Journal of Medicinal Chemistry

Article

Identification and Structure-Activity Relationships of Diarylhydrazides as Novel Potent and Selective Human Enterovirus Inhibitors

Xin Han, Ningyuan Sun, Haoming Wu, Deyin Guo, Po Tien, Chune Dong, Shuwen Wu, and Hai-Bing Zhou

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.5b01803 • Publication Date (Web): 17 Feb 2016

Just Accepted

ACS Publications

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Identification and Structure-Activity Relationships of Diarylhydrazides as Novel Potent and Selective Human Enterovirus Inhibitors

Xin Han,[a] Ningyuan Sun,[b] Haoming Wu,[b] Deyin Guo,[b] Po Tien,[b] Chune Dong,[a] Shuwen Wu*[b] and Hai-Bing Zhou*[a]

[a] *State Key Laboratory of Virology, Key Laboratory of Combinatorial Biosynthesis and Drug Discovery (Wuhan University), Ministry of Education, Wuhan University School of Pharmaceutical Sciences, Wuhan 430071, China*

[b] *College of Life Sciences, Wuhan University, Wuhan 430072, China*

zhouhb@whu.edu.cn; shuwenwu@hotmail.com

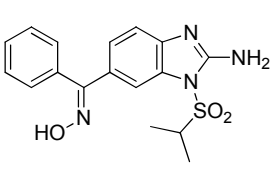
ABSTRACT

Enterovirus 71 (EV71) plays an important role in hand-foot-and-mouth disease. In this study, a series of diarylhydrazide analogues was synthesized, and the systematic exploration of SAR led to potent enterovirus inhibitors, of which compound **15** exhibits significant improvements in inhibition potency, with an EC_{50} value of 0.02 μM against EV71. It is very interesting that this class of diarylhydrazides exhibits activities against a series of human enteroviruses at the picomolar level, including EV71 and Coxsackieviruses B1 (CVB1), CVB2, CVB3, CVB4, CVB5 and CVB6 (EC_{50} as low as 0.5 nM). Compared with the reference anti-enterovirus drug **1** (enviroxime) and known inhibitor **5** (WIN 51711), the four highly selective compounds **15**, **27**, **41** and **47** inhibited EV71 replication with EC_{50} values of 0.17-0.02 μM and SI values in a range of 978.4-12338. A preliminary mechanistic study indicated that VP1 might be the target site for this type of compound.

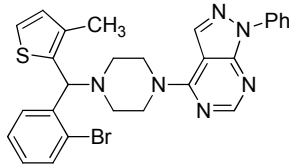
Introduction

Hand-foot-and-mouth disease (HFMD), which was first reported as an outbreak in Taiwan, China, in 1998, is one of the most common infectious diseases among children.[1,2] In recent years, HFMD has become a prevalent disease in many countries in the Asian-Pacific region. Enterovirus 71 (EV71) and coxsackievirus 16 are the most common etiologic agents of HFMD. EV71, first isolated in 1969 as a member of the piconaviridae family, is also associated with some other serious diseases such as mild childhood exanthema, herpangina, aseptic meningitis, encephalitis, and poliomyelitis-like paralysis.[3,4] Recently, there have been a number of enterovirus-associated HFMD breakouts, leading to high mortality and making the disease a serious public health issue in China.[5-8] Several approaches have been attempted to combat enterovirus infections, but there are no effective antiviral drugs against human enterovirus presently in clinical use.[9-11] Therefore, the development of novel anti-enteroviral agents for HFMD therapy is urgently needed. EV71 is a single-stranded RNA virus in the genus enterovirus, and its open reading frame (ORF) includes three consecutive parts, P1, P2 and P3. The viral capsid has icosahedral symmetry and is composed of 60 identical units, each consisting of four structural proteins VP1-VP4. These VPs (viral proteins) are important capsid proteomes that form the enterovirus and contain significant numbers of cell-recognition sites that could be used to guide the antivirus drug discovery.[12-14] To date, a number of molecules have been reported to inhibit enterovirus, the earliest and the most widely studied of which is enviroxime **1** (Figure 1).[15-19] Vinylacetylene analogues of **1** (enviroxime) have also been utilized by the Spitzer group as poliovirus and coxsackie A21 inhibitors in tissue culture.[20,21] In 2003, 2-aminoimidazo[1,2-*b*] pyridazines were reported to exhibit broad spectrum activity against human picornaviruses in a plaque reduction assay and a cytopathic effect assay.[22] More recently, Chern and co-workers developed pyrazolo[3,4-*d*]pyrimidine (**2**, Figure 1) as an enterovirus inhibitor, in particular, an anti-coxsackievirus agent.[23] In 2010, Pérez-Pérez and co-workers developed 9-arylpurines (**3**, Figure 1) that elicit activity against a variety of enteroviruses with EC_{50} values of 5-8 μM.[15,16] In 2013, MacLeod *et al.* identified

