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Synthesis and antipicornavirus activity of (*R*)- and (*S*)-1-[5-(4′-chlorobiphenyl-4-yloxy)-3-methylpentyl]-3-pyridin-4-yl-imidazolidin-2-one

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Abstract—The new pyridyl imidazolidinone derivative, 1-[5-(4′-chlorobiphenyl-4-yloxy)-3-methylpentyl]-3-pyridin-4-yl-imidazolidin-2-one (±)-**1a**, was synthesized and found to have an excellent antiviral activity against EV71 (IC_{50} = 0.009 μM). Therefore, both the enantiomers, (*S*)-(+)-**1a** and (*R*)-(−)-**1a**, have been prepared starting from readily available monomethyl (*R*)-3-methylglutarate (**7**) as a useful chiral building block and their antiviral activity was evaluated in a plaque reduction assay. Interestingly, we observed that the enantiomer (*S*)-(+)-**1a** was 10-fold more active against enterovirus71 (EV71) (IC_{50} = 0.003 μM) than the corresponding enantiomer (*R*)-(−)-**1a** (IC_{50} = 0.033 μM). Similar results were found against all five strains (1743, 2086, 2231, 4643, and BrCr) of EV71 tested. This demonstrated that the absolute configuration of the chiral carbon atom at the 3-position of the alkyl linker considerably influenced the anti-EV71 activity of these pyridyl imidazolidinones.

1. Introduction

Enterovirus 71 (EV71) was first recognized in 1969 in California, USA, when it was isolated from the feces of an infant suffering from encephalitis.[1] EV71 belongs to the human Enterovirus A species of the *Enterovirus* genus within the family of *Picornaviridae*.[2] Virions consist of a non-enveloped capsid surrounding a core of single-stranded, positive-polarity RNA approximately 7.5 kb in size. The viral capsid is icosahedral in symmetry and is composed of 60 identical units each consisting of the four structural proteins VP1–VP4. The complete nucleotide sequence of the EV71 prototype strain BrCr has been determined.[3] Symptoms for EV71 infections range from non-specific upper respiratory infection and mild fever to central nervous system infections particularly viral meningitis, encephalitis, and severe myocarditis.[4] Children are considered to be relatively immunodeficient, therefore EV71 infections of neonates can be lethal and life-threatening, with high risk for morbidity and mortality. Many epidemic outbreaks have been reported from Asian countries.[5] A major outbreak of EV71 infection in Taiwan in 1998 caused many severe by infected cases and 78 deaths.[6] Currently, there is no specific antiviral therapy to treat or prevent enterovirus disease.

Pleconaril[7] is a typical and systemically acting small-molecule inhibitor of enteroviruses and rhinoviruses, but this compound exhibited no activity against EV71. In our laboratory, many structurally related pyridyl imidazolidinones were recently found to have strong activity against EV71.[8] Among this series of compounds synthesized, the pyridyl imidazolidinone with a tether chain length of five carbons, DBPR103 (Fig. 1), was identified as the most potent enterovirus 71 inhibitor (IC_{50} = 0.054 μM) with no apparent cytotoxic effect toward RD (rhabdomyosarcoma) cell lines (CC_{50} > 25 μM).[8] Although DBPR103 is a new agent with potent antiviral activity against EV71, the mechanism of action is still unclear. As part of our continuous efforts toward the identification of more potent anti-EV71 agents, we made a drastic change at the

