Design, Synthesis, and Antipicornavirus Activity of 1-[5-(4-Arylphenoxy)alkyl]-3-pyridin-4-ylimidazolidin-2-one Derivatives

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A series of pyridylimidazolidinone derivatives was synthesized and tested in vitro against enterovirus 71 (EV71). On the basis of compound **33** (DBPR103), introduction of a methyl group at the 2- or 3-position of the linker between the imidazolidinone and the biphenyl resulted in markedly improved antiviral activity toward EV71 with IC_{50} values of 5.0 nM (**24b**) and 9.3 nM (**14a**), respectively. Increasing the branched chain to propyl resulted in a progressive decrease in activity, while inserting different heteroatoms entirely rendered the compound only weakly active. The introduction of a bulky group (cyclohexyl, phenyl, or benzyl) led to loss of activity against EV71. The 4-chlorophenyl moiety in **14a** was replaced with bioisosteric groups such as oxadiazole (**28a**−**d**) or tetrazole (**32a**,**b**), dramatically improving anti-EV71 activity and selectivity indices. Compounds **14a**, **24b**, **28b**, **28d**, and **32a** exhibited a strong activity against lethal EV71, and no apparent cellular toxicity was observed. Three of the more potent imidazolidinone compounds, **14a**, **28b**, and **32b**, were subjected to a large group of picornaviruses to determine their spectrum of antiviral activity.

Introduction

Human enterovirus 71 (EV71), a genus of the Picornaviridae family first isolated in 1969,[1] is most often associated with outbreaks of the mild childhood exanthema, herpangina, and hand-foot-mouth disease (HFMD). It is also associated with the cause of acute neurologic disease including aseptic meningitis, encephalitis, and poliomyelitis-like paralysis.[2] It is transmitted by fecal−oral contamination and less commonly via respiratory droplets and has the potential to cause outbreaks or epidemics. EV71 has been associated with sporadic cases of outbreaks in various parts of the world, including the United States,[3] Australia,[4] and Europe.[5,6] Since 1997 there has been a significant increase in EV71 epidemic activity in the Asia−Pacific region,[7−11] often associated with severe encephalitis and high mortality. During the past 5 years severe outbreaks have occurred in Malaysia (30 deaths in 1997)[12] and in Taiwan (34 deaths in 1998, 25 deaths in 2000, and 26 deaths in 2001).[13−16] A seroepidemiological study in Taiwan demonstrated that the epidemic EV71 seroprevalence rates in adults and children older than 6 years ranged from 57% to 67%, in children younger than 3 years old ranged from 0% to 36%, and for 3−6-year-old children ranged from 26% to 51%.[17] The EV71 seropositive rate among 12−15-year-old Brazilian children was 69.2%.[18] A seroepidemiological study in Singapore also shown that the EV71 seroprevalence rate in the general population was as high as 60%−70%.[19] Therefore, outbreaks of EV71 infection could spread to other parts of the world and cause substantial illness and death. EV71 infection may have a fulminant clinical course, and fatal cases were in children <5 years of age.[20] According to the study, the spinal cord and brain stem were the main targets of EV71 in the fatal cases of these outbreaks;[21] however, heart and pancreas might also be involved.[22] After the eradication of poliomyelitis, EV71 will become the most important enterovirus that affects children.

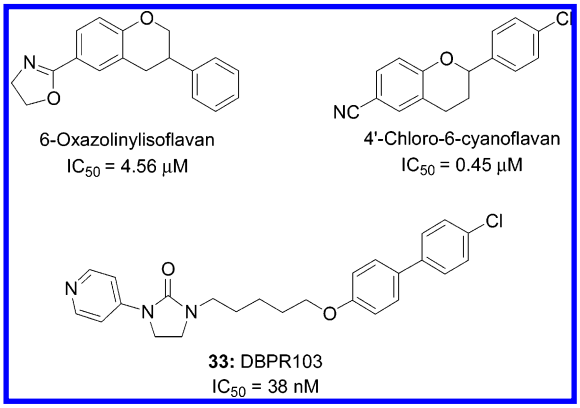

Figure 1. The anti-enterovirus 71 Compounds.

EV71 consists of a nonenveloped capsid surrounding a core of single-stranded, positive-polarity RNA approximately 7.5 kb in size. The viral capsid is icosahedral ($T = 1$) in symmetry and is composed of 60 identical units, each consisting of the four structural proteins VP1−VP4.[23] Although a number of promising antiviral agents with activity against enteroviruses are currently being developed,[24−27] including the potent and broad antiviral WIN compounds,[28−32] rare published papers reveal activity against EV71.[33] In literature research, the structure−activity relationships (SAR) of EV71 inhibitors is still limited. There are only three kinds of small molecules that possess activity against EV71, as shown in Figure 1. Additionally, both the large molecules of lactoferrin[34] and allophycocyanin[35] also reveal anti-EV71 activity by cytopathic effect assay.

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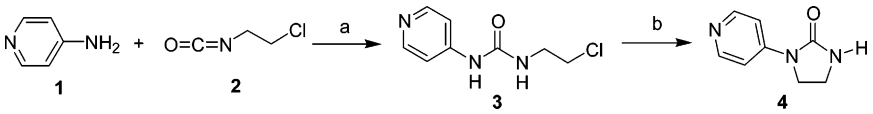
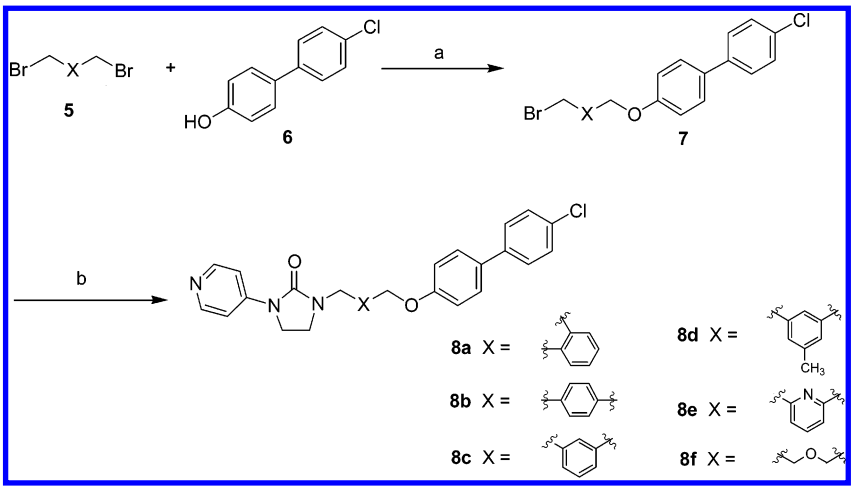
Scheme 1[a]



[a] Reaction conditions: (a) toluene, rt; (b) NaH, DMF/THF (1:1), rt.

Scheme 2[a]



[a] Reaction condition: (a) K_2CO_3, KI, NMP, 60 °C; (b) **4**, NaH, DMF, rt.

Therein, oxazolinylisoflavan[36] is more active against rhinovirus 1B (IC_{50} = 1.01 μM) than against EV71 (IC_{50} = 4.56 μM). 4′-Chloro-6-cyanoflavan[37] possesses moderate activities against coxsackievirus B4 (IC_{50} = 0.32 μM), echovirus 6 (IC_{50} = 0.48 μM), and EV71 (IC_{50} = 0.45 μM). Lactoferrin is an iron-binding glycoprotein presented in milk, saliva, mucous secretions, and other biological fluids of mammals. Allophycocyanin is a red fluorescent protein that can be isolated from the marine algae *Spirulina platensis*. Compound **33** is an imidazolidinone derivative with broad-spectrum antiviral activity against EV71 (IC_{50} = 38 nM),[38] coxsackieviruses, and echoviruses. The series of imidazolidinone compounds has been found to effectively inhibit the EV71 activity by directly targeting the VP1 capsid protein through analysis of the resistant EV71.[39] These compounds are able to block viral uncoating and/or attachment to host cell receptors and then inhibit the viral infection. The X-ray crystallographic structure of the capsid for EV71 has not been solved yet. Till now, there is no effective antiviral treatment for severe EV71 infections, and no vaccine is available. This fact further reinforces the need to develop antiviral agents against EV71.

As the result of our previous work, the imidazolidinone derivatives demonstrated antiviral activity. Therein, compound **33** possessed good anti-EV71 activity and moderate selectivity index.[38] Moreover, our preliminary study showed that compound **33** displayed good oral efficacy and low cytotoxicity in animal models. It brought an opportunity to develop more portent anti-EV71 agents. On the basis of compound **33**, we attempted to modify the linker and the biphenyl moiety to improve the anti-EV71 activity, selectivity index, and oral bioavailability. Herein, we report the discovery of more active agents against EV71 that dramatically increase activity against all serotypes of EV71, possess broad antiviral spectrum, and have significantly decreased cytotoxicity.

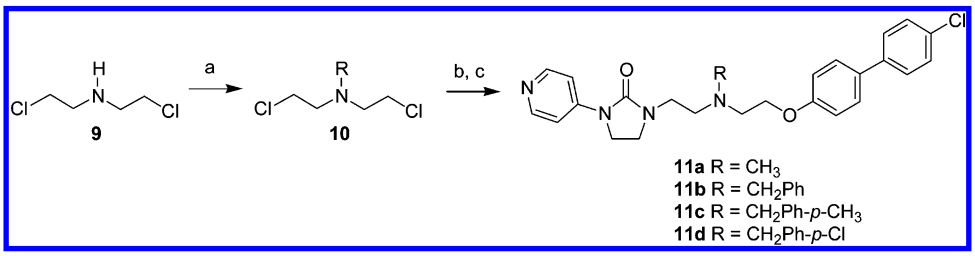
Chemistry

Considerable efforts to develop structure−activity relationships have been made over the past several years to investigate the distance between the imidazolidinone and the substituted phenyl rings.[38] The five-carbon chain is an appropriate length for anti-EV71 activity. Currently, we focus on the modification of the hydrophobic linker between the heterocyclic and aromatic moieties based on the skeleton of compound **33**. The following substituents were adopted as linkers: (1) an aromatic ring, (2) a heteroatom aliphatic spacer, and (3) a branched hydrophobic chain. Furthermore, the use of a heteroaromatic ring surrogated the biphenyl moiety to improve activity against EV71.

Several methods were developed to synthesize analogues in this series, depending upon the substitution pattern. The preparation of the core structure of pyridyl imidazolidinone is outlined in Scheme 1. Compound **4**[38] was synthesized starting with the coupling of 4-aminopyridine and 2-chloroethyl isocyanate to give urea **3**, followed by cyclization by the treatment with NaH in a mixture of DMF/THF (1:1) at room temperature. Serving as a key synthetic intermediate, compound **4** underwent substitution reactions with a variety of alkylating agents to provide compounds in high to excellent yields. Compounds **8a**−**f** were synthesized by combining several kinds of commercially available dibromo compounds **5** and 4′-chloro-4-hydroxybiphenyl as starting materials with K_2CO_3 and KI in *N*-methylpyrolidinone at 60 °C to give monosubstituted **7** followed by *N*-alkylation with **4** (Scheme 2).

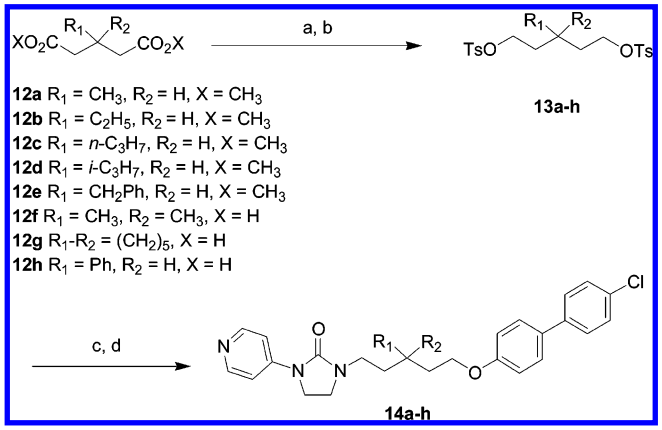
The synthesis of the linker replaced with an aliphatic chain containing a nitrogen atom (**11a**−**d**) is illustrated in Scheme 3. The bis(2-chloroethyl)amine hydrochloride was treated with formaldehyde and formic acid at 100 °C to introduce the methyl group at the nitrogen atom (**10a**). Treatment of **9** with substituted benzyl bromide in the presence of K_2CO_3 in acetonitrile at 60 °C gave

Scheme 3[a]



[a] Reaction conditions: (a) formaldehyde, formic acid, 100 °C or RBr, K_2CO_3, CH_3CN, 60 °C; (b) **6**, KOH, KI, CH_3CN, 60 °C; (c) **4**, NaH, DMF, 40 °C.