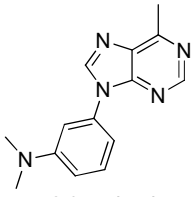
imidazopyrazine derivatives as interesting inhibitors of EV71, with EC_{50} values as low as 11 nM (**4**, Figure 1).[24] Some natural products have also been found to have potent enterovirus inhibitory activity.[25-27] Although anti-HFMD drugs mainly include site-combining blockers that contain scavenger receptor B2 (SCARB2), P-selectin glycoprotein ligand-1 (PSGL1),[28,29] 3C protease inhibitors,[1,30] and RNA interference,[31] because of their low bioactivities and bioavailabilities, as well as the complex structures of the existing human enterovirus inhibitors, novel enterovirus inhibitors with simplified scaffolds, higher bioactivities and bioavailabilities are urgently needed from the point of view of new drug discovery.[32-35] Hence, it is highly desirable to develop more effective EV71 inhibitors for the treatment of HFMD and enterovirus-related diseases. Some recent examples including WIN 51711 (**5**, Figure 1), a picornavirus inhibitor, were found to have a broad spectrum of activities against enteroviruses by interfering with the capsid-receptor binding site through inhibiting the virus attachment to the cells and the uncoating of the viral RNA.[36] In 2015, itraconazole, a clinical drug for antifungal therapy, was found to be a broad spectrum inhibitor of enteroviruses through acting on the novel targets oxy-sterol-binding protein (OSBP) and OSBP-related protein 4 (ORP4) by Van Kuppeveld and co-workers.[37] Very recently, we have developed thiophene-2-carboxamides (**6**, Figure 1) as anti-EV71 agents, which showed low micromolar activity against EV71 (EC_{50} = 1.42 μM) compared with the reference drug **1** (enviroxime).[38]



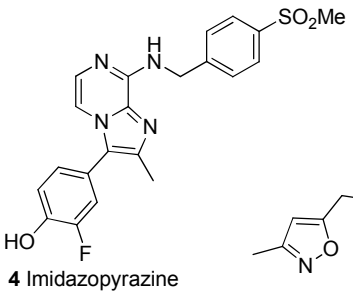
1 Enviroxime



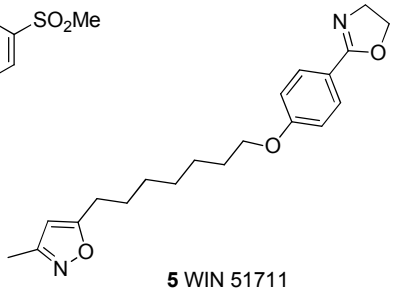
2 pyrazolo[3,4-*d*]pyrimidines



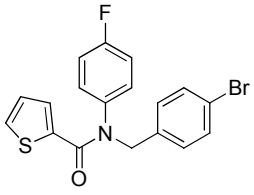
3 9-arylpurine



4 Imidazopyrazine



5 WIN 51711



6 Thiophene-carboxamide

Figure 1. Structures of known enterovirus inhibitors

As a part of our long-term interest in the development of antiviral agents,[39-41] as well as the further exploration of anti-EV71 drugs that might have superior efficacy and fewer side effects than existing therapeutic agents, diarylhydrazide derivatives with significant antiviral activity were identified based on the result of our previous work. Two hit compounds **10** and **11** based on a 2,6-dichlorosubstituted phenyl hydrazide scaffold showed sub-micromolar potency against EV71, with EC_{50} values of 0.47 μM and 0.94 μM, respectively, but suffer from poor selectivity indexes (Figure 2). The subsequent chemical optimization led to the potential compound **15** with improved bioactive properties and a higher selectivity index. It is worth noting that these compounds are easy to synthesize. As far as we are aware, a hydrazide scaffold has not been studied as an EV71 inhibitor, although certain hydrazide derivatives are known to exhibit antibacterial, antimycotic, algicidal and anthelmintic activities (Figure 2).[42-44] Hence, an attempt has been made to explore the potential of these newly discovered hydrazides as leads for anti-EV71 drug development.