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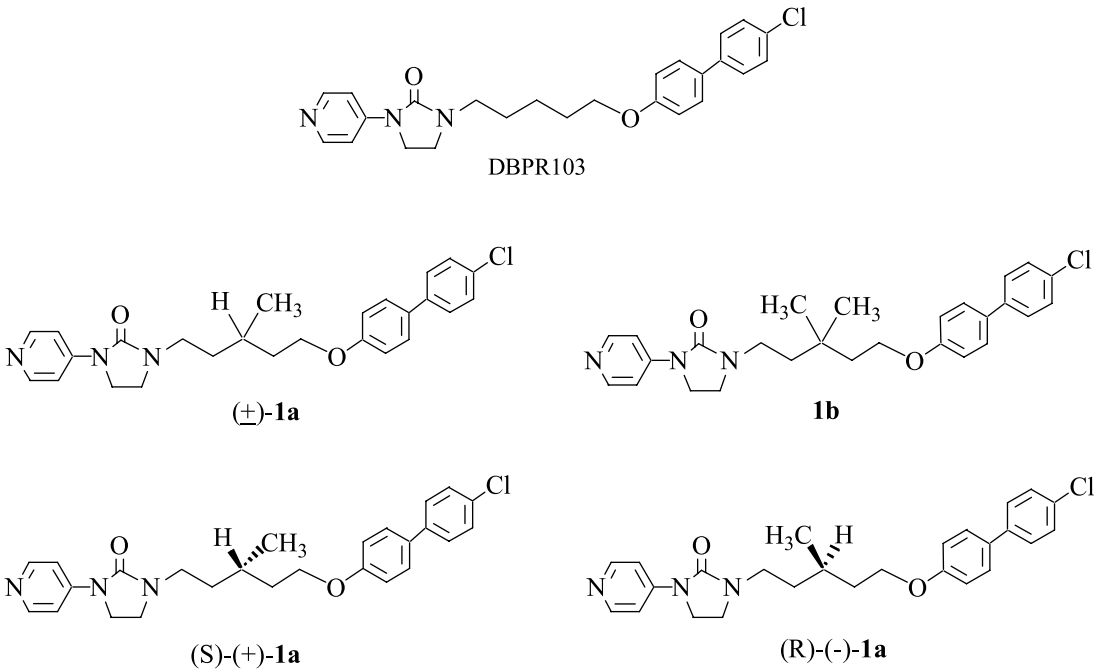


Figure 1. Structures of DBPR103, (±)-**1a**,**b**, (*S*)-(+)-**1a**, and (*R*)-(−)-**1a**.

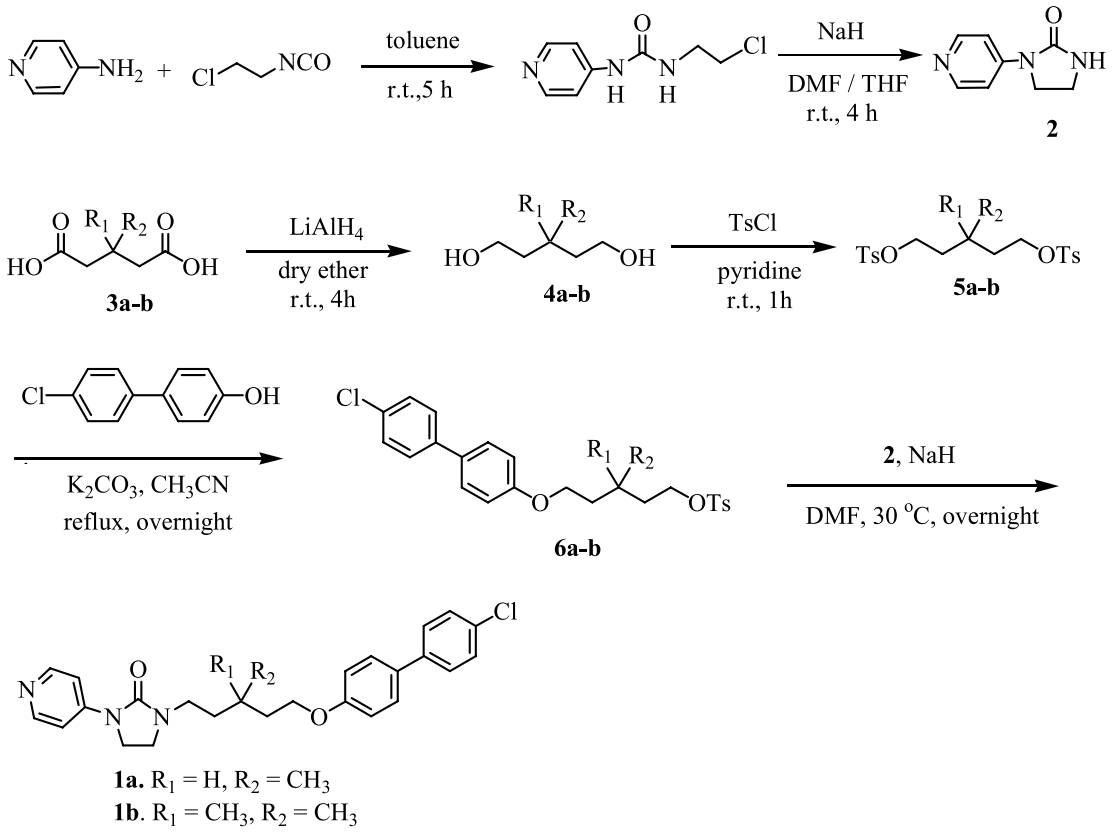
3-position of the alkyl linker of DBPR103. However, the racemic 3-methyl analogue (±)-**1a**, its corresponding enantiomers, (*S*)-(+)-**1a** and (*R*)-(−)-**1a**, and the 3,3-dimethyl analogue **1b** (Fig. 1) were successfully synthesized and assayed for antiviral activity in a plaque reduction assay.[9] It is noteworthy to mention that the chiral 3-methyl analogue (*S*)-(+)-**1a** has shown significant improvement in potency relative to the reference compound DBPR103 as well as broad-spectrum activity against a variety of enterovirus serotypes. Details of this investigation will be described herein.