Scheme 4[a]



[a] Reaction conditions: (a) LAH, THF, reflux; (b) *p*-tosyl chloride, pyridine, rt; (c) **6**, K_2CO_3, CH_3CN, 60 °C; (d) **4**, NaH, DMF, rt.

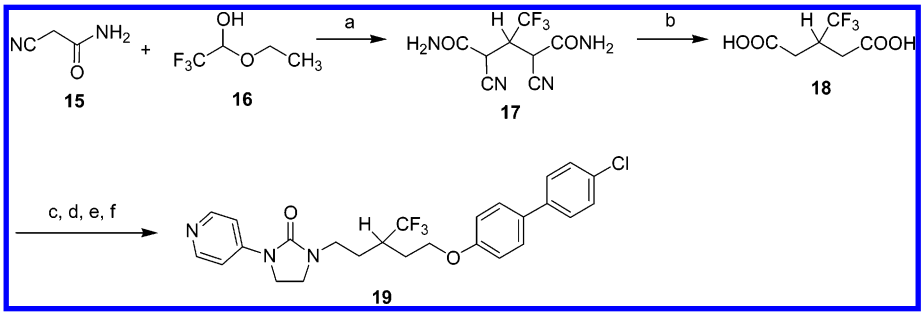
the *N*-substituted **10b**−**d**. The *O*-alkylation of biphenyl **6** with **10a**−**d** in the presence of KOH and KI afforded monosubstituted intermediates, which were coupled with **4** to give **11a**−**d**.

The synthesis of the linker replaced with a branched hydrophobic chain (**14a**−**h**) is shown in Scheme 4. Compounds **14a**−**h** were synthesized by using the commercially available ester **12a** and acids **12f**−**h** as starting materials or using the Michael addition to prepare 3-substituted glutarate diesters **12b**−**e**. Compounds **12b**−**e** were prepared by reacting dimethyl glutaconate with the appropriate Grignard reagents in the presence of catalytic CuI and excess TMSCl at −78 °C following Welmaker's synthetic method.[40] Reduction of **12a**−**h** with lithium aluminum hydride gave the corresponding alcohols, which were tosylated with *p*-tosyl chloride to afford **13a**−**h**. Reaction of tosylates **13a**−**h** with biphenyl **6** in the presence of K_2CO_3 at 60 °C followed by coupling with imidazolidinone **4** gave **14a**−**h**. The construction of trifluoromethyl **18** adopted the modified Kent's method (Scheme 5).[41] Condensation of cyanoacetamide and trifluoroacetaldehyde ethyl hemiacetal in the presence of piperidine/H_2O followed by hydrolysis of intermediate **17** by heating with concentrated HCl at 130 °C gave acid **18**. The dicarboxylic acid **18** was subjected to the same sequential reactions as in the preparation of **14a**−**h** (Scheme 4) to afford the desired trifluoromethyl compound **19**.

The position variation of the branched methyl group of the linker is illustrated in Scheme 6. The commercially available **20** was tosylated with *p*-toluenesulfonyl chloride in pyridine at room temperature to provide **21**. Due to one tosyl group being less hindered, **21** was reacted with **6** to afford biphenyl **22**, which was coupled with **4** in the presence of NaH to yield **24a**−**c**. Similarly, compound **21** was *N*-alkylated primarily with imidazolidinone **4** to give **23** and then *O*-alkylated with biphenyl **6** to afford **25a**−**c**.

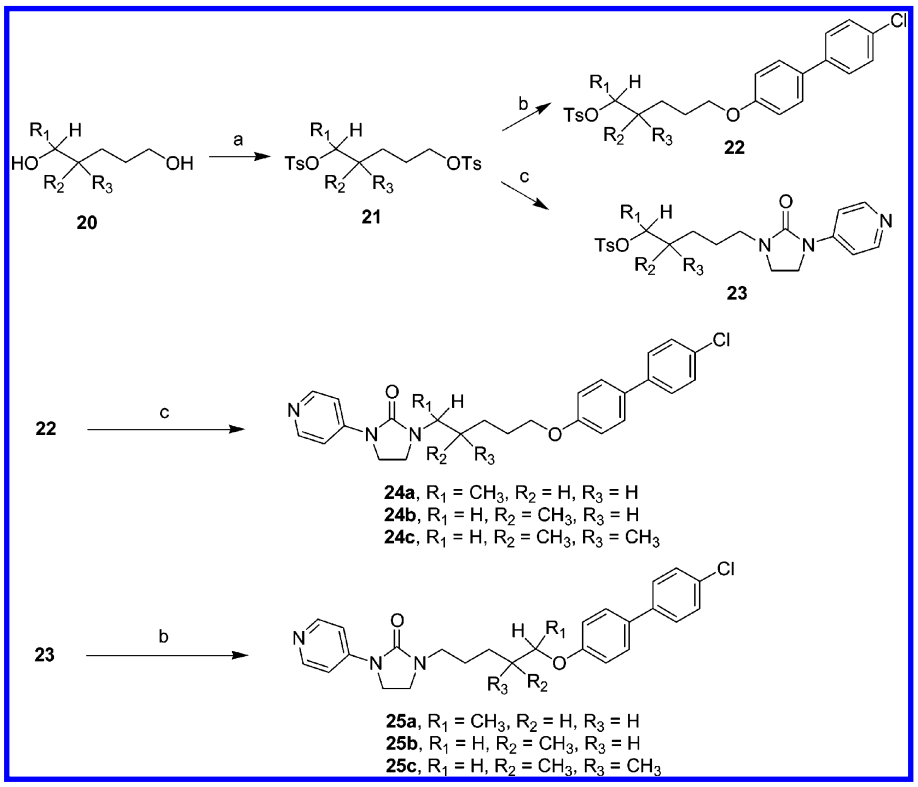
The syntheses of oxadiazoles **28a**−**d** and tetrazoles **32a**,**b** are described in Schemes 7 and 8, respectively. The tosylate **13a** was treated with 4-cyanophenol followed by *N*-alkylation with **4** to give **27** (Scheme 7). Treatment of **27** with hydroxylamine hydrochloride and K_2CO_3 gave amidoxime intermediate, which was acylated with the appropriate acid anhydride in pyridine at reflux followed by cyclization to give the corresponding 5-substituted oxadiazoles **28a**−**d**. The protection of phenolic **26** with methyl iodide followed by treatment with sodium azide in the presence of ammonium chloride[42] generated tetrazole **29** (Scheme 8), which was treated with the appropriate alkyl iodide to give 2-substituted tetrazoles **30**. The demethylation of **30** with boron tribromide and then *O*-alkylation with **13a** provided **31**. Finally, compound **31** was reacted with imidazolidinone **4** in the presence of NaH at room temperature to give the corresponding tetrazole **32a**,**b**.

Scheme 5[a]



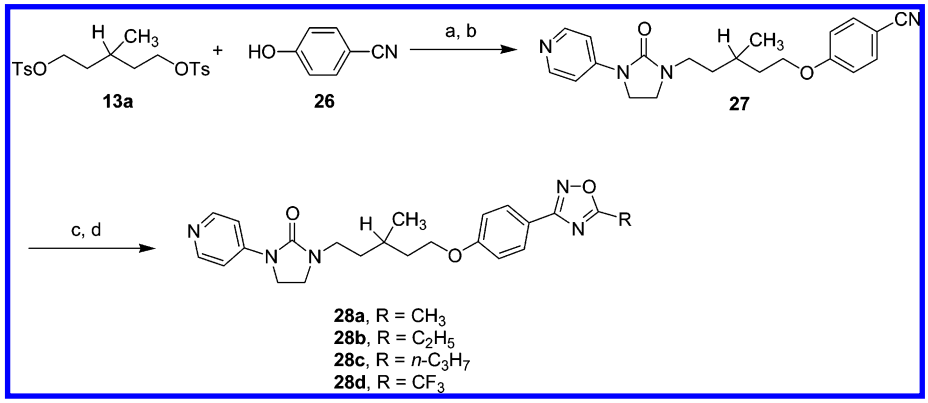
[a] Reaction conditions: (a) piperidine, H_2O, rt; (b) concd HCl, H_2O, 130 °C; (c) LAH, THF, reflux; (d) *p*-tosyl chloride, pyridine, rt; (e) **6**, K_2CO_3, CH_3CN, 60 °C; (f) **4**, NaH, DMF, r.t.

Scheme 6[a]



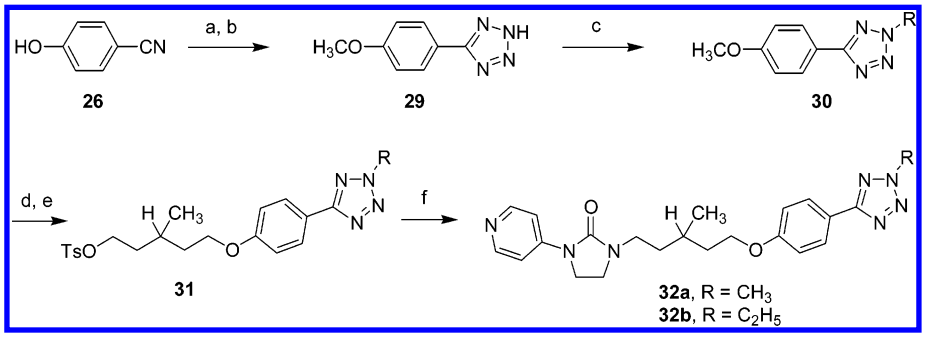
[a] Reaction conditions: (a) *p*-tosyl chloride, pyridine, rt; (b) **6**, K_2CO_3, CH_3CN, 60 °C; (c) **4**, NaH, DMF, rt.

Scheme 7[a]



[a] Reaction conditions: (a) K_2CO_3, CH_3CN, 60 °C; (b) **4**, NaH, DMF, rt; (c) $NH_2OH{\cdot}HCl$, K_2CO_3, EtOH, reflux; (d) RCOOCOR, pyridine, reflux.

Scheme 8[a]

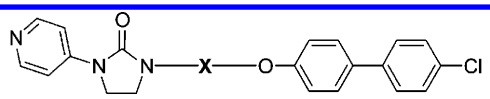


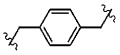
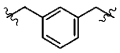
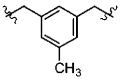
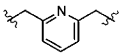
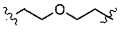
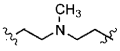
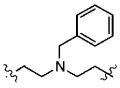
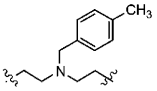
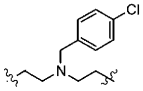
[a] Reaction conditions: (a) CH_3I, K_2CO_3, acetone, reflux; (b) NaN_3, DMF, NH_4Cl, 110 °C; (c) RI, K_2CO_3, CH_3CN, reflux; (d) BBr_3, CH_2Cl_2, rt; (e) **13a**, K_2CO_3, CH_3CN, 60 °C; (f) **4**, NaH, DMF, rt.