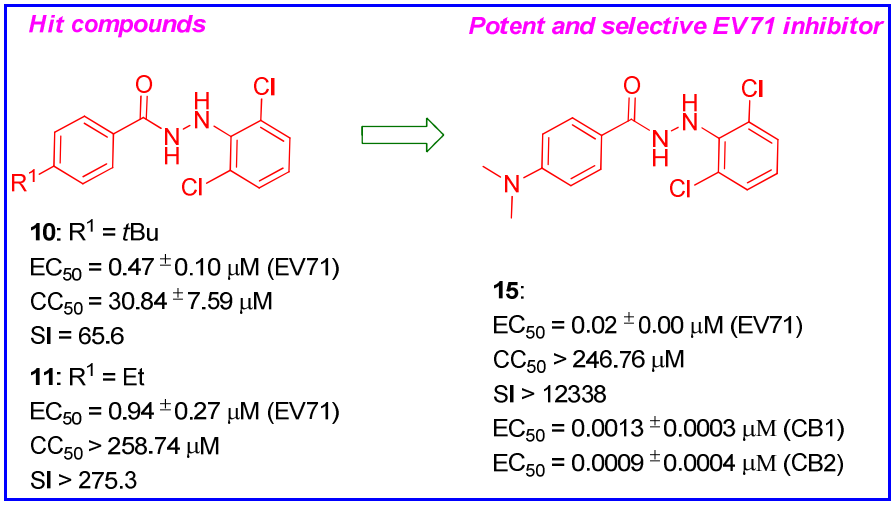


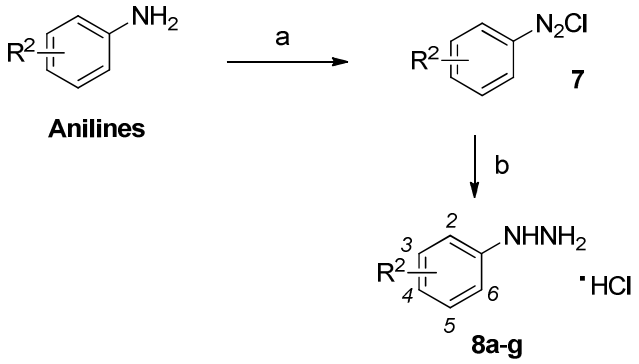
Figure 2. Identification of diarylhydrazide **15** as a potent and highly selective EV71 inhibitor from the systematic SAR study of the diarylhydrazide analogues. EC_{50}: effective concentration (μM) for 50% inhibition of EV71, as evaluated by an MTS-based CPE reduction assay in Vero cells; CC_{50}: cytotoxic concentration (μM) to induce 50% death of noninfected cells, as evaluated by an MTS-based CPE reduction

assay in Vero cells; SI: selectivity index calculated as CC_{50}/EC_{50} ratio; EV71: Enterovirus 71; CVB1: Coxsackie virus B1; CVB2: Coxsackie virus B2.

RESULTS AND DISCUSSION

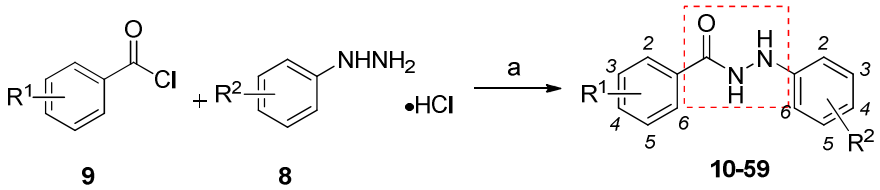
Chemical Synthesis

The general synthetic route for the preparation of all of the designed hydrazides **10-59** is illustrated in Schemes 1 and 2. The key intermediate arylhydrazine hydrochloride **8** was prepared through the simple $SnCl_2$ reduction of the corresponding aryldiazenes **7** derived from various anilines (Schemes 1). According to the reported method, the arylhydrazine hydrochloride **8** coupling with acyl chloride **9** in the presence of triethylamine in CH_2Cl_2 at room temperature afforded the final products **10-59** in 75-95% yields (Scheme 2).[45]



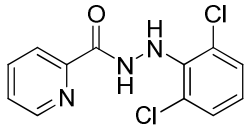
Cmpd	R^2	Yield (%)
8a	2,6-Br_2	90
8b	2,6-Et_2	88
8c	2,6-Pr_2	85
8d	2,6-OMe_2	82
8e	2-Cl-6-Me	87
8f	2,4,6-Cl_3	91
8g	4-OEt	83

Scheme 1. Synthesis of arylhydrazine hydrochlorides. Reagents and reaction conditions: (a) $NaNO_2$ (1.1 equiv), H_2O, HCl (3 equiv), -5 °C - rt; (b) $SnCl_2$ (2 equiv), H_2O, HCl, rt, 12 h.