2. Chemistry

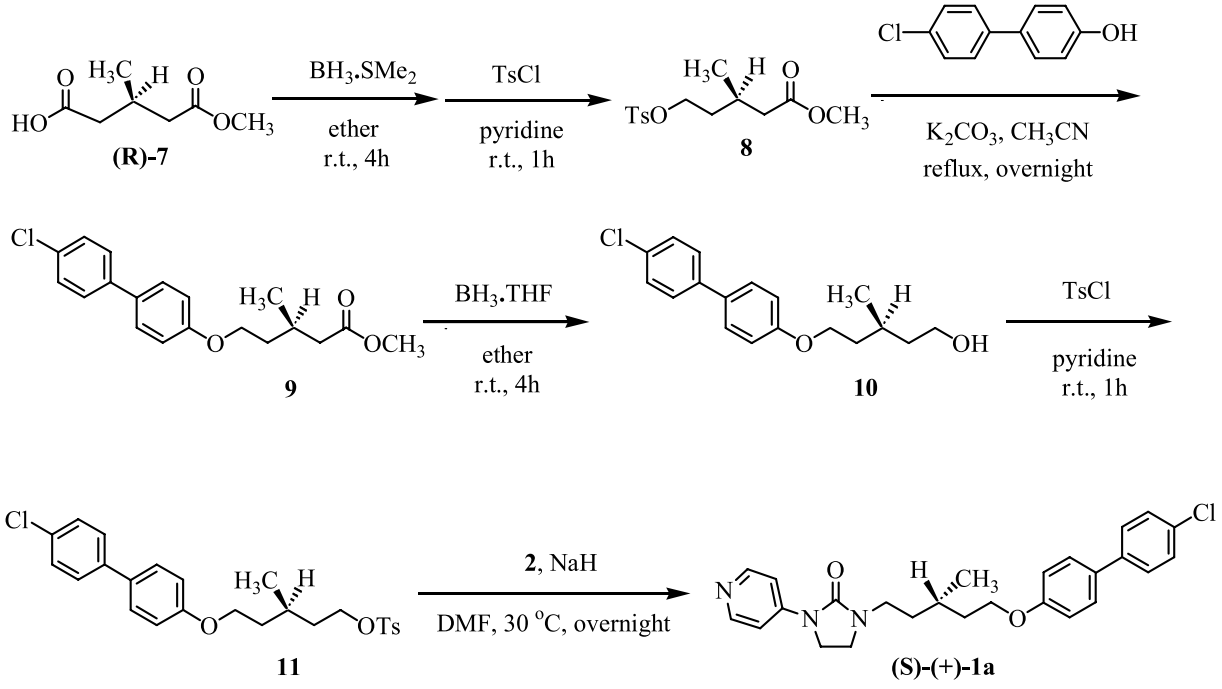
Three different routes were employed to prepare the pyridyl imidazolidinones (±)-**1a**,**b**, (*S*)-(+)-**1a**, and (*R*)-(−)-**1a**. Compounds (±)-**1a** and **b** were synthesized by the method summarized in Scheme 1. For the preparation of 1-(4-pyridyl)-2-imidazolidinone **2** (Scheme 1), 4-aminopyridine was first coupled with 2-chloroethylisocyanate to give the corresponding urea intermediate in 80% yield. Subsequent intramolecular cyclization of the intermediate by treatment with sodium hydride in the THF/DMF (1:1) cosolvent system at room temperature resulted in the formation of cyclic urea **2** in 92% yield. Reduction of the glutaric acids **3a** and **b** with lithium aluminum hydride in dry THF gave the diols **4a** and **b**, which were then reacted with *p*-toluenesulfonyl chloride in pyridine to give the corresponding ditosylates **5a**and **b** (74 and 71% for two steps). Subsequent nucleophilic substitution of 4-chloro-4′-hydroxybiphenyl with 2 equiv of the ditosylates **5a** and **b** using potassium carbonate as a base at refluxing acetonitrile gave compounds **6a** and **b**, which were coupled with 1-(4-pyridyl)-2-imidazolidinone **2** in the presence of sodium hydride in DMF to give the target compounds (±)-**1a** and **b** (65% and 58% for two steps).