Results and Discussion

Compounds were tested against two EV71 serotypes in a neutralization test. EV71-2231 strain was isolated from a patient with mild HFMD and EV71-4643 strain was isolated from the spinal cord of a fatal case. Human neurons are vulnerable to EV71-4643 infection and are

Table 1. Anti-EV71 Activity and Cytotoxicity of Imidazolidinones **8a**−**f** and **11a**−**d**



Compound	X	IC_{50} (μM)[a] ± SD		CC_{50} (μM)[c]
		EV71 (2231)[b]	EV71 (4643)[b]	
8a		> 1.25	> 1.25	> 1.25
8b		> 1.25	> 1.25	> 1.25
8c		1.17 ± 0.01	0.16 ± 0.02	> 6.25
8d		> 25	22.88 ± 0.48	> 25
8e		> 25	> 25	> 25
8f		7.30 ± 3.08	0.83 ± 0.31	25
11a		1.18 ± 0.31	0.40 ± 0.08	25
11b		> 5.0	> 5.0	> 5.0
11c		> 12.5	> 12.5	> 12.5
11d		> 25	> 25	> 25
33		0.076 ± 0.004	0.038 ± 0.001	6.25

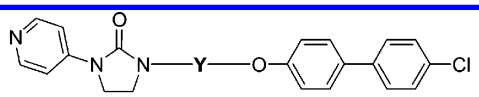
[a] Mean of triplicate well values. All experiments were performed against each EV strain at least twice. Neutralization test was employed. [b] Both EV71-2231 and EV71-4643 strains were isolated from patients in Taiwan. [c] Cellular toxicity/RD cell.

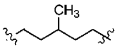
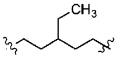
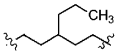
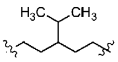
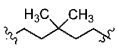
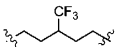
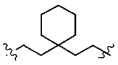
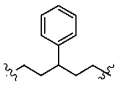
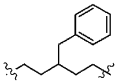
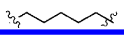
consistent with the clinical observation that EV71-4643 targets mainly neuronal cells but is also found in many organs in conjunction with an inflammatory reaction.[43] The concentration of a test compound required to reduce cell viability to 50% of the tested control culture was expressed as CC_{50}.

To investigate an appropriate orientation between the imidazolidinone and the biphenyl ring of compound **33**, the results of varying the substituents with rigid moieties on the linker are shown in Table 1. The data revealed that either an ortho (**8a**) or a para (**8b**) substitution on the phenyl ring resulted in inactivity while the meta substitution (**8c**) demonstrated moderate activity against both the EV71-2231 strain (IC_{50} = 1.17 μM) and the EV71-4643 strain (IC_{50} = 0.16 μM). However, an additional methyl group on the phenyl ring (**8d**) or a replacement with pyridine as a spacer (**8e**) led to the loss of antiviral activity.

We inserted different heteroatoms (N in **8e** or **11a**−**d**, O in **8f**) in the aliphatic linker to explore whether the active sites exhibited hydrogen-bonding interaction with the linker of imidazolidinones. Both **8f** and **11a** exhibited less potent antiviral activity in comparison to that of compound **33**, with IC_{50} values of 0.83 and 0.40 μM, respectively (Table 1). This effect was understandable when considering the hydrophobic nature of the drug-binding site. In addition, several hydrophobic moieties were introduced on the nitrogen atom to increase hydrophobic characteristics. Derivatives **11b**−**d**, with a benzyl group at the nitrogen of the linker, lost the inhibitory activity. It appeared that there were no strong hydrogen bonding and $\pi-\pi$ interactions with the active binding site due to the linker, and the steric bulk of the branched moiety was not tolerated in the binding site. Particularly, the introduction of the methyl group (**11a**) at the nitrogen resulted in a moderately active

Table 2. Anti-EV71 Activity and Cytotoxicity of Imidazolidinones **14a**−**h** and **19**



Compound	Y	IC_{50} (μM)[a] ± SD		CC_{50} (μM)[c]
		EV71 (2231)[b]	EV71 (4643)[b]	
14a		0.038 ± 0.003	0.0093 ± 0.0001	> 25
14b		0.60 ± 0.02	0.17 ± 0.01	> 25
14c		> 25	0.96 ± 0.05	> 25
14d		> 25	2.11 ± 0.12	> 25
14f		0.14 ± 0.02	0.041 ± 0.003	> 25
19		2.54 ± 0.03	0.74 ± 0.18	> 12.5
14g		> 25	> 25	> 25
14h		> 25	> 25	> 25
14e		> 10	> 10	> 10
33		0.076 ± 0.004	0.038 ± 0.001	6.25

[a] Mean of triplicate well values. All experiments were performed against each EV strain at least twice. Neutralization test was employed. [b] Both EV71-2231and EV71-4643 strains were isolated from patients in Taiwan. [c] Cellular toxicity/RD cell.

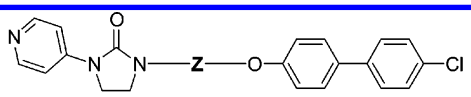
compound. The results appeared to indicate that the small aliphatic group might be a key element for activity.

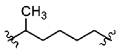
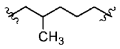
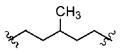
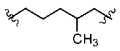
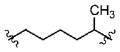
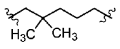
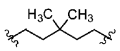
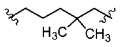
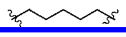
The effects of introducing aliphatic chains to the central position of linker were also examined. Substitution of a methyl group at the central hydrophobic linker (**14a**) exhibited 4-fold increased inhibitory potency (IC_{50} = 9.3 nM) in the 4643 strain and 2-fold greater in the 2231 strain (IC_{50} = 38 nM) as compared to compound **33** (Table 2). However, a decrease in activity was found in the continuing increase of the branched chain length from one carbon to three carbons (**14a**−**c**). The dimethyl-substituted derivative **14f** exhibited a potency comparable to that of compound **33**. The trifluoromethyl group (**19**) reduced the inhibitory activity toward EV71. Similarly, the compounds employing a bulky group like isopropyl (**14d**), cyclohexyl (**14g**), phenyl (**14h**), or benzyl (**14e**) were devoid of any antiviral activity. The results indicated that a methyl group might undergo a hydrophobic interaction with a small pocket located at the VP1 binding site to enhance the affinity, while bulkier groups at the linker might suffer from steric hindrance with the pocket, leading to the loss in antiviral activity.

The effect on anti-EV71 activity of introducing the methyl group at different positions of the linker is identified in Table 3. The results indicated that the introduction of the methyl group at the 2-position (**24b**) of the linker improved the activity against both the 2231 and 4643 strains, with IC_{50} values of 20 and 5 nM, respectively, while shifting the methyl group in the direction of the imidazolidinone ring (**24a**) or the biphenyl moiety (**25a**) resulted in a decrease in activity toward EV71. A methyl group introduced at the 4-position (**25b**) resulted in comparable activity to **14a**. Both **24b** and **25b** were significantly less cytotoxic than the corresponding analogues of **24a** and **25a**. Replacement of the methyl group of **24b** and **25b** with a dimethyl group to form **24c** and **25c**, respectively, dramatically reduced activity. However, the dimethyl **14f** still possessed anti-EV71 activity comparable to that of lead compound **33**. The above results revealed that the introduction of the methyl group at the 2- or 3-position of the linker significantly improved activity against EV71. The bulky group substitution on the linker rendered the compound only weakly active.

The pyridylimidazolidinone derivatives effectively inhibit virus replication in the early stages, implying that these compounds can inhibit viral adsorption and/or viral RNA uncoating. The virological investigation demonstrated that the compounds of this series target EV71 capsid protein VP1.[39] It is also very interesting to note that pleconaril[44] developed by Viropharma Inc.

Table 3. Anti-EV71 Activity and Cytotoxicity of Imidazolidinones **24a**−**c** and **25a**−**c**



Compound	Z	IC_{50} (μM)[a] ± SD		CC_{50} (μM)[c]
		EV71 (2231)[b]	EV71 (4643)[b]	
24a		0.23 ± 0.03	0.036 ± 0.006	12.5
24b		0.020 ± 0.001	0.005 ± 0.001	> 25
14a		0.038 ± 0.003	0.0093 ± 0.0001	> 25
25b		0.057 ± 0.005	0.015 ± 0.002	> 25
25a		0.60 ± 0.05	0.053 ± 0.001	> 12.5
24c		0.72 ± 0.05	0.094 ± 0.003	20
14f		0.14 ± 0.02	0.041 ± 0.003	> 25
25c		> 25	0.13 ± 0.07	> 25
33		0.076 ± 0.004	0.038 ± 0.001	6.25

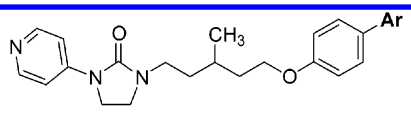
[a] Mean of triplicate well values. All experiments were performed against each EV strain at least twice. Neutralization test was employed. [b] Both EV71-2231and EV71-4643 strains were isolated from patients in Taiwan. [c] Cellular toxicity/RD cell.

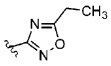
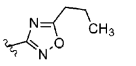
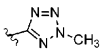
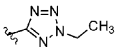
has been shown to have a broad spectrum of activities against rhinoviruses and enteroviruses. Moreover, it can occupy the hydrophobic binding pocket within VP1 as a capsid-binder inhibitor.[45] On one hand, we retain the methyl group attached to the center of the linker in order to improve activity and simplify synthesis; on the other hand, we attempt to modify the biphenyl moiety with the oxadiazole based on pleconaril to enhance antiviral spectrum. The results are shown in Table 4. The oxadiazole analogues **28a**−**d** exhibited good activities against both EV71 strains. Interestingly, we have found that both the 5-methyl- and 5-ethyloxadiazoles (**28a**, **28b**) display excellent antiviral activity against the lethal EV71-4643 strain with subnanomolar potency (**28a**, IC_{50} = 0.9 nM; **28b**, IC_{50} = 0.5 nM). Although **28a** possessed good inhibitory activity, it demonstrated a considerable cytotoxic effect. However, compound **28b** possessed 18-fold and 2.5-fold potency in 4643 strain and in 2231 strain, respectively, compared to **14a**. Replacement of the ethyl group in **28b** with a trifluoromethyl (**28d**) was only slightly less active (IC_{50} = 1.4 nM). Compounds **28b** and **28d** dramatically improved the antiviral activity against lethal EV71-4643 strain and enjoy low cytotoxicity (CC_{50} > 25 μM). Moreover, increasing the size of the chain to *n*-propyl (**28c**) resulted in decreased activity against EV71.

Compounds **32a**,**b** illustrated that the tetrazole moiety acts as a metabolically stable bioisostere for the oxadiazole functionality (Table 4).[46] Both **32a** and **32b** exhibited good activity comparable to that of the corresponding oxadiazole derivatives. The ethyl tetrazole **32b** had better activity against EV71-4643 strain (IC_{50} = 0.9 nM) and higher selectivity index (SI > 13 888) than **14a**. Indeed, a considerably increased activity was observed when the 4′-chlorophenyl in compound **14a** was replaced with its corresponding oxadiazole or tetrazole ring. In all cases examined, **28b** in the oxadiazole series and **32b** in the tetrazole series, in terms of potency and selectivity index, appeared to be the most promising candidates for further development as anti-EV71 agents.

In addition to screening imidazolidinones against EV71 (2 serotypes), we examined the broad-spectrum activity of this scaffold. The three kinds of different chemical platforms in **14a**, **28b**, and **32b** were selected and individually subjected to bioevaluation against a variety of viruses, including human enteroviruses 68 and 71 (five serotypes), coxsackieviruses (CV; 10 serotypes), echoviruses (Echo; two serotypes), and human rhinoviruses (HRV; two serotypes). The results are illustrated in Table 5. As shown, all of these compounds exhibited significant activity against EV71 (IC_{50} = 0.0005−0.32 μM), CVA10 (IC_{50} = 0.24−1.71 μM), CVA16 (IC_{50} = 0.09−0.90 μM), CVA24 (IC_{50} = 0.14−0.25 μM), CVB1 (IC_{50} = 0.035−2.89 μM), CVB4 (IC_{50} = 1.24−2.74 μM), CVB5 (IC_{50} = 1.32−10.4 μM), Echo9 (IC_{50} = 0.28−2.63 μM), Echo29 (IC_{50} = 0.04−3.23 μM), as well as HRV14 (IC_{50} = 0.83−7.91 μM). Moreover, compound **14a** showed extended potency against CVA9 (IC_{50} = 0.033 μM). In addition, both **28b** and **32b** also possessed inhibitory activity toward HRV2. We were pleased to find that these compounds exhibited dramatic potency against all serotypes of EV71 and broad-spectrum inhibitory activity compared to lead compound **33**.