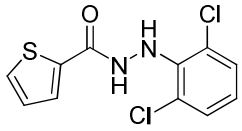


9 8 10-59

Cmpd	R^1	R^2	Yield (%)	Cmpd	R^1	R^2	Yield (%)
10	4-*t*-Bu	2,6-Cl_2	92	**35**	4-Me	2-Cl-6-Me	90
11	4-Et	2,6-Cl_2	93	**36**	4-OMe	2-Cl-6-Me	92
12	4-Me	2,6-Cl_2	91	**37**	4-OEt	2-Cl-6-Me	94
13	4-OEt	2,6-Cl_2	94	**38**	4-*n*-Pr	2-Cl-6-Me	92
14	4-*n*-Pr	2,6-Cl_2	95	**39**	4-N(Me)$_2$	2-Cl-6-Me	78
15	4-N(Me)$_2$	2,6-Cl_2	83	**40**	4-SMe	2-Cl-6-Me	88
16	4-OMe	2,6-Cl_2	88	**41**	4-*t*-Bu	2,6-Br_2	93
17	2-OMe	2,6-Cl_2	88	**42**	4-Et	2,6-Br_2	94
18	H	2,6-Cl_2	90	**43**	4-Me	2,6-Br_2	91
19	4-Cl	2,6-Cl_2	92	**44**	4-OMe	2,6-Br_2	93
20	–	2,6-Cl_2	75	**45**	4-OEt	2,6-Br_2	95
21	–	2,6-Cl_2	85	**46**	4-*n*-Pr	2,6-Br_2	92
22	4-OEt	2,4,6-Cl_3	90	**47**	4-N(Me)$_2$	2,6-Br_2	81
23	2,6-Cl_2	4-OEt	88	**48**	H	2,6-Br_2	90
24	4-*t*-Bu	2,6-OMe_2	87	**49**	2-OMe	2,6-Br_2	88
25	4-*t*-Bu	2,6-Et_2	84	**50**	4-*t*-Bu	2,6-F_2	94
26	4-*t*-Bu	2,6-Pr_2	83	**51**	4-OMe	2,6-F_2	93
27	4-*t*-Bu	2,6-Me_2	92	**52**	4-OEt	2,6-F_2	95
28	4-Et	2,6-Me_2	91	**53**	4-*n*-Pr	2,6-F_2	92
29	4-Me	2,6-Me_2	93	**54**	4-N(Me)$_2$	2,6-F_2	80
30	4-OMe	2,6-Me_2	90	**55**	4-F	2,6-Cl_2	81
31	4-*n*-Pr	2,6-Me_2	93	**56**	4-Br	2,6-Cl_2	84
32	4-N(Me)$_2$	2,6-Me_2	81	**57**	4-CN	2,6-Cl_2	89
33	4-*t*-Bu	2-Cl-6-Me	93	**58**	4-NO_2	2,6-Cl_2	85
34	4-Et	2-Cl-6-Me	91	**59**	3-Cl	2,6-Cl_2	91



20



21

Scheme 2. Synthesis of the target compounds **10-59**. Reagents and reaction conditions: (a) triethylamine (2.6 equiv), CH_2Cl_2, rt, 2 h.

Structure-Activity Relationship Analysis

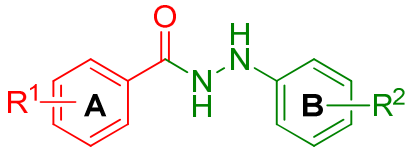
The activities of all new hydrazide compounds were tested against diversified enterovirus serotypes in a neutralization test. In addition, the references anti-enterovirus drug **1** (enviroxime) and known inhibitor **5** (WIN 51711) were tested for comparison. The results of this extensive biological evaluation are shown in Table 1. As a global observation, it is noteworthy that the two phenyl rings A and B

displayed distinct SARs found in most of the anti-enterovirus agents. Initially, compounds **10** and **11** showed good activities against EV71 (EC_{50} = 0.47 μM and 0.94 μM, respectively) compared to **1** (enviroxime) (EC_{50} = 0.15 μM) and **5** (WIN 51711) (EC_{50} = 19.48 μM) (entries 1-4). Based on the structures of **10** and **11**, in the subsequent chemical optimization, diverse substituents including electron-withdrawing and electron-donating groups on the acyl phenyl ring A were screened in the presence of the same 2,6-dichloro on the hydrazine phenyl ring B. The results revealed that electron-donating groups are favorable substituents in the control of the bioactivity, and the corresponding hydrazides exhibited potent anti-enterovirus activity (entries 3-9), with the best one (**15**) possessing a $N(Me)_2$ at the *para*-position of the acyl phenyl ring A, showing the lowest EC_{50} of 0.02 μM (entry 8). On the other hand, the unsubstituted or chloro-substituted hydrazide on the acyl phenyl ring A and some other heterocyclic rings, e.g., pyridine (**20**) and thiophene (**21**), showed no activity against EV71 (entries 11-14). Other substrates, for example, transferring the OMe from the *para*- to *ortho*-position, adding a chlorine atom onto the hydrazine phenyl ring B, or exchanging the position of rings A and B, also showed no activities against EV71 (entries 9, 10, 15 and 16). In the presence of the *t*-Bu on phenyl ring A, phenyl ring B with different groups, e.g., 2,6-OMe_2, 2,6-Et_2, 2,6-Pr_2, 2,6-Me_2, 2,6-Br_2, 2,6-F_2 or 2-Cl-6-Me, has an inhibitory activity that decreases with the increase in the side chain by a large margin (entries 17-20, 26, 34 and 43). This observation could be verified by computer modeling (Figure 7), which indicated that there is not enough space between the 2,6-disubstituted phenyl ring B and the acting site in the protease to accommodate a bulky substituent. Moreover, upon introducing 2,6-dimethyl groups instead of 2,6-dichloro groups on phenyl ring B, reduced inhibitory activities were obtained, except for with a *t*-Bu group at the *para*-position of phenyl ring A (entries 20-25). On the other hand, the replacement of phenyl ring B with unsymmetric 2-Cl-6-Me groups at the same position led to comparable activity to the corresponding 2,6-dimethyl substituted ring (entries 26-32). There was no obvious change in activity upon altering the 4-OMe to 4-SMe in the presence of the same phenyl ring B (entries 29 and 33). The 2,6-dibromo substituted ring B usually led to increased anti-EV71