On the other hand, both the enantiomers of pyridyl imidazolidinone, (*S*)-(+)-**1a** and (*R*)-(−)-**1a**, have been prepared starting from enantiomerically pure monomethyl (*R*)-3-methylglutarate **7**[10] (Schemes 2 and 3). It has been reported that (*R*)-**7** is a useful chiral building block for the synthesis of many biologically active compounds.[11] In this paper, compound (*S*)-(+)-**1a** was prepared stereospecifically as shown in Scheme 2. Selective reduction of monomethyl (*R*)-3-methylglutarate **7** with borane–disulfide followed by tosylation of the resulting hydroxyester afforded the tosylate **8** (54% for two steps). Subsequent nucleophilic substitution of 4-chloro-4′-hydroxybiphenyl with tosylate **8** in the presence of potassium carbonate in acetonitrile gave compound **9**, which was further reduced with borane–tetrahydrofuran to give the alcohol **10** (65% for two steps). Tosylation of the alcohol **10** with *p*-toluenesulfonyl chloride in pyridine gave the corresponding tosylate **11** (93%), which was then coupled with 1-(4-pyridyl)-2-imidazolidinone **2** in the presence of sodium hydride in DMF to give the desired compound (*S*)-(+)-**1a** ($[\alpha]_D$ +11.1 (*c* 0.5, $CHCl_3$)) in 80% yield.

For the preparation of the other enantiomer (*R*)-(−)-**1a**, a different synthetic approach was followed (Scheme 3). The tosylate **8** was first coupled with 1-(4-pyridyl)-2-imidazolidinone **2** to give the corresponding pyridyl imidazolidinone **12** (89%). Subsequent reduction of **12** with borane–tetrahydrofuran in dry ether afforded alcohol **13** (73%). Tosylation of **13** with *p*-toluenesulfonyl chloride followed by the nucleophilic substitution of the resulting tosylate **14** with 4-chloro-4′-hydroxybiphenyl



Scheme 1. Synthesis of pyridyl imidazolidinones (±)-**1a** and **b**.