Table 4. Anti-EV71 Activity and Cytotoxicity of Imidazolidinones **28a**−**d** and **32a**,**b**



Compound	Ar	IC_{50} (μM)[a] ± SD		CC_{50} (μM)[c]	Selectivity Index (SI)[d]	
		EV71 (2231)[b]	EV71 (4643)[b]		EV71(2231)	EV71(4643)
28a		0.010 ± 0.001	0.0009 ± 0.0001	> 6.25	> 625	> 6944
28b		0.015 ± 0.001	0.0005 ± 0.0001	> 25	> 1666	> 50000
28c		0.055 ± 0.001	0.019 ± 0.001	> 12.5	> 227	> 657
28d		0.024 ± 0.004	0.0014 ± 0.0001	> 25	> 1041	> 17857
32a		0.043 ± 0.001	0.0028 ± 0.0001	> 25	> 581	> 8928
32b		0.014 ± 0.001	0.0009 ± 0.0005	> 12.5	> 892	> 13888
14a		0.038 ± 0.003	0.0093 ± 0.0001	> 25	> 657	> 2688

[a] Mean of triplicate well values. All experiments were performed against each EV strain at least twice. Neutralization test was employed. [b] Both EV71-2231and EV71-4643 strains were isolated from patients in Taiwan. [c] Cellular toxicity/RD cell. [d] Selectivity index is the value of CC_{50}/IC_{50}.

Table 5. Comparative Evaluation of DBPR103, **28b**, and **32b** against Picornavirus

	IC_{50} (μM)[a]			
picornavirus	33	14a	28b	32b
EV71 (1743) genotype B	0.092 ± 0.010	0.016 ± 0.001	0.015 ± 0.003	0.018 ± 0.006
EV71 (2086) genotype C	0.043 ± 0.013	–	0.004 ± 0.001	0.003 ± 0.001
EV71 (2231) genotype C	0.076 ± 0.004	0.038 ± 0.003	0.015 ± 0.001	0.014 ± 0.001
EV71 (4643) genotype C	0.038 ± 0.001	0.0093 ± 0.0001	0.0005 ± 0.0001	0.0009 ± 0.0005
EV71 (BrCr) genotype A	0.130 ± 0.004	0.069 ± 0.0004	0.010 ± 0.002	0.015 ± 0.001
EV68	>12.5	2.68 ± 0.01	5.46 ± 0.69	2.87 ± 0.32
coxsackievirus A9	0.071 ± 0.004	0.033 ± 0.013	>25	>25
coxsackievirus A10	>12.5	1.71 ± 0.04	0.56 ± 0.07	0.24 ± 0.03
coxsackievirus A16	>12.5	0.90 ± 0.02	0.087 ± 0.013	0.13 ± 0.03
coxsackievirus A24	1.30 ± 0.94	0.25 ± 0.09	0.15 ± 0.02	0.14 ± 0.01
coxsackievirus B1	>12.5	2.89 ± 0.14	0.046 ± 0.005	0.035 ± 0.004
coxsackievirus B2	>12.5	>12.5	4.79 ± 0.51	>25
coxsackievirus B3	>12.5	>12.5	>25	>25
coxsackievirus B4	1.30 ± 0.79	1.24 ± 0.22	1.27 ± 0.16	2.74 ± 0.62
coxsackievirus B5	1.14 ± 0.56	1.32 ± 0.06	4.50 ± 1.41	10.4 ± 1.96
coxsackievirus B6	>12.5	>12.5	>25	>25
echovirus 9	1.31 ± 0.13	2.63 ± 0.21	0.50 ± 0.05	0.28 ± 0.02
echovirus 29	0.39 ± 0.06	0.040 ± 0.001	2.63 ± 0.33	3.23 ± 0.38
human rhinovirus 2	>12.5	>12.5	1.58 ± 0.20	1.45 ± 0.15
human rhinovirus 14	>12.5	7.91 ± 0.62	0.83 ± 0.10	0.85 ± 0.11

[a] Mean of triplicate well values. All experiments were performed at least twice. Neutralization test was employed.

Conclusion

The pyridylimidazolidinone derivative is the first compound reported to possess the potent activity against EV71. On the basis of the SAR studies, we successfully explored the linked spacer between imidazolidinone and biphenyl on the basis of lead compound **33**. The linker replacement with the aromatic ring should be meta-substituted orientation. The results of introducing an oxygen atom or the nitrogenated side chain on the linker were detrimental to the antiviral activity against EV71. Increasing the length of the branched chain on the linker resulted in a progressive decrease in activity, while replacement with the hydrophobic aromatic moiety rendered the compound only weakly active or inactive. The methyl group was the right substitution on the linker. Moreover, introducing the methyl group on the 2- or 3-position of the linker substantially improved activity against EV71. Furthermore, the replacement of the chlorophenyl ring of **12a** with the oxadiazole ring or the tetrazole ring resulted in dra-

matically improved inhibitory activity. The 5-ethyloxadiazole **28b** possessed excellent activity against lethal EV71-4643 strain with significant low cytotoxicity. Compounds **14a**, **28b**, and **32b** provided remarkable evidence that they were much more specific for human enteroviruses, in particular, EV71. These compounds were dramatically active against EV71 with a broad spectrum of potent activity against three genotypes (A, B, and C) of EV71. Therefore, imidazolidinone compounds **14a**, **28b**, and **32b** are well-qualified to serve as drug candidates for the further development of anti-EV71 agents.

Experimental Section

A. Synthetic Methods and Spectroscopic Details. Reagents were purchased from Aldrich, Acros, TCI, and Lancaster. Solvents, including dry ether and tetrahydrofuran (THF), were obtained by distillation from the sodium ketyl of benzophenone under nitrogen. Other solvents, including chloroform, dichloromethane, ethyl acetate, and hexane, were distilled over CaH_2 under nitrogen. Absolute methanol and ethanol were purchased from Merck and used as received. A commercial sample of CuI was obtained from Fisher Scientific Co. The colored impurities were removed from these salts by dissolving them in a saturated aqueous solution of KI followed by treatment with charcoal, filtration, and dilution with cold water to reprecipitate CuI halide. Analytical TLC was performed on precoated plates purchased from Merck (silica gel 60 F_{254}). Compounds were visualized by using UV light, I_2 vapor, or 2.5% phosphomolybdic acid in ethanol with heating. Melting points were obtained with a Yanaco (MP-500D) melting point apparatus. Fourier transform IR (FTIR) spectra were recorded on a Perkin-Elmer RX-I instrument (neat; ν, cm^{-1}). The proton NMR spectra were obtained on a Varian Mer-Vx-300 (300 MHz) spectrometer. Chloroform-*d* was used as solvent and the spectra were referenced to the residual protonated solvent signal. All NMR chemical shifts are reported as δ values in parts per million (ppm), and coupling constants (J) are given in hertz (Hz). Mass spectra were carried out on a Hewlett-Packard (model 1100 MSD) mass spectrometer. Microanalyses were performed on a Heraeus CHN−O rapid microanalyzer. Purification on silica gel refers to flash column chromatography on Merck silica gel 60 (particles size 230−400 mesh).

A.1. Preparation of 1-(4-Pyridyl)-2-imidazolidinone (4). To a stirred solution of 4-aminopyridine (5.0 g, 53.12 mmol) dissolved in toluene (20 mL) was added 2-chloroethyl isocyanate (9.69 g, 79.68 mmol) dropwise over 30 min in an ice bath. The mixture was reacted at room temperature for 12 h. The precipitate was collected by filtration and washed with toluene (20 mL) to afford *N*-(2-chloroethyl)-*N*′-(4-pyridyl)urea **3** (9.49 g, 83%).

To an ice-cooled solution of **3** (9.0 g, 41.73 mmol) in a mixture of dry THF (40 mL) and DMF (40 mL) was added slowly NaH (1.05 g, 43.82 mmol). The resulting mixture was stirred at room temperature for 5 h under argon and then quenched with MeOH (20 mL). After evaporation under vacuum, the crude residue was extracted with chloroform (30 mL × 2). The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The solid residue was recrystallized from MeOH to give compound **4** as a white solid (6.51 g, 96%).

A.2. General Procedure for the Synthesis of Compounds 8a−f. To a stirred suspension of desired dibromo compound (2.0 mmol), potassium bicarbonate (1.9 mmol), and potassium iodide (0.1 mmol) in *N*-methylpyrolidinone (3 mL) was added 4-chloro-4′-hydroxybiphenyl (1.0 mmol) at 60 °C for 2.5 h under argon. After cooling, the mixture was quenched with water (30 mL) followed by extraction with ethyl acetate (30 mL × 3). The organic layers were collected, washed with brine, and then concentrated under vacuum. The residue was subjected to flash chromatography on silica gel to give 1-substituted **7** (yield 50−85%).

A suspension of 1-(4-pyridyl)-2-imidazolidinone (0.50 mmol) and sodium hydride (75% dispersion in mineral oil, 0.55 mmol) in anhydrous DMF (7 mL) was reacted for 30 min under nitrogen followed by addition of a solution of **7** (0.50 mmol) in anhydrous DMF (5 mL). After 12 h, the suspension was quenched with water followed by extraction with ethyl acetate (100 mL × 3). The organic layers were combined, washed with brine, dried over anhydrous magnesium sulfate, and concentrated under vacuum. The crude residue was subjected to flash chromatography on silica gel to afford the target **8**.

1-[2-(4′-Chlorobiphenyl-4-yloxymethyl)benzyl]-3-pyridin-4-ylimidazolidin-2-one (8a): yield 87%; white solid; mp 209−211 °C; IR ν_{max} (neat) 2889, 1710, 1594, 1482, 1260 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.22 (br, 2H), 7.25−7.41 (m, 12H), 6.85 (d, J = 8.7 Hz, 2H), 4.99 (s, 2H), 4.52 (s, 2H), 3.59−3.64 (m, 2H), 3.27−3.32 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 157.8, 155.9, 149.7, 146.5, 138.8, 135.0, 134.8, 132.7, 132.5, 130.3, 129.6, 128.7, 128.6, 128.3, 127.8, 127.7, 114.7, 110.7, 67.9, 45.7, 41.4, 40.9; ESMS *m/z* 470.1 (MH^+). Anal. ($C_{28}H_{24}ClN_3O_2$) C, H, N.

1-[4-(4′-Chlorobiphenyl-4-yloxymethyl)benzyl]-3-pyridin-4-ylimidazolidin-2-one (8b): yield 93%; white solid; mp 215−216 °C; IR ν_{max} (neat) 2895, 1704, 1596, 1482 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.44 (d, J = 6.0 Hz, 2H), 7.54 (d, J = 6.3 Hz, 2H), 7.42−7.48 (m, 6H), 7.24−7.37 (m, 4H), 7.02 (d, J = 8.7 Hz, 2H), 5.10 (s, 2H), 4.52 (s, 2H), 3.78−3.83 (m, 2H), 3.41−3.46 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 158.1, 156.4, 150.0, 146.6, 138.9, 136.3, 135.9, 132.6, 132.5, 128.6, 128.3, 127.9, 127.8, 127.7, 115.0, 110.8, 69.7, 47.8, 41.4, 41.0; ESMS *m/z* 470.1 (MH^+). Anal. ($C_{28}H_{24}ClN_3O_2$) C, H, N.

1-[3-(4′-Chlorobiphenyl-4-yloxymethyl)benzyl]-3-pyridin-4-ylimidazolidin-2-one (8c): yield 88%; white solid; mp 171−172 °C; IR ν_{max} (neat) 1704, 1597, 1481, 1252 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.43 (br, 2H), 7.42−7.52 (m, 6H), 7.34−7.39 (m, 6H), 7.0 (d, J = 8.7 Hz, 2H), 5.09 (s, 2H), 4.51 (s, 2H), 3.75−3.81 (m, 2H), 3.39−3.44 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 158.0, 156.4, 150.0, 146.6, 138.8, 137.3, 136.4, 132.6, 132.5, 128.9, 128.7, 127.8, 127.7, 127.7, 127.0, 126.8, 115.1, 110.8, 69.8, 47.9, 41.4, 41.0; ESMS *m/z* 470.1 (MH^+). Anal. ($C_{28}H_{24}ClN_3O_2$) C, H, N.