activity; most notably, compounds **41** and **49** gave EC_{50} values of 0.08 μM and 0.86 μM, respectively (entries 34-42). Interestingly, the 2,6-difluoro substituted ring B exhibited a severely reduced activity compared with the corresponding 2,6-dichloro and 2,6-dibromo analogues (entries 43-47). Moreover, analogues **55-59** with electron-withdrawing substituents, i.e., 4-F (**55**), 4-Br (**56**), 4-CN (**57**), 4-NO_2 (**58**), 3-Cl (**59**), on phenyl ring A show no activity against EV71. One can conclude that the installation of an electron-withdrawing group on phenyl ring A usually leads to no activity. Among all of the novel anti-EV71 hydrazide agents, there were three compounds (**15**, **41** and **47**) having a better inhibitory activity than the reference anti-EV71 agent **1** (enviroxime) (entries 1, 8, 34 *vs* 40) and eight compounds possessing comparable activities to that of **1** (entries 3, 6, 7, 20, 25, 30, 32 and 38).

These diarylhydrazide analogues were further tested using another cell line, RD cells. All of the compounds, except for **12** and **39**, which show slightly better inhibitory activities, showed a smaller inferior inhibitory activity for EV71 in RD cells than those of Vero cells (Table 1). The most potent compound **15** was the most remarkable, with an EC_{50} value of 0.03 μM in RD cells (entry 8). Most of the compounds had no or little cellular toxicity and high selectivity. These biological results demonstrate that this series of hydrazides consists of potent and versatile anti-EV71 agents with a high selectivity index and potential for pharmaceutical use.

Table 1. Antiviral Evaluation of the Hydrazides against EV71[a]



Entry	Cmpd	R^1	R^2	EC_{50} (μM)[b]		CC_{50} (μM)[c]		SI[d]	
				Vero Cells	RD Cells	Vero Cells	RD Cells	Vero Cells	RD Cells
1	**1**	Enviroxime		0.15 ± 0.06	0.24 ± 0.07	>223.21	>223.21	>1488.1	>949.8
2	**5**	WIN 51711		19.48 ± 6.57	15.32 ± 4.64	147.77 ± 38.84	167.39 ± 47.84	7.6	10.9
3	**10**	4-*t*-Bu	2, 6-Cl_2	0.47 ± 0.10	0.85 ± 0.24	30.84 ± 7.59	36.72 ± 9.3	65.6	43.2
4	**11**	4-Et	2, 6-Cl_2	0.94 ± 0.27	1.68 ± 0.53	>258.74	>258.74	>275.3	>154
5	**12**	4-Me	2, 6-Cl_2	1.28 ± 0.48	0.96 ± 0.34	>271.04	>271.04	>211.8	>281.3
6	**13**	4-OEt	2, 6-Cl_2	0.33 ± 0.07	0.37 ± 0.10	>246.01	>246.01	>745.5	>664.9
7	**14**	4-*n*-Pr	2, 6-Cl_2	0.42 ± 0.08	0.44 ± 0.15	>247.51	>247.51	>589.3	>562.5
8	**15**	4-$N(Me)_2$	2, 6-Cl_2	0.02 ± 0.00	0.03 ± 0.01	>246.76	>246.76	>12338	>8225.3
9	**16**	4-OMe	2, 6-Cl_2	0.68 ± 0.13	1.36 ± 0.37	>257.07	>257.07	>378	>189
10	**17**	2-OMe	2, 6-Cl_2	>100	>100	ND[e]	ND	--[f]	--
11	**18**	H	2, 6-Cl_2	>100	>100	ND	ND	--	--
12[g]	**19**	4-Cl	2, 6-Cl_2	>100	>100	ND	ND	--	--
13	**20**	--	2, 6-Cl_2	>100	>100	ND	ND	--	--
14	**21**	--	2, 6-Cl_2	>100	>100	ND	ND	--	--
15	**22**	4-OEt	2, 4, 6-Cl_3	>100	>100	ND	ND	--	--
16	**23**	2,6-Cl_2	4-OEt	>100	>100	ND	ND	--	--