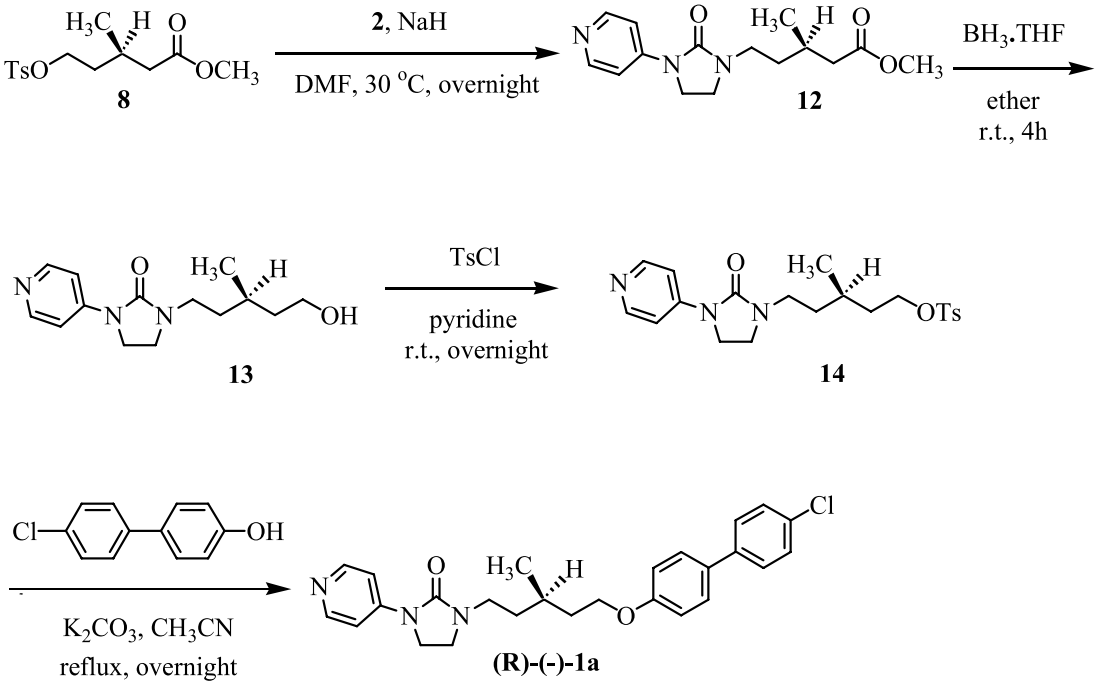


Scheme 2. Synthesis of pyridyl imidazolidinone (*S*)-(+)-**1a**.

afforded the desired compound (*R*)-(−)-**1a** ($[\alpha]_D$ −10.6 (*c* 0.5, $CHCl_3$)) in 56% yield. All the new pyridyl imidazolidinones gave satisfactory spectral data consistent with their proposed structures.[12]

3. Results and discussion

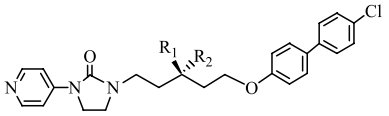
The pyridyl imidazolidinones described herein were tested in a plaque reduction assay[9] under a standard



Scheme 3. Synthesis of pyridyl imidazolidinone (*R*)-(−)-**1a**.

procedure. Compounds (±)-**1a**,**b**, (*S*)-(+)-**1a**, and (*R*)-(−)-**1a** were submitted for anti-EV71 testing as well as cytotoxicity evaluation in the RD cell lines. The results are shown in Table 1 and are compared to the reference compound DBPR103. In this study, DBPR103 showed inhibitory activity against EV71 with IC_{50} value of 0.054 μM. We first examined the skeleton of DBPR103 (Table 1). Introduction of a methyl group at the 3-position of the alkyl linker ((±)-**1a** vs DBPR103) resulted in a 6-fold increase in inhibitory activity against EV71 (IC_{50} = 0.009 μM) with very low cytotoxicity toward RD cell lines (CC_{50} > 25 μM). However, when two methyl groups were introduced at the 3-position of the alkyl linker (**1b** vs DBPR103), compound **1b** showed similar antiviral activity (IC_{50} = 0.040 μM) as the unsubstituted lead compound. This effect might be due to their drastically conformational change and steric requirement of the alkyl linker of these pyridyl imidazolidinones. On the other hand, both the enantiomers of pyridyl imidazolidinone, (*S*)-(+)-**1a** and (*R*)-(−)-**1a**, were synthesized and their anti-EV71 activities were measured (Table 1). Interestingly, we have found that the enantiomer (*S*)-(+)-**1a** was 3-fold more potent (IC_{50} = 0.003 μM) against EV71 than the racemic compound (±)-**1a** (IC_{50} = 0.009 μM). In contrast, the corresponding enantiomer (*R*)-(−)-**1a** was considerably less active (IC_{50} = 0.033 μM). These significant results demonstrated that the absolute configuration of the chiral carbon atom at the 3-position of the alkyl linker considerably influenced the anti-EV71 activity of this series of compounds. It was therefore of interest to explore this potential enantiomerically pure anti-EV71 compound (*S*)-(+)-**1a**.