1-[3-(4′-Chlorobiphenyl-4-yloxymethyl)-5-methylbenzyl]-3-pyridin-4-ylimidazolidin-2-one (8d): yield 85%; white solid; mp 170−172 °C; IR ν_{max} (neat) 2918, 1713, 1595, 1483, 1262 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.43 (d, J = 6.0 Hz, 2H), 7.34−7.49 (m, 7H), 7.17 (d, J = 15.0 Hz, 2H), 7.04 (d, J = 15.0 Hz, 2H), 7.0 (d, J = 8.4 Hz, 2H), 5.04 (s, 2H), 4.45 (s, 2H), 3.73−3.78 (m, 2H), 3.36−3.42 (m, 2H), 2.37 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 158.1, 156.4, 150.0, 146.6, 138.8, 137.2, 136.4, 136.4, 132.5, 132.5, 128.7, 128.5, 127.8, 127.7, 127.6, 124.2, 115.1, 110.8, 69.9, 47.9, 41.4, 41.0, 21.5; ESMS *m/z* 484.4 (MH^+). Anal. ($C_{29}H_{26}ClN_3O_2$) C, H, N.

1-[6-(4′-Chlorobiphenyl-4-yloxymethyl)-pyridin-2-ylmethyl]-3-pyridin-4-ylimidazolidin-2-one (8e): yield 82%; yellow solid; mp 210−212 °C; IR ν_{max} (neat) 2906, 1708, 1593, 1480, 1278 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.44 (d, J = 6.3 Hz, 2H), 7.25−7.74 (m, 11H), 7.02 (d, J = 8.7 Hz, 2H), 5.21 (s, 2H), 4.62 (s, 2H), 3.79−3.85 (m, 2H), 3.58−3.63 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 157.8, 156.6, 156.6, 155.8, 150.0, 146.6, 138.8, 137.6, 132.8, 132.6, 128.7, 127.9, 127.7, 121.0, 120.1, 115.1, 110.8, 70.7, 49.6, 41.8, 41.6; ESMS *m/z* 471.4 (MH^+). Anal. ($C_{27}H_{23}ClN_4O_2$) C, H, N.

1-{2-[2-(4′-Chlorobiphenyl-4-yloxy)ethoxy]ethyl}-3-pyridin-4-ylimidazolidin-2-one (8f): yield 75%; white solid; mp 128−130 °C; IR ν_{max} (neat) 2976, 1710, 1594, 1483, 1276 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.31 (d, J = 5.4 Hz, 2H), 7.27−7.34 (m, 8H), 6.84 (d, J = 8.4 Hz, 2H), 4.03−4.06 (m, 2H), 3.74−3.76 (m, 2H), 3.60−3.68 (m, 2H), 3.54−3.59 (m, 4H), 3.41−3.44 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 158.1, 156.4, 149.9, 146.6, 138.7 132.5, 132.4, 128.6, 127.7, 127.6, 114.7, 110.6, 70.0, 69.4, 67.5, 43.8, 42.9, 41.6; ESMS *m/z* 438.5 (MH^+). Anal. ($C_{24}H_{24}ClN_3O_3$) C, H, N.

A.3. General Procedure for the Synthesis of Compounds 11a−d. 1-{2-[2-(4′-Chlorobiphenyl-4-yloxy)ethyl-*N*-methylamino]ethyl}-3-pyridin-4-ylimidazolidin-2-one (11a).

A mixture of bis(2-chloroethyl)amine hydrochloride (2.0 g, 11.21 mmol), 37% formaldehyde (0.54 g, 16.82 mmol), and formic acid (1 mL) was heated at 75 °C for 18 h in a sealed bottle. After cooling, the mixture was taken up in ethyl ether and washed with saturated $NaHCO_3$ solution until basic pH. The organic layer was washed with water and dried over $MgSO_4$, and the solvent was removed in vacuo. The crude product was purified by flash chromatography on silica gel using hexanes/ethyl acetate (9:1) as eluant to give **10a** (1.6 g, 91%).

To a stirred solution of **10a** (0.16 g, 1.0 mmol) dissolved in dry CH_3CN was added compound **6** (0.20 g, 1.0 mmol), KOH (0.11 g, 2.0 mmol), and catalytic KI (17 mg, 0.1 mmol) under argon. The mixture was heated at 60 °C for 12 h. After cooling, the suspension was concentrated to remove CH_3CN and then partitioned with CH_2Cl_2/H_2O. The organic layer was collected and concentrated. The residue was subjected to chromatography on silica gel to give monosubstituted intermediate (0.22 g, 68%). Sequentially, this intermediate (0.20 g, 0.61 mmol) was treated with a mixture of **4** (0.10 g, 0.61 mmol) and NaH (44 mg, 1.83 mmol) in dry DMF (2 mL). The suspension was reacted at 40 °C under argon. After cooling, the suspension was quenched with distilled water (20 mL) and then extracted with ethyl acetate (20 mL × 3). The organic layer was collected, washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was subjected to flash chromatograph on silica gel to give **11a** as a yellow solid (0.23 g, 85%): mp 130−131 °C; IR ν_{max} (neat) 2933, 1704, 1598, 1485, 1274 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.37 (br, 2H), 7.32−7.42 (m, 8H), 6.90 (d, J = 8.7 Hz, 2H), 4.06 (t, J = 5.3 Hz, 2H), 3.61−3.68 (m, 4H), 3.42 (t, J = 6.4 Hz, 2H), 2.86 (t, J = 5.3 Hz, 2H), 2.70 (t, J = 6.4 Hz, 2H), 2.40 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 158.2, 156.5, 149.7, 146.7, 138.8, 132.5, 132.3, 128.6, 127.7, 127.6, 114.6, 110.7, 66.3, 56.5, 55.5, 42.9, 42.1, 41.7, 41.5; ESMS *m/z* 451.2 (M^+). Anal. ($C_{25}H_{27}ClN_4O_2$) C, H, N.

1-{2-[*N*-Benzyl-2-(4′-chlorobiphenyl-4-yloxy)ethylamino]ethyl}-3-pyridin-4-ylimidazolidin-2-one (11b). To a stirred suspension of bis(2-chloroethyl)amine hydrochloride (0.18 g, 1.0 mmol) and K_2CO_3 (0.42 g, 3.0 mmol) in CH_3CN (10 mL) was added benzyl bromide (0.17 g, 1.0 mmol) at reflux for 8 h. After cooling, the solid material was filtered out and the filtrate was concentrated in a vacuum. The residue was subjected to flash chromatography on silica gel to afford **10b** (0.20 g, 87%). To a stirred solution of **10b** (0.15 g, 0.65 mmol) dissolved in dry CH_3CN were added **6** (0.13 g, 0.65 mmol), KOH (73 mg, 1.30 mmol), and catalytic KI (11 mg, 0.065 mmol) at 60 °C for 24 h under argon. After cooling, the mixture was concentrated, diluted with distilled water (20 mL), and partitioned with CH_2Cl_2 (20 mL × 3). The organic layer was collected, washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The oil residue was subjected to chromatography on silica gel to give monosubstituted intermediate (0.13 g, 51%). Sequentially, the intermediated (0.10 mg, 0.25 mmol) was treatment with the mixture of **4** (41 mg, 0.25 mmol) and NaH (18 mg, 0.75 mmol) in dry DMF (1.5 mL). The suspension was reacted at 40 °C for 24 h under argon. After cooling, the suspension was quenched with distilled water (15 mL) with vigorous stirring and extracted with ethyl acetate (15 mL × 3). The organic layer was collected, washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The oil residue was subjected to flash chromatography on silica gel to afford **11b** as a colorless oil (95 mg, 72%). IR ν_{max} (neat) 2926, 1714, 1596, 1482, 1274 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.36 (d, J = 5.1 Hz, 2H), 7.25−7.41 (m, 13H), 6.86 (d, J = 8.4 Hz, 2H), 4.06 (t, J = 5.3 Hz, 2H), 3.73 (s, 2H), 3.50−3.55 (m, 2H), 3.34−3.43 (m, 4H), 2.95 (t, J = 5.3 Hz, 2H), 2.78 (t, J = 5.9 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 158.2, 156.4, 149.8, 146.7, 139.1, 138.8, 132.5, 132.2, 128.7, 128.6, 128.0, 127.8, 127.6, 126.9, 114.5, 110.6, 66.9, 59.8, 53.1, 52.3, 42.0, 41.9, 41.4; ESMS *m/z* 527.4 (M^+). Anal. ($C_{31}H_{31}ClN_4O_2$) C, H, N.

The desired compounds **11c**−**d** were prepared using the similar manner as described in the synthetic method of **11b**.

1-{2-[2-(4′-Chlorobiphenyl-4-yloxy)-*N*-(4-methylbenzyl-)ethylamino]ethyl}-3-pyridin-4-ylimidazolidin-2-one (11c): yield 67%; yellow oil; ^1H NMR ($CDCl_3$) δ 8.38 (dd, J = 5.0, 1.3 Hz, 2H), 7.34−7.42 (m, 8H), 7.19 (d, J = 7.8 Hz, 2H), 7.05 (d, J = 7.8 Hz, 2H), 6.88 (d, J = 8.7 Hz, 2H), 4.06 (t, J = 5.4 Hz, 2H), 3.70 (s, 2H), 3.52−3.53 (m, 2H), 3.48−3.50 (m, 2H), 3.37 (t, J = 5.7 Hz, 2H), 2.95 (t, J = 5.4 Hz, 2H), 2.79 (t, J = 5.7 Hz, 2H), 2.27 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 158.2, 156.4, 149.9, 146.7, 138.8, 136.5, 135.9, 132.5, 132.2, 128.7, 128.7, 128.7, 127.8, 127.6, 114.5, 110.6, 66.9, 59.5, 53.1, 52.3, 42.1, 41.5, 31.1, 21.2; ESMS *m/z* 541.1 (M^+). Anal. ($C_{32}H_{33}ClN_4O_2$) C, H, N.

1-{2-[*N*-(4-Chlorobenzyl)-2-(4′-chlorobiphenyl-4-yloxy)-ethylamino]ethyl}-3-pyridin-4-ylimidazolidin-2-one (11d): yield 72%; yellow solid; mp 118−119 °C; IR ν_{max} (neat) 2927, 1711, 1594, 1483, 1271 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.41 (dd, J = 5.1, 1.2 Hz, 2H), 7.29−7.44 (m, 6H), 7.22−7.29 (m, 6H), 6.89 (d, J = 8.7 Hz, 2H), 4.08 (t, J = 5.3 Hz, 2H), 3.74 (s, 2H), 3.56−3.61 (m, 2H), 3.44−3.48 (m, 2H), 3.40 (t, J = 5.9 Hz, 2H), 2.97 (t, J = 5.3 Hz, 2H), 2.81 (t, J = 5.9 Hz, 2H); ESMS *m/z* 561.3 (M^+). Anal. ($C_{31}H_{30}Cl_2N_4O_2$) C, H, N.

A.4. General Procedure for the Synthesis of Compounds 14a−h. (1) Preparation of 3-Substituted Glutarate Diesters (12a−e). To a stirred suspension of CuI (0.3 mmol) in THF (0.1 M) was added dropwise a desired Grignard reagent (4.0 mmol) under argon. The resulting mixture was stirred at room temperature until a dark color persisted (5 min). The mixture then was cooled to −78 °C, and TMSCl (4.0 mmol) and the glutarate diester (1.0 mmol) were introduced sequentially. The mixture was stirred at −78 °C for 2 h and then allowed to warm to ambient temperature over 4 h. The reaction then was quenched with saturated aqueous NH_4Cl and diluted with ethyl acetate. The phases were separated, and the aqueous phase was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The crude product was purified by flash chromatograph on silica gel to give 3-substituted glutarate diester **12a**−**e** (yield about 56−85%).