17	**24**	4-*t*-Bu	2, 6-OMe_2	10.41 ± 3.78	27.39 ± 8.52	>243.60	>243.60	>23.4	>8.9
18	**25**	4-*t*-Bu	2, 6-Et_2	>100	>100	ND	ND	--	--
19	**26**	4-*t*-Bu	2, 6-Pr_2	>100	>100	ND	ND	--	--
20	**27**	4-*t*-Bu	2, 6-Me_2	0.17 ± 0.04	0.42 ± 0.15	166.33 ± 31.81	188.49 ± 50.27	978.4	454.2
21	**28**	4-Et	2, 6-Me_2	1.57 ± 0.43	2.75 ± 0.87	135.27 ± 36.63	164.31 ± 42.37	86.2	59.7
22	**29**	4-Me	2, 6-Me_2	5.31 ± 1.35	13.56 ± 3.27	>314.56	>314.56	>59.2	>23.2
23	**30**	4-OMe	2, 6-Me_2	12.32 ± 4.14	28.97 ± 7.64	>295.94	>295.94	>24	>10.2
24	**31**	4-*n*-Pr	2, 6-Me_2	0.95 ± 0.44	2.64 ± 0.85	>283.31	>283.31	>298.2	>107.2
25	**32**	4-$N(Me)_2$	2, 6-Me_2	0.29 ± 0.08	0.69 ± 0.21	>282.32	>282.32	>973.5	>410.9
26	**33**	4-*t*-Bu	2-Cl-6-Me	0.79 ± 0.20	0.97 ± 0.37	49.24 ± 13.16	63.23 ± 20.48	62.3	65.1
27	**34**	4-Et	2-Cl-6-Me	6.37 ± 1.46	10.52 ± 2.59	81.74 ± 17.87	105.42 ± 27.53	12.8	10.0
28	**35**	4-Me	2-Cl-6-Me	12.96 ± 4.62	26.87 ± 8.56	>291.18	>291.18	>22.5	>10.8
29	**36**	4-OMe	2-Cl-6-Me	5.74 ± 1.82	15.38 ± 4.34	>275.16	>275.16	>47.9	>17.9
30	**37**	4-OEt	2-Cl-6-Me	0.39 ± 0.07	0.82 ± 0.30	>262.49	>262.49	>673.1	>319.3
31	**38**	4-*n*-Pr	2-Cl-6-Me	0.92 ± 0.19	2.16 ± 0.43	80.25 ± 22.85	113.28 ± 31.38	87.2	52.4
32	**39**	4-$N(Me)_2$	2-Cl-6-Me	0.28 ± 0.08	0.16 ± 0.07	92.83 ± 25.05	106.43 ± 28.72	331.5	682.3
33	**40**	4-SMe	2-Cl-6-Me	6.13 ± 2.17	15.43 ± 4.26	106.58 ± 27.44	134.42 ± 31.58	17.4	8.7
34	**41**	4-*t*-Bu	2, 6-Br_2	0.08 ± 0.03	0.29 ± 0.07	>187.73	>187.73	2346.6	>640.7
35	**42**	4-Et	2, 6-Br_2	0.93 ± 0.61	1.58 ± 0.73	>200.96	>200.96	>216.1	>127.0
36	**43**	4-Me	2, 6-Br_2	10.81 ± 6.17	24.65 ± 8.53	146.33 ± 27.08	163.27 ± 30.61	13.5	6.6
37	**44**	4-OMe	2, 6-Br_2	3.32 ± 0.71	2.63 ± 0.84	>199.97	>199.97	60.2	>76.1
38	**45**	4-OEt	2, 6-Br_2	0.37 ± 0.10	0.53 ± 0.24	>193.19	>193.19	>522.1	>361.8
39	**46**	4-*n*-Pr	2, 6-Br_2	3.54 ± 0.92	4.62 ± 1.57	>194.12	>194.12	>54.8	>42.0
40	**47**	4-$N(Me)_2$	2, 6-Br_2	0.03 ± 0.01	0.07 ± 0.03	>193.65	>193.65	>6455	>2934.2
41	**48**	H	2, 6-Br_2	0.86 ± 0.44	2.43 ± 0.72	>216.19	>216.19	>251.4	>89.1
42	**49**	2-OMe	2, 6-Br_2	54.24 ± 16.42	>100	109.48 ± 17.05	ND	2	--

43	**50**	4-*t*-Bu	2, 6-F_2	2.19 ± 0.81	4.27 ± 0.92	149.84 ± 28.69	183.84 ± 26.35	68.4	43.0
44	**51**	4-OMe	2, 6-F_2	74.03 ± 24.22	>100	>287.51	ND	3.9	--
45	**52**	4-OEt	2, 6-F_2	66.72 ± 25.39	>100	>273.71	ND	4.1	--
46	**53**	4-*n*-Pr	2, 6-F_2	27.38 ± 5.80	51.44 ± 8.53	>275.57	>275.57	10.1	>5.4
47	**54**	4-$N(Me)_2$	2, 6-F_2	17.95 ± 12.98	23.46 ± 11.47	>274.64	>274.64	15.3	>11.7