Table 1. Anti-EV71 activity and cytotoxicity for DBPR103, (±)-**1a**,**b**, (*S*)-(+)-**1a**, and (*R*)-(−)-**1a**



Compound	R_1	R_2	Melting point (°C)	$[\alpha]_D$[e]	IC_{50} (μM)[a] EV71[c]	CC_{50} (μM)[b] RD[d]
DBPR103	H	H	167–169	—	0.054 ± 0.010	>25
(±)-**1a**	H	CH_3	166–167	—	0.009 ± 0.001	>25
1b	CH_3	CH_3	150–152	—	0.040 ± 0.003	>25
(*S*)-(+)-**1a**	H	CH_3	166–167	+11.1	0.003 ± 0.001	>25
(*R*)-(−)-**1a**	CH_3	H	166–167	−10.6	0.033 ± 0.001	>25

[a] Mean of triplicate well values. All experiments were performed at least twice. Plaque reduction assay was employed.
[b] Mean of triplicate well values. All experiments were performed at least twice.
[c] EV71: human enterovirus 71 strain 4643.
[d] RD: human rhabdomyosarcoma cells.
[e] (*c* 0.5, $CHCl_3$).

Table 2. Comparative evaluation of DBPR103, (*S*)-(+)-**1a**, and (*R*)-(−)-**1a** against various viruses

	IC_{50} (μM)[a]		
	DBPR103	(*S*)-(+)-**1a**	(*R*)-(−)-**1a**
Enterovirus 71 (1743) B	0.092 ± 0.010	0.026 ± 0.003	0.17 ± 0.01
Enterovirus 71 (2086) C	0.043 ± 0.013	0.008 ± 0.001	0.049 ± 0.003
Enterovirus 71 (2231) C	0.092 ± 0.010	0.024 ± 0.002	0.16 ± 0.02
Enterovirus 71 (4643) C	0.054 ± 0.010	0.003 ± 0.001	0.033 ± 0.001
Enterovirus 71 (BrCr) A	0.13 ± 0.01	0.033 ± 0.001	0.32 ± 0.14
Enterovirus 68	>25	>25	>25
Coxsackievirus A9	0.071 ± 0.004	0.019 ± 0.001	0.15 ± 0.01
Coxsackievirus A10	>25	1.32 ± 0.03	>25
Coxsackievirus A16	>25	6.92 ± 6.17	>25
Coxsackievirus A24	1.30 ± 0.94	>25	>25
Coxsackievirus B1	>25	>25	>25
Coxsackievirus B2	>25	>25	>25
Coxsackievirus B3	>25	>25	>25
Coxsackievirus B4	1.30 ± 0.79	1.25 ± 0.03	2.05 ± 0.12
Coxsackievirus B5	1.14 ± 0.56	4.85	2.08 ± 0.19
Coxsackievirus B6	>25	>25	>25
Echovirus 9	1.31 ± 0.13	>25	>25
Echovirus 29	0.39 ± 0.06	1.30 ± 0.02	1.47 ± 0.13
Human rhinovirus 2	>25	>25	>25
Human rhinovirus 14	>25	>25	>25

[a] Mean of triplicate well values. All experiments were performed at least twice. Plaque reduction assay was employed.

In addition to screening pyridyl imidazolidinones against EV71, we examined the broad-spectrum nature of this chemical platform. Both the enantiomers, (*S*)-(+)-**1a** and (*R*)-(−)-**1a**, were selected for further screening against a panel of 15 additional viruses that was chosen as representative of the large number of human enteroviruses and human rhinoviruses. A comparison of the activity of DBPR103, (*S*)-(+)-**1a**, and (*R*)-(−)-**1a** against 16 serotypes is illustrated in Table 2. These compounds were individually subjected to evaluation against a variety of viruses, including coxsackieviruses (10 serotypes), echoviruses (2 serotypes), and human enteroviruses 68 and 71, and human rhinoviruses 2 and 14. As shown, (*S*)-(+)-**1a** was found, in addition to strongly inhibiting all the genotypes (A, B, and C) of EV71, to possess antiviral activity against coxsackieviruses A9, A10, A16, B4, B5, and echovirus 29. However, compound (*S*)-(+)-**1a** showed no activity against human rhinoviruses 2 and 14 up to the concentration of 25 μM. On the basis of these significant results, we observed that the enantiomer (*S*)-(+)-**1a** was considerably more active against the majority of human enterovirus serotypes tested, in particular, enterovirus 71. We were pleased to find that compound (*S*)-(+)-**1a** exhibited potency and broad-spectrum activity against most of these viruses. Therefore, for our purpose, (*S*)-(+)-**1a** is well qualified to serve as a lead compound for the further development of anti-EV71 agent.