(2) General Procedure of the Synthesis of 14a−h from 12a−h. To a stirred solution of the synthetic diester (1.0 mmol, i.e., **12a**−**e**) or the commercially available acid (1 mmol, i.e., **12f**−**h**) dissolved in THF (20 mL) was added lithium aluminum hydride (3 mmol) at reflux for 24 h. After cooling the mixture was quenched with distilled water (50 mL) under vigorous stirring and the precipitate filtered out. The filtrate was concentrated under vacuum and extracted with ethyl acetate (30 mL × 3). The organic layer was collected, washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The oil residue was subjected to flash chromatograph on silica gel to give pure diol as a colorless oil. The diol (1.0 mmol) was treated with *p*-tosyl chloride (2.0 mmol) in pyridine (3 mL) at room temperature for 2 h. The resulting mixture was concentrated under vacuum and subjected to flash chromatography on silica gel to afford an appropriately tosylated **13** as a white powder (yield about 90−95%). Compound **13** (2.0 mmol) dissolved in CH_3CN (10 mL) was treated with **6** (1.0 mmol) and K_2CO_3 (1.0 mmol) at 60 °C for 24 h. After cooling, the mixture was concentrated under vacuum and diluted with CH_2Cl_2/H_2O (1:1). The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was subjected to flash chromatography on silica gel to give the 3-substituted 5-(4′-chlorobiphenyl-4-yloxy)pentyl *p*-toluenesulfonate (yield about 65−82%). The intermediate (1.0 mmol) was treated with the mixture of **4** (1.0 mmol) and NaH (1.5 mmol) in dry DMF (2 mL) at room temperature. After reaction for 24 h, the mixture was quenched with distilled water (20 mL) and extracted with ethyl acetate (20 mL × 3). The organic layer was collected, washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was subjected to flash chromatography on silica gel to give the target **14a**−**h**.

1-[5-(4′-Chlorobiphenyl-4-yloxy)-3-methylpentyl]-3-pyridin-4-ylimidazolidin-2-one (14a): yield 92%; white solid; mp 166−167 °C; IR ν_{max} (neat) 2931, 1704, 1482 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.41 (d, J = 5.7 Hz, 2H), 7.43−7.46 (m, 6H), 7.34−7.36 (m, 2H), 6.92 (d, J = 8.4 Hz, 2H), 4.01−4.06 (m, 2H), 3.75−3.80 (m, 2H), 3.48−3.55 (m, 2H), 3.34−3.42 (m, 2H), 1,-

75−1.90 (m, 2H), 1.69−1.73 (m, 2H), 1.44−1.48 (m, 1H), 1.03−1.05 (d, J = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 158.4, 156.5, 149.9, 146.7, 139.0, 132.5, 132.2, 128.6, 127.8, 127.7, 114.7, 110.7, 65.9, 42.0, 41.5, 41.5, 36.2, 34.4, 27.7, 19.7; ESMS *m/z* 450.5 (MH^+). Anal. ($C_{26}H_{28}ClN_3O_2$) C, H, N.

1-[5-(4′-Chlorobiphenyl-4-yloxy)-3-ethylpentyl]-3-pyridin-4-ylimidazolidin-2-one (14b): yield 86%; white solid; mp 141−142 °C; IR ν_{max} (neat) 2933, 1710, 1594, 1483, 1248 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.41 (d, J = 4.8 Hz, 2H), 7.42−7.45 (m, 6H), 7.34−7.36 (m, 2H), 6.93 (d, J = 8.7 Hz, 2H), 4.03 (t, J = 6.5 Hz, 2H), 3.74−3.79 (m, 2H), 3.49−3.55 (m, 2H), 3.36 (t, J = 7.2 Hz, 2H), 1.81−1.84 (m, 2H), 1.59−1.65 (m, 3H), 1.44−1.48 (m, 2H), 0.93 (t, J = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 158.4, 156.4, 149.9, 146.7, 139.0, 132.4, 132.2, 128.6, 127.8, 127.7, 114.7, 110.7, 66.0, 41.9, 41.5, 41.5, 33.8, 32.6, 30.8, 26.0, 10.8; ESMS *m/z* 464.1 (MH^+). Anal. ($C_{27}H_{30}ClN_3O_2$) C, H, N.

1-[5-(4′-Chlorobiphenyl-4-yloxy)-3-propylpentyl]-3-pyridin-4-ylimidazolidin-2-one (14c): yield 80%; white solid; mp 142−143 °C; IR ν_{max} (neat) 2930, 1710, 1595, 1483, 1249 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.41 (dd, J = 5.1, 1.8 Hz, 2H), 7.42−7.45 (m, 6H), 7.34−7.37 (m, 2H), 6.91−6.94 (d, J = 8.7 Hz, 2H), 4.03 (t, J = 6.5 Hz, 2H), 3.73−3.78 (m, 2H), 3.49−3.54 (m, 2H),3.36 (t, J = 7.4 Hz, 2H), 1.81−1.84 (m, 2H), 1.59−1.64 (m, 3H), 1.36−1.39 (m, 4H), 0.90−0.94 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 158.4, 156.4, 150.0, 146.7, 139.0, 132.4, 132.1, 128.6, 127.8, 127.7, 114.7, 110.7, 66.0, 41.9, 41.5, 41.5, 36.0, 33.1, 32.4, 31.3, 19.8, 14.6; ESMS *m/z* 478.1 (MH^+). Anal. ($C_{28}H_{32}ClN_3O_2$) C, H, N.

1-[5-(4′-Chlorobiphenyl-4-yloxy)-3-isopropylpentyl]-3-pyridin-4-ylimidazolidin-2-one (14d): yield 75%; white solid; mp 141−142 °C; IR ν_{max} (neat) 2957, 1710, 1594, 1483, 1268 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.40 (d, J = 6 Hz, 2H), 7.40−7.45 (m, 6H), 7.34−7.37 (m, 2H), 6.91−6.93 (d, J = 8.7 Hz, 2 H), 4.02 (t, J = 6.6 Hz, 2H), 3.71−3.76 (m, 2H), 3.49−3.54 (m, 2H), 3.35 (t, J = 7.2 Hz, 2H), 1.83−1.87 (m, 2H), 1.66−1.73 (m, 2H), 1.49−1.54 (m, 2H),0.91 (d, J = 6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 158.4, 156.4, 149.9, 146.6, 138.9, 132.4, 132.1, 128.6, 127.8, 127.6, 114.6, 110.7, 66.7, 42.6, 41.5, 41.4, 38.2, 30.2, 29.7, 28.4, 19.1; ESMS *m/z* 478.1 (MH^+). Anal. ($C_{28}H_{32}ClN_3O_2$) C, H, N.

1-[3-Benzyl-5-(4′-chlorobiphenyl-4-yloxy)pentyl]-3-pyridin-4-yl-imidazolidin-2-one (14e): yield 59%; colorless oil; ^{1}H NMR ($CDCl_3$) δ 8.41 (dd, J = 5.1, 1.2 Hz, 2H), 7.34−7.45 (m, 8H), 7.16−7.29 (m, 5H), 6.90 (d, J = 8.7 Hz, 2H), 4.05 (t, J = 6.3 Hz, 2H), 3.64−3.70 (m, 2H), 3.31−3.41 (m, 2H), 3.21−3.30 (m, 2H), 2.61−2.76 (m, 2H), 1.94−2.03 (m, 1H), 1.84−1.90 (m, 2H), 1.57−1.64 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 158.3, 156.4, 149.9, 146.7, 140.2, 138.9, 132.5, 132.2, 129.0, 128.6, 128.2, 127.8, 127.7, 125.9, 114.7, 110.7, 66.0, 41.7, 41.4, 41.1, 40.7, 34.8, 33.0, 29.9; ESMS *m/z* 526.2 (M^+). Anal. ($C_{32}H_{32}ClN_3O_2$) C, H, N.

1-[5-(4′-Chlorobiphenyl-4-yloxy)-3,3-dimethylpentyl]-3-pyridin-4-ylimidazolidin-2-one (14f): yield 85%; white solid; mp 150−152 °C; IR ν_{max} (neat) 2927, 1704, 1606, 1482 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.42 (d, J = 5.1 Hz, 2H), 7.44−7.46 (m, 6H), 7.34−7.37 (m, 2H), 6.94 (d, J = 8.7 Hz, 2H), 4.07 (t, J = 6.9 Hz, 2H), 3.77−3.82 (m, 2H), 3.51−3.56 (m, 2H), 3.35−3.41 (m, 2H), 1.81 (t, J = 6.9 Hz, 2H), 1.55−1.60 (m, 2H), 1.06 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 158.3, 156.3, 149.9, 146.7, 138.9, 132.4, 132.1, 128.6, 127.8, 127.7, 114.7, 110.6, 64.8, 41.4, 40.6, 40.4, 40.2, 39.0, 32.0, 27.6; ESMS *m/z* 464.1 (MH^+). Anal. ($C_{27}H_{30}ClN_3O_2$) C, H, N.

1-{2-{1-[2-(4′-Chlorobiphenyl-4-yloxy)ethyl]cyclohexyl}-ethyl}-3-pyridin-4-ylimidazolidin-2-one (14g): yield 91%; white solid; mp 162−163 °C; IR ν_{max} (neat) 2929, 1713, 1594, 1483, 1248 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.42 (d, J = 6.3 Hz, 2H), 7.43−7.46 (m, 6H), 7.34−7.37 (m, 2H), 6.95 (d, J = 8.4 Hz, 2H), 4.08 (t, J = 6.9 Hz, 2H), 3.75−3.81 (m, 2H), 3.51−3.57 (m, 2H), 3.34−3.39 (m, 2H), 1.88 (t, J = 6.9 Hz, 2H), 1.61−1.66 (m, 2H), 1.24−1.49 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 158.4, 156.3, 150.0, 146.7, 139.0, 132.5, 132.2, 128.6, 127.8, 127.7, 114.7, 110.7, 64.3, 41.6, 41.5, 39.5, 36.2, 35.9, 34.5, 34.3, 26.4, 21.7; ESMS *m/z* 504.2 (MH^+). Anal. ($C_{30}H_{34}ClN_3O_2$) C, H, N.

1-[5-(4′-Chlorobiphenyl-4-yloxy)-3-phenylpentyl]-3-pyridin-4-ylimidazolidin-2-one (14h). yield 67%; white solid; mp 175−177 °C; IR ν_{max} (neat) 2933, 1712, 1594, 1482, 1271 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.41 (dd, J = 5.0, 1.3 Hz, 2H), 7.32−7.91 (m, 7H), 7.17−7.28 (m. 6H), 6.83 (d, J = 8.7 Hz, 2H), 3.69−3.87 (m, 2H), 3.51−3.67 (m, 2H), 3.31−3.48 (m, 2H), 3.19−3.29 (m, 2H), 2.88−3.0 (m, 1H), 2.17−2.28 (m, 2H), 1.95−2.08 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 158.3, 156.2, 149.9, 146.6, 143.2, 139.0, 132.4, 132.2, 128.6, 128.5, 127.7, 127.7, 127.3, 126.4, 114.7, 110.7, 65.6, 42.7, 41.5, 41.3, 40.5, 36.5, 34.2; ESMS *m/z* 512.2 (MH^+). Anal. ($C_{31}H_{30}ClN_3O_2$) C, H, N.

A.5. General Procedure for the Synthesis of Compounds 19. 1-[5-(4′-Chlorobiphenyl-4-yloxy)-3-trifluoromethylpentyl]-3-pyridin-4-ylimidazolidin-2-one (19). To a stirred solution of cyanoacetamide (5.38 g, 64.0 mmol) dissolved in water (35 mL) was added successively trifluoroacetaldehyde ethyl hemiacetal (4.60 g, 32.0 mmol) and piperidine (0.2 mL, 2.1 mmol) at 10 °C. After stirring for 3 h at room temperature, the reaction mixture was cooled in an ice bath and stirred for another 1 h. After 15 min, the mixture was allowed to come to room temperature and then filtered with suction and washed thoroughly with cold distilled water to yield α,α′-dicyano-β-trifluoromethylglutamide **17** as a white solid (2.10 g, 27%).