[a] All data are mean values ± standard deviation for at least three independent experiments; [b] EC_{50}: effective concentration (μM) for 50% inhibition of EV71, as evaluated by an MTS-based CPE reduction assay in corresponding cells; [c] CC_{50}: cytotoxic concentration (μM) to induce 50% death of noninfected cells, as evaluated by an MTS-based CPE reduction assay in corresponding cells; [d] SI: selectivity index calculated as CC_{50}/EC_{50} ratio; [e] ND: Not determined; [f] Not calculated because the EC_{50} was too high; [g] Other electron-withdrawing substituents, e.g., 4-F (**55**), 4-Br (**56**), 4-CN (**57**), 4-NO_2 (**58**), and 3-Cl (**59**), were also found to have no activities.

An analysis of the anti-EV71 activities of alternate substituents at the 4-position of phenyl ring A with a 2,6-Cl_2 substituted phenyl ring B is presented in Figure 3A. The results reveal that the derivatives with substituents $N(Me)_2$ (**15**), OEt (**13**), *t*-Bu (**10**) and *n*-Pr (**14**) have better anti-EV71 activities compared to others, with $N(Me)_2$ being the best in the series. However, with no substituent or the electron-withdrawing group Cl at the 4-position of phenyl ring A, there is almost no activity, with EC_{50} values greater than 100 μM. $N(Me)_2$ is also a favorable active moiety in the determination of anti-EV71 activity in the presence of an 2,6-Cl_2, 2,6-Me_2, 2-Cl-6-Me or 2,6-Br_2 substituted phenyl ring B, although not the 2,6-F_2 substituted ring (Figure 3B). These results verify that aryl phenylhydrazides that have electron-donating groups at the 4-position of phenyl ring A and disubstituted groups on phenyl ring B usually have excellent inhibitory activities for EV71.

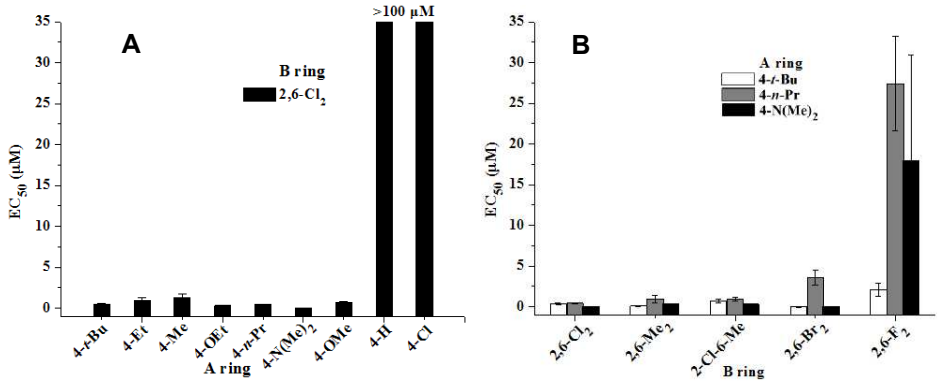


Figure 3. A) and **B**) Graphical exhibit of the effect of substituents of phenyl rings A and B on the anti-EV71 activity of the compounds in Vero cells.

The four most potent anti-EV71 agents, compounds **15**, **27**, **41** and **47**, were further selected for evaluation against a panel of other human enteroviruses, including Coxsackie viruses B1 (CVB1), CVB2, CVB3, CVB4, CVB5 and CVB6. We were pleased to find that these compounds exhibited inspiring activity against a variety of human enteroviruses. The results are illustrated in Table 2, showing that all of the compounds exhibited significant activity against Coxsackievirus, with EC_{50} values ranging from lower micromolar to nanomolar concentrations. Compounds **15** and **47** proved to be the two most potent anti-enterovirus agents against CVB2, with EC_{50} values as low as picomolar, 0.0009 μM and 0.0005 μM, respectively. In terms of

CVB1, antiviral activity in the low nanomolar range was also found in the presence of compounds **15**, **27** and **47** (EC_{50} = 0.0013-0.005 μM). Although these compounds showed weaker activities against CVB3, CVB4, CVB5 and CVB6 than against CVB1 and CVB2, the EC_{50} values were still in a range of low micromolar to nanomolar concentrations (EC_{50} = 6.52-0.02 μM). It is noteworthy that for all enteroviruses tested, compound **47** showed much better antiviral activity than reference drug **1** (enviroxime), while compound **15** showed superior activity against CVB1–CVB4 than **1** (enviroxime).