4. Conclusion

In summary, the new pyridyl imidazolidinones (±)-**1a** and**b** were first synthesized and their anti-EV71 activity was evaluated in a plaque reduction assay. It is very interesting to note that the 3-methyl analogue (±)-**1a** of DBPR103 was found to have excellent antiviral activity against EV71 (IC_{50} = 0.009 μM). In contrast, the corresponding 3,3-dimethyl analogue **1b** was less active (IC_{50} = 0.040 μM) than (±)-**1a**. These significant results demonstrated that the methyl group at the 3-position of the alkyl linker of pyridyl imidazolidinones seems to play a very important role in influencing anti-EV71 activity. However, this unexpected biological result is not fully understood and is worthy of further study. On the other hand, both the enantiomers, (*S*)-(+)-**1a** and (*R*)-(−)-**1a**, have been synthesized and then submitted for anti-EV71 testing as well as cytotoxicity evaluation in the RD cell lines. In this study, we observed that the enantiomer (*S*)-(+)-**1a** was 10-fold more active against EV71 (IC_{50} = 0.003 μM) than the corresponding enantiomer (*R*)-(−)-**1a** (IC_{50} = 0.033 μM). Similar results were found against all five strains (1743, 2086, 2231, 4643, and BrCr) of EV71 tested. This demonstrated that the absolute configuration of the chiral carbon atom at the 3-position of the alkyl linker considerably influenced the anti-EV71 activity of these pyridyl imidazolidinones. Further SAR studies and mechanistic studies on these new antiviral compounds are currently under active investigation and will be reported in due course.

Acknowledgment

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12. All the new compounds gave satisfactory spectral data consistent with their proposed structures. Selected spectral data for compounds **1a** and **b**. Compounds (±)-**1a**, (*S*)-(+)-**1a**, and (*R*)-(−)-**1a**: white solid; mp 166–167 °C; IR ($CHCl_3$) ν_{max} 1704, 1591, 1507, 1481, 1423, 830, 815, 746 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.41 (d, J = 5.7 Hz, 2H), 7.46–7.42 (m, 6H), 7.35 (d, J = 8.4 Hz, 2H), 6.92 (d, J = 8.4 Hz, 2H), 4.06–4.01 (m, 2H), 3.80–3.75 (m, 2H), 3.54–3.45 (m, 2H), 3.42–3.33 (m, 2H), 1.90–1.60 (m, 4H), 1.48–1.43 (m, 1H), 1.04 (d, J = 6.6 Hz, 3H); ESMS 450.5 (M+1). Anal. Calcd for $C_{26}H_{28}ClN_3O_2$: C, 69.40; H, 6.27; N, 9.34. Found: C, 69.35; H, 6.21; N, 9.30. Compound **1b**: white solid; mp 150–152 °C; IR ($CHCl_3$) ν_{max} 1703, 1606, 1594, 1482, 1428, 1392, 1377, 1285, 1252, 817, 747 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.42 (brs, 2H), 7.46–7.43 (m, 6H), 7.35 (d, J = 8.7 Hz, 2H), 6.94 (d, J = 8.7 Hz, 2H), 4.07 (t, J = 6.9 Hz, 2H), 3.82–3.76 (m, 2H), 3.56–3.50 (m, 2H), 3.41–3.35 (m, 2H), 1.81 (t, J = 6.9 Hz, 2H), 1.58 (t, J = 6.9 Hz, 2H), 1.06 (s, 6H); ESMS 464.1 (M+1). Anal. Calcd for $C_{27}H_{30}ClN_3O_2$: C, 69.89; H, 6.52; N, 9.06. Found: C, 69.81; H, 6.47; N, 8.99.