To a stirred suspension of diamide (2.0 g, 8.1 mmol) was added concentrated HCl (4.4 mL) at room temperature. The suspension was warmed to 50 °C, and distilled water (4.4 mL) was added. The mixture was heated at 130 °C for 8 h. After cooling, the resulting solution was saturated with NaCl and extracted with ethyl ether (30 mL × 3). The organic layer was collected, dried over $MgSO_4$ and concentrated under vacuum. The residue was recrystallized from cosolvent (ethyl acetate and ethanol) to obtain trifluoromethylglutaric acid **18** as a pale yellow solid (0.62 g, 39%). Compound **19** was prepared using the similar manner as described in method A.4, employing the required **18** as a starting material: yield 25%; colorless oil; IR ν_{max} (neat) 2943, 1713, 1594, 1483, 1262 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.39 (d, J = 6.3 Hz, 2H), 7.33−7.42 (m, 8H), 6.90 (d. J = 8.7 Hz, 2H), 4.07−4.11 (m, 2H), 3.64−3.71 (m, 2H), 3.35−3.54 (m, 4H), 2.49−2.52 (m, 1H), 2.18−2.45 (m, 1H), 1.91−2.00 (m, 2H), 1.77−1.84 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 157.9, 156.5, 149.1, 146.4, 138.7, 132.5, 132.5, 128.6, 128.0 (J_{CF} = 277.1 Hz), 127.8, 127.6, 114.5, 110.7, 64.7, 42.0, 41.8, 41.4, 37.4 (J_{CF} = 25.6 Hz), 28.0, 26.1; ESMS *m/z* 504.1 (MH^+). Anal. ($C_{26}H_{25}ClF_3N_3O_2$) C, H, N.

A.6. General Procedure for the Synthesis of Compounds 24a−c and 25a−c. 1-[5-(4′-Chlorobiphenyl-4-yloxy)-1-methylpentyl]-3-pyridin-4-ylimidazolidin-2-one (24a). To a stirred solution of **20a** (1.00 g, 8.46 mmol) dissolved in pyridine (10 mL) was added *p*-toluenesulfonyl chloride (3.55 g, 18.61 mmol) at 0 °C under argon. After reaction for 3 h, the mixture was evaporated off under vacuum. The oil residue was subjected to flash chromatograph on silica gel to give **21a** as a white solid (3.48 g, 96%). Compound **21a** (0.85 g, 2 mmol) was treated with **6** (0.20 g, 1 mmol) and K_2CO_3 (0.14 g, 1 mmol) in CH_3CN (20 mL) at 60 °C for 6 h. After cooling the mixture was concentrated to remove solvent and partitioned with CH_2Cl_2/H_2O (1:1). The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was subjected to flash chromatography on silica gel to give **22a** as a white solid (0.42 g, 91%). Compoound **22a** (0.27 g, 0.59 mmol) was treated with the mixture of **4** (96 mg, 0.59 mmol) and NaH (42 mg, 1.77 mmol) in dry DMF (1 mL). The resulting mixture was reacted at room temperature for 12 h under argon and then quenched with distilled water (10 mL) and extracted with ethyl acetate (10 mL × 3). The organic layers were collected, washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was subjected to flash chromatography on silica gel to give **24a** as a white solid (0.23 g, 80%): mp 127−128 °C; ^{1}H NMR ($CDCl_3$) δ 8.41 (d, J = 5.4 Hz, 2H), 7.21−7.47 (m, 6H), 7.33−7.36 (m, 2H), 6.91 (d, J = 8.4 Hz, 2H), 4.10−4.17 (m, 2H), 3.96−4.00 (m, 2H), 3.77−3.82 (m, 2H), 3.37−3.53 (m, 4H), 1.75−1.90 (m, 2H), 1.48−1.64 (m, 2H), 1.20 (d, J = 6.6 Hz,

3H); ^{13}C NMR ($CDCl_3$) δ 158.4, 156.1, 149.9, 146.7, 139.0, 132.4, 132.1, 128.6, 127.8, 127.7, 114.6, 110.7, 67.6, 47.8, 41.6, 36.4, 33.6, 29.0, 23.2, 18.1; ESMS *m/z* 450.2 (MH^+). Anal. ($C_{26}H_{28}ClN_3O_2$) C, H, N.

The desired compounds **24b,c** were prepared using a similar manner as described for the synthesis of **24a**. The synthetic procedure of **25a**−**c** employed the same starting material **20** but primarily *N*-alkylation with **4** in the presence of NaH and then coupling with **6** by K_2CO_3.

1-[5-(4′-Chlorobiphenyl-4-yloxy)-2-methylpentyl]-3-pyridin-4-ylimidazolidin-2-one (24b):

yield 86%; white solid; mp 145−146 °C; IR ν_{max} (neat) 2947, 1706, 1604, 1483, 1425, 1273 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.42 (dd, J = 4.9, 1.7 Hz, 2H), 7.41−7.47 (m, 6H), 7.32−7.37 (m, 2H), 6.92 (d, J = 8.7 Hz, 2H), 3.98 (t, J = 6.3 Hz, 2H), 3.55−3.81 (m, 2H), 3.49−3.53 (m, 2H), 3.11−3.23 (m, 2H), 1.77−1.99 (m, 3H), 1.57−1.64 (m, 1H), 1.30−1.33 (m, 1H), 0.98 (d, J = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 158.4, 156.8, 150.0, 146.6, 139.0, 132.4, 132.1, 128.6, 127.8, 127.7, 114.6, 110.7, 68.0, 50.4, 42.3, 41.5, 31.7, 30.6, 26.8, 17.6; ESMS *m/z* 450.2 (MH^+). Anal. ($C_{26}H_{28}ClN_3O_2$) C, H, N.

1-[5-(4′-Chlorobiphenyl-4-yloxy)-2,2-dimethylpentyl]-3-pyridin-4-ylimidazolidin-2-one (24c). yield 83%; colorless oil; IR ν_{max} (neat) 2946, 1698, 1593, 1483, 1420, 1388, 1248 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.43 (d, J = 6.3 Hz, 2H), 7.42−7.48 (m, 6H), 7.33−7.37 (m, 2H), 6.93 (d, J = 8.7 Hz, 2H), 3.96−4.00 (m, 2H), 3.78−3.83 (m, 2H), 3.61−3.66 (m, 2H), 3.11 (s, 2H), 1.82−1.87 (m, 2H), 1.42−1.47 (m, 2H), 0.99 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 158.4, 157.7, 149.9, 146.6, 139.0, 132.4, 132.1, 128.6, 127.7, 127.6, 114.6, 110.8, 68.6, 56.0, 45.3, 41.5, 36.5, 35.8, 25.8, 24.2; ESMS *m/z* 464.1 (MH^+). Anal. ($C_{27}H_{30}ClN_3O_2$) C, H, N.

1-[5-(4′-Chlorobiphenyl-4-yloxy)-5-methylpentyl]-3-pyridin-4-ylimidazolidin-2-one (25a): yield 79%; white solid; mp 143−144 °C; IR ν_{max} (neat) 2935, 1710, 1593, 1482, 1425, 1268 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.41 (d, J = 6.0 Hz, 2H), 7.41−7.46 (m, 6H), 7.32−7.37 (m, 2H), 6.91 (d, J = 9.0 Hz, 2H), 4.36−4.42 (m, 1H), 3.74−3.80 (m, 2H), 3.47−3.53 (m, 2H), 3.29−3.33 (m, 2H), 1.78−1.83 (m, 2H), 1.59−1.75 (m, 2H), 1.41−1.56 (m, 2H), 1.32 (d, J = 6.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 157.5, 156.4, 149.8, 146.7, 139.0, 132.4, 132.0, 128.6, 127.8, 127.6, 115.9, 110.7, 73.6, 43.8, 41.5, 41.4, 36.2, 27.9, 22.9, 19.9; ESMS *m/z* 450.2 (MH^+). Anal. ($C_{26}H_{28}ClN_3O_2$) C, H, N.

1-[5-(4′-Chlorobiphenyl-4-yloxy)-4-methylpentyl]-3-pyridin-4-ylimidazolidin-2-one (25b): yield 89%; white solid; mp 140−142 °C; IR ν_{max} (neat) 2916, 1712, 1598, 1485, 1427, 1276 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.42 (br, 2H), 7.43−7.45 (m, 6H), 7.34−7.36 (m, 2H), 6.91−6.94 (m, 2H), 3.77−3.82 (m, 4H), 3.50−3.56 (m, 2H), 3.31−3.35 (m, 2H), 1.99−2.01 (m, 2H), 1.56−1.70 (m, 2H), 1.25−1.31 (m, 1H), 1.05 (d, J = 6.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 158.6, 156.5, 149.9, 146.7, 139.0, 132.4, 132.1, 128.6, 127.8, 127.7, 114.7, 110.8, 72.9, 44.2, 41.5, 41.5, 33.1, 30.6, 25.0, 17.2; ESMS *m/z* 450.2 (MH^+). Anal. ($C_{26}H_{28}ClN_3O_2$) C, H, N.

1-[5-(4′-Chlorobiphenyl-4-yloxy)-4,4-dimethylpentyl]-3-pyridin-4-ylimidazolidin-2-one (25c): yield 85%; colorless oil; IR ν_{max} (neat) 2963, 1704, 1602, 1485, 1425, 1252 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.42 (s, 2H), 7.43−7.49 (m, 6H), 7.34−7.36 (m, 2H), 6.93 (d, J = 8.7 Hz, 2H), 3.76−3.81 (m, 2H), 3.64 (s, 2H), 3.48−3.53 (m, 2H), 3.28−3.33 (m, 2H), 1.56−1.61 (m, 2H), 1.40−1.46 (m, 2H), 1.02 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 158.8, 156.4, 149.4, 147.1, 139.0, 132.4, 132.1, 128.6, 127.8, 127.7, 114.7, 110.8, 76.0, 44.5, 41.5, 41.4, 35.9, 34.4, 24.8, 22.1; ESMS *m/z* 464.1 (MH^+). Anal. ($C_{27}H_{30}ClN_3O_2$) C, H, N.

A.7. General Procedure for the Synthesis of Compounds 28a−d. 1-{3-Methyl-5-[4-(5-methyl-1,2,4-oxadiazol-2-yl)phenoxy]pentyl}-3-pyridin-4-ylimidazolidin-2-one (28a). To a stirred solution of **13a** (2.0 g, 4.69 mmol) dissolved in dried CH_3CN were added 4-cyanophenol (0.28 g, 2.35 mmol) and K_2CO_3 (0.65 g, 4.69 mmol) at 60 °C for 24 h. After cooling, the solvent was removed in vacuo and the residue was diluted with distilled water and then extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was subjected to flash chromatography to provide 5-(4-cyanophenoxy)-3-methylpentyl *p*-toluenesulfonate (0.83 g, 2.23 mmol). The material was treated with NaH in a similar manner as described in method A.4 to provide **27** (0.38 g, 91%). To a mixture of hydroxylamine hydrochloride (0.40 g, 5.80 mmol) and K_2CO_3 (0.81 g, 5.80 mmol) in ethanol (35 mL) was added **27** (0.62 g, 1.70 mmol) at reflux for 2 h. After cooling, the resulting mixture was concentrated in vacuo, and the solid residue was triturated with distilled water (5 mL). The resulting mixture was filtered, and the cake was washed with CH_2Cl_2/MeOH (8:1) to provide crude amidoxime as a white solid (0.52 g, 77%). The material (0.17 g, 0.43 mmol) was dissolved in pyridine (7 mL) and acetic anhydride (0.1 mL, 1.08 mmol) was added. The mixture was heated at 110 °C for 1 h. After cooling the mixture was concentrated in vacuo to give the crude solid. The residue was subjected to flash chromatography to yield **28a** as a colorless oil (0.12 g, 67%): ^{1}H NMR ($CDCl_3$) δ 8.42 (d, J = 6.0 Hz, 2H), 7.94 (d, J = 8.7 Hz, 2H), 7.47 (d, J = 6.0 Hz, 2H), 6.93 (d, J = 8.7 Hz, 2H), 4.03−4.09 (m, 2H), 3.75−3.80 (m, 2H), 3.48−3.54 (m, 2H), 3.31−3.43 (m, 2H), 2.62 (s, 3H), 1.76−1.93 (m, 2H), 1.63−1.74 (m, 2H), 1.39−1.51 (m, 1H), 1.03 (d, J = 6.3 Hz, 3H); ESMS *m/z* 422.2 (MH^+). Anal. ($C_{23}H_{27}N_5O_3$) C, H, N.