Table 2. Antiviral Evaluation of Compounds **15**, **27**, **41, 47** and **1** (enviroxime) against a Selected Panel of Enteroviruses[a]

Virus (strain)	EC_{50} (μM)[b]				
	1 (enviroxime)	**15**	**27**	**41**	**47**
CVB1	0.75 ± 0.20	0.0013 ± 0.0003	0.0016 ± 0.0005	2.61 ± 0.99	0.005 ± 0.002
CVB2	0.15 ± 0.03	0.0009 ± 0.0004	0.0016 ± 0.0006	1.57 ± 0.60	0.0005 ± 0.0004
CVB3	2.48 ± 0.65	0.95 ± 0.23	0.09 ± 0.03	0.87 ± 0.35	0.03 ± 0.01
CVB4	> 186	2.57 ± 1.07	5.62 ± 1.79	6.52 ± 1.70	0.34 ± 0.10
CVB5	0.12 ± 0.04	2.27 ± 0.73	3.19 ± 0.92	0.98 ± 0.48	0.06 ± 0.02
CVB6	0.10 ± 0.02	0.94 ± 0.47	0.56 ± 0.21	0.92 ± 0.44	0.02 ± 0.01

[a] All data are mean values ± standard deviation for at least three independent experiments; [b] EC_{50}: effective concentration (μM) for 50% inhibition of EV71, as evaluated by an MTS-based CPE reduction assay in Vero cells.

Proposed Targets of Hydrazides Acting on the Enterovirus

Based on the enterovirus 71 genome structure, in the single long open reading frame (ORF), there are three or four consecutive parts P1, P2, P3, and P1'.[14] The enterovirus entering into the host cell leads to the translation of polyprotein. The synthesized polyprotein could be hydrolyzed into 4 types of structural protein, i.e., VP1, VP2, VP3, and VP4 at P1, and 7 types of non-structural protein, i.e., 2A-2C at P2 and 3A-3D at P3, in the presence of its own 3C protease. These VPs are important capsid

proteomes that form the enterovirus and contain a significant number of cell-recognition sites. As such, if there is a small molecule that can enter into the hydrophobic pocket of the VP, it should be an effective anti-enterovirus inhibitor. Preliminary mechanistic studies indicated that our compounds inhibit the virus replication at the early stages, referring to the attachment or uncoating of the virion, by testing compound **15** and the two reference agents **5** (WIN 51711) and **1** (enviroxime) through a time-course experiment (Figure 4). Similar to **5** (WIN 51711), a capsid-binding inhibitor that is different from **1** (enviroxime), a 3A protease inhibitor, it is likely that compound **15** acts on the capsid to prevent the uncoating of the virion.[11,36,46]

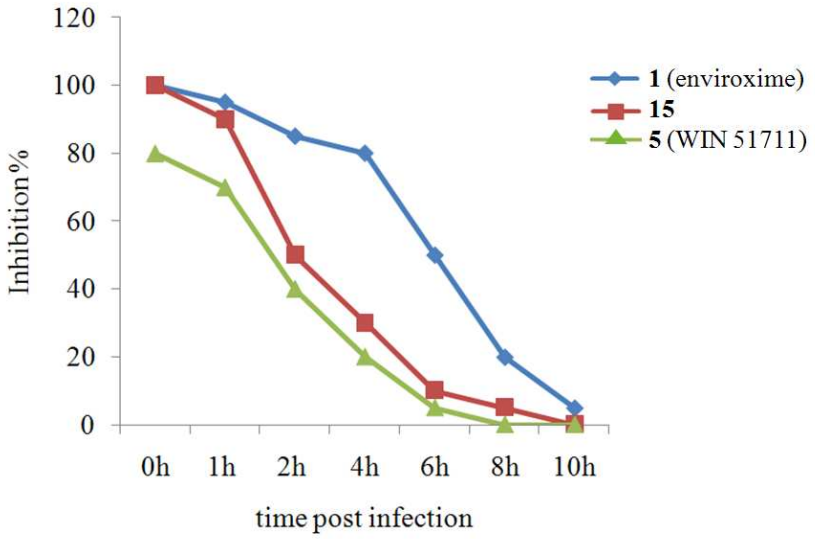


Figure 4. Time course experiment. Compound **15** (c = 0.05 μg/mL), **1** (enviroxime) (c = 0.53 μg/mL) and **5** (WIN 51711) (c = 20 μg/mL) were added to the RD cells after being infected by 0.01 MOI EV71 at the corresponding time.

Fluorescence-based thermal stability assays were also conducted to verify the above proposed mechanism.[36,47] The result indicated that when the RNA genome in EV71 virions is incubated with 5 μg/mL compound **15**, 2 °C higher temperatures are needed to achieve the equal staining of the genome compared to native virions without compound **15** (Figure 5). A similar phenomenon occurred when the positive control **5** (WIN 51711) instead of compound **15** was utilized in the same system (Figure 5). Thus, the possible mechanism of inhibition of these ligands is the

stabilizing effect of compound **15** on the EV71 capsid, thereby preventing the uncoating of the virion.

