**17**)	H	8-Me	Me	0.35	7.7	22
SK5M9M (**18**)	H	9-Me	Me	0.29	6.4	22
SK1M4M5M (**19**)	1,4-Me_2	H	Me	0.063	2.2	35
SK3M4M5M (**20**)	3,4-Me_2	H	Me	0.0035	7.2	2057

[a]$SI = CC_{50}/EC_{50}$

To interpret the qualitative structure-activity relationship (SAR) mentioned above, we examined the quantitative structure-activity relationship (QSAR) for anti-BVDV activity of the compounds shown in Table 1 and the mono-methylated γ-carbolines described in our previous report.[13] The QSAR analysis was performed by using the QSAR applications of the molecular operating environment (MOE 2006)[16] with the genetic algorithm analysis applied to all descriptors of MOE (184 kinds). The correlation plot between pEC_{50} values obtained from observed EC_{50} values and calculated pEC_{50} values according to eq. 1 is displayed in Figure 3.

pEC_{50} (μM) = -0.30177+0.40904×(wienerPol)-0.07763×(vdw_area)+0.04366×(PEOE_VSA_HYD) -0.0691×(PEOE_VSA+1) (1)

The meanings of the parameters of eq. 1 are as follows: (i) wienerPol[17]: Wiener polarity number, (ii) vdw_area: van der Waals surface area, (iii) PEOE_VSA_HYD[18]: total hydrophobic van der Waals surface area, (iv) PEOE_VSA+1[18]: total positive 1 van der Waals surface area.

The observed pEC_{50} values were positively correlated with calculated pEC_{50} values with the correlation coefficient value (R^2) of ca. 0.84. In addition, the positive coefficients of wienerPol and PEOE_VSA_HYD in Eq. 1 indicate that the anti-BVDV activity tends to be enhanced when the molecule has a hydrophobic functional group and/or becomes more polarized. This result is broadly consistent with our qualitative SAR. In fact, EC_{50} values of γ-carboline derivatives with one or more methyl groups on the pyridine moiety were found to decrease in proportion to the number of methyl groups possibly due to the hydrophobic and electron-donating property of methyl group.

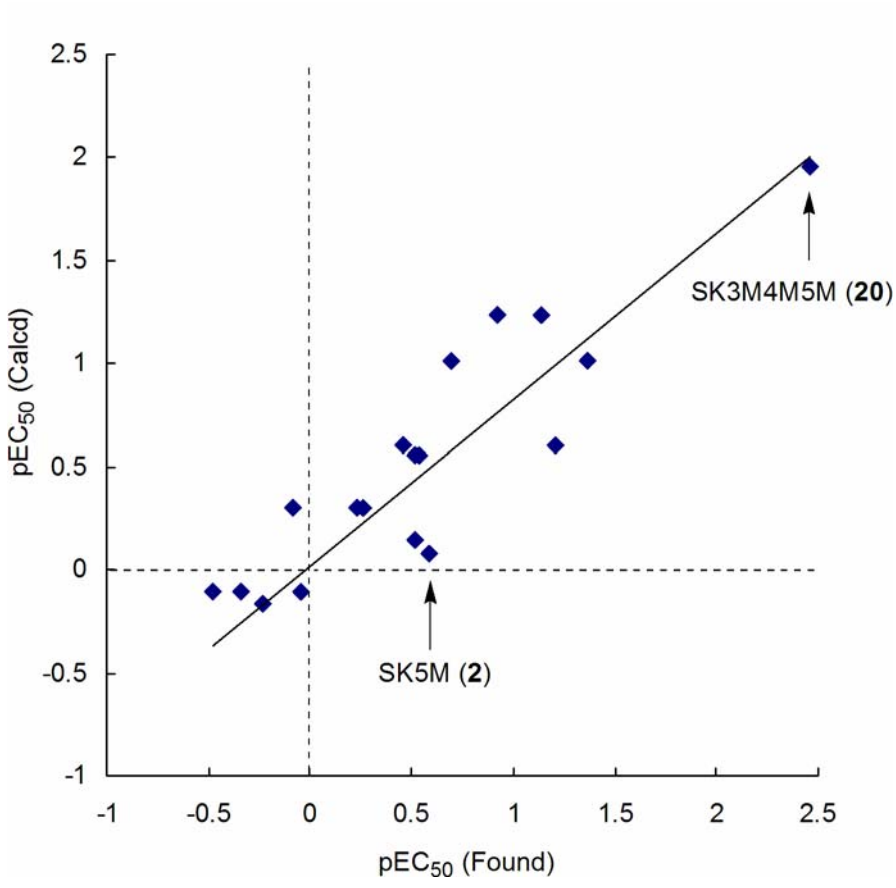


Figure 3. Correlation of observed and calculated (with eq. 1) pEC_{50} values for polymethylated γ-carbolines.

In conclusion, we have synthesized several polymethylated γ-carbolines, and found that 3,4,5-trimethyl-γ-carboline (SK3M4M5M: **20**) exhibits two-orders-of-magnitude higher anti-viral activity than the lead compound SK5M (**2**), with the EC_{50} value of 3.5 nM. The result of QSAR analysis were

consistent with our SAR data. Further structural development based on the QSAR analysis is in progress, together with biological studies.

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¶The first two authors contributed equally.

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