1-{5-[4-(5-Ethyl-1,2,4-oxadiazol-2-yl)phenoxy]-3-methylpentyl}-3-pyridin-4-ylimidazolidin-2-one (28b): yield 62%; yellow oil; IR ν_{max} (neat) 2928, 1713, 1613, 1594, 1481, 1426, 1377, 1253 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.40 (d, J = 6.0 Hz, 2H), 7.95 (d, J = 9.0 Hz, 2H), 7.44 (d, J = 6.0 Hz, 2H), 6.93 (d, J = 9.0 Hz, 2H), 4.01−4.08 (m, 2H), 3.74−3.79 (m, 2H), 3.47−3.53 (m, 2H), 3.33−3.41 (m, 2H), 2.94 (q, J = 7.6 Hz, 2H), 1.76−1.93 (m, 2H), 1.62−1.74 (m, 2H), 1.41−1.48 (m, 1H), 1.43 (t, J = 7.6 Hz, 3H), 1.03 (d, J = 6.3 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 180.1, 167.6, 160.9, 156.4, 149.8, 146.7, 128.7, 119.1, 114.5, 110.7, 65.9, 41.9, 41.4, 41.4, 36.0, 34.3, 27.7, 20.5, 19.6, 11.1; ESMS *m/z* 436.2 (MH^+). Anal. ($C_{24}H_{29}N_5O_3$) C, H, N.

1-{3-Methyl-5-[4-(5-*n*-propyl-1,2,4-oxadiazol-2-yl)phenoxy]pentyl}-3-pyridin-4-ylimidazolidin-2-one (28c): yield 57%; yellow oil; IR ν_{max} (neat) 2933, 1714, 1593, 1481, 1426, 1254 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.42 (d, J = 5.7 Hz, 2H), 7.96 (d, J = 9.0 Hz, 2H), 7.47 (d, J = 5.7 Hz, 2H), 6.93 (d, J = 9.0 Hz, 2H), 4.03−4.09 (m, 2H), 3.75−3.81 (m, 2H), 3.48−3.54 (m, 2H), 3.33−3.42 (m, 2H), 2.89 (t, J = 7.5 Hz, 2H), 1.83−1.95 (m, 2 H), 1.76−1.91 (m, 2H), 1.63−1.74 (m, 2H), 1.39−1.51 (m, 1H), 1.04 (t, J = 7.4 Hz, 3H), 1.04 (d, J = 6.3 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 179.2, 167.6, 160.9, 156.4, 149.5, 147.0, 128.8, 119.2, 114.5, 110.7, 65.9, 42.0, 41.5, 41.5, 36.0, 34.3, 28.6, 27.7, 20.4, 19.6, 13.9; ESMS *m/z* 450.2 (MH^+). Anal. ($C_{25}H_{31}N_5O_3$) C, H, N.

1-{3-Methyl-5-[4-(5-trifluoromethyl-1,2,4-oxadiazol-2-yl)phenoxy]pentyl}-3-pyridin-4-ylimidazolidin-2-one (28d): yield 58%; colorless oil; IR ν_{max} (neat) 2928, 1713, 1608, 1596, 1481, 1427, 1259, 1161 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.41 (d, J = 6.3 Hz, 2H), 8.00 (d, J = 9.0 Hz, 2H), 7.45 (d, J = 6.3 Hz, 2H), 6.96 (d, J = 9.0 Hz, 2H), 4.04−4.09 (m, 2H), 3.75−3.90 (m, 2H), 3.49−3.54 (m, 2H), 3.34−3.41 (m, 2H), 1.81−1.94 (m, 2H), 1.65−1.74 (m, 2H), 1.43−1.50 (m, 1H), 1.04 (d, J = 6.3 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 168.4, 165.1 (q, J_{CF} = 44.6 Hz), 161.8, 156.4, 149.9, 146.6, 129.2, 117.0, 115.8 (q, J_{CF} = 270.8 Hz), 114.8, 110.7, 66.1, 41.9, 41.5, 41.5, 35.9, 34.4, 27.7, 19.6; ESMS *m/z* 476.1 (MH^+). Anal. ($C_{23}H_{24}F_3N_5O_3$) C, H, N.

A.8. General Procedure for the Synthesis of Compounds 32a,b. 1-{3-Methyl-5-[4-[5-(2-methyl-2*H*-tetrazolyl)]-phenoxy]pentyl}-3-pyridin-4-ylimidazolidin-2-one (32a). To a stirred solution of **26** (1.19 g, 10 mmol) dissolved in dry acetone (30 mL) was added K_2CO_3 (1.67 g, 12 mmol) at room temperature for 10 min under argon. Methyl iodide (1.70 g, 12 mmol) was added dropwise, and the mixture was heated at reflux for 3 h. After cooling the mixture was concentrated in vacuo and partitioned between distilled water and ethyl ether. The organic layer was collected, washed with 10% NaOH and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was subjected to flash chromatography to give 4-methoxybenzonitrile as a white solid (1.21 g, 91%). The material

(1.0 g, 7.51 mmol) was dissolved in DMF (10 mL) and NaN_3 (0.54 g, 8.26 mmol), and NH_4Cl (0.10 g, 1.88 mmol) was added under argon. The mixture was heated at 110 °C for 16 h. After cooling, the reaction mixture was poured into crashed ice with vigorous stirring and extracted with CH_2Cl_2 (30 mL × 3). The aqueous solution was acidified carefully with 6 N HCl. A white solid precipitate was filtered and recrystallized from CH_3CN to give **29** (0.71 g, 54%). To a stirred suspension of **29** (0.60 g, 3.41 mmol) in CH_3CN (20 mL) were added CH_3I (0.58 g, 4.09 mmol) and K_2CO_3 (0.57 g, 4.09 mmol), and the mixture was heated at reflux for 1 h. After cooling, the mixture was concentrated in vacuo, and the residue was partitioned between ethyl acetate and distilled water. The organic layers were collected, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was subjected to flash chromatography to give **30a** (0.37 g, 57%). To a stirred solution of **30a** (0.32 g, 1.68 mmol) dissolved in dry CH_2Cl_2 (15 mL) was added dropwise BBr_3 (5.04 mmol, 1 M solution in CH_2Cl_2) at room temperature under argon. After 3 h the mixture was poured into ice−water with vigorous stirring and the resultant white precipitate was collected and recrystallized from ethanol to give 4-[5-(2-methyl-2*H*-tetrazolyl)]phenol (0.28 g, 94%). The phenol material was *O*-alkylation by treatment with **13a** to provide tosylate **31**, which was coupled with imidazolidine **4** to give target **32a** (the procedure was similar to the synthesis of **14** from **13** as described in method A.4):

yield 45%; yellow oil; ^{1}H NMR ($CDCl_3$) δ 8.41 (d, J = 5.4 Hz, 2H), 8.01 (d, J = 9.0 Hz, 2H), 7.45 (d, J = 5.4 Hz, 2H), 6.95 (d, J = 9.0 Hz, 2H), 4.36 (s, 3H), 4.03−4.10 (m, 2H), 3.74−3.80 (m, 2H), 3.42−3.54 (m, 2H), 3.34−3.39 (m, 2H), 1.79−1.92 (m, 2H), 1.64−1.73 (m, 2H), 1.40−1.51 (m, 1H), 1.04 (d, J = 6.3 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 164.8, 160.3, 156.4, 149.9, 146.7, 128.1, 119.7, 114.6, 110.8, 65.9, 41.9, 41.5, 41.5, 39.5, 36.1, 34.3, 27.7, 19.7; ESMS *m/z* 422.2 (MH^+). Anal. ($C_{22}H_{27}N_7O_2$) C, H, N.

1-{5-[4-[5-(2-Ethyl-2*H*-tetrazolyl)]phenoxy]-3-methylpentyl}-3-pyridin-4-ylimidazolidin-2-one (32b): yield 67%; yellow solid; mp 77−79 °C; IR ν_{max} (neat) 2928, 1714, 1615, 1594, 1506, 1480, 1462, 1428, 1392, 1251 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.36 (d, J = 6.3 Hz, 2H), 7.98 (d, J = 8.7 Hz, 2H), 7.40 (d, J = 6.3 Hz, 2H), 6.91 (d, J = 8.7 Hz, 2H), 4.02 (q, J = 7.3 Hz, 2H), 3.99−4.04 (m, 2H), 3.68−3.73 (m, 2H), 3.41−3.48 (m, 2H), 3.26−3.39 (m, 2H), 1.67−1.88 (m, 4H), 1.62 (t, J = 7.3 Hz, 3H), 1.38−1.47 (m, 1H), 1.00 (d, J = 6.3 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 164.0, 159.8, 155.9, 149.4, 146.3, 127.6, 119.4, 114.2, 110.2, 65.5, 48.0, 41.4, 41.0, 40.9, 35.6, 33.8, 27.2, 19.2, 14.4; ESMS *m/z* 436.2 (MH^+). Anal. ($C_{23}H_{29}N_7O_2$) C, H, N.

B. Biological Evaulation. B.1. Cells and Viruses. EV 71 isolates from the 1998 outbreak were obtained from the Clinical Virology Laboratory in Chang Gang Children's Hospitals (Taipei, Taiwan). BrCr, the prototype of EV 71, was obtained from the American Type Culture Collection (ATCC accession no. VR 784). EV 71-1743, EV 71-2234, and EV 71-4643 were isolated from throat swabs, while EV 71-2086 was isolated from the skin lesion of an implicated HFMD (hand, foot, and mouth disease) patient. RD cells (rhabdomyosarcoma cells; incubate HEV), Vero cells (African green monkey kidney cells; ATCC accession no. CCL-81; incubate HSV), MDCK cells (canine kidney epithelial cells; incubate influenza virus), and MRC-5 cells (normal fetal lung cells; ATCC accession no. CCL-171; incubate HRV) were used for virus isolation and propagation.

B.2. Neutralization Test. This assay measured the ability of a test compound to inhibit the cytopathic effect induced by a picornavirus on RD cells. The 96-well tissue culture plates were seeded with 200 μL of RD cells at a concentration of 3 × 10^5 cells/mL in DMEM with 10% FBS. The plates were incubated for 24−30 h at 37 °C and were used at about 90% confluency. Virus (100 $TCID_{50}$) mixed with different concentrations of test compounds was added to the cells and incubated at 37 °C for 1 h. After adsorption, the infected cell plates were overlayed with 50 μL of DMEM plus 5% FBS and 2% DMSO. The plate was wrapped in Parafilm and incubated at 37 °C for 64 h. At the end of incubation, the plates were fixed by the addition of 100 μL of 0.5% glutaraldehyde for 1 h at room temperature. After the removal of glutaldehyde, the plates were stained with 0.1% crystal violet for 15 min at room temperature. The plates were washed and dried, and the density of the well was measured at 570 nm. The concentration required for a test compound to reduce the virus-induced cytopathic effect (CPE) by 50% relative to the virus control was expressed as IC_{50}. All assays were performed in triplicate and at least twice.

B.3. Cytotoxicity Assay. Twenty-three test compounds at various concentrations were added to RD cells. The cells were then incubated at 37 °C for 96 h. After incubation, the cells were harvested and viable cells were counted by trypan blue staining. All experiments were performed in triplicate and at least twice. The concentration of a test compound required to reduce cell viability to 50% of the tested control culture was expressed as CC_{50}.

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Supporting Information Available: Protocols for biological assays, synthetic procedures, and spectral data for **8a**−**f**, **11a**−**d**, **14a**−**h**, **19**, **24a**−**c**, **25a**−**c**, **28a**−**d**, **32a**−**b** and elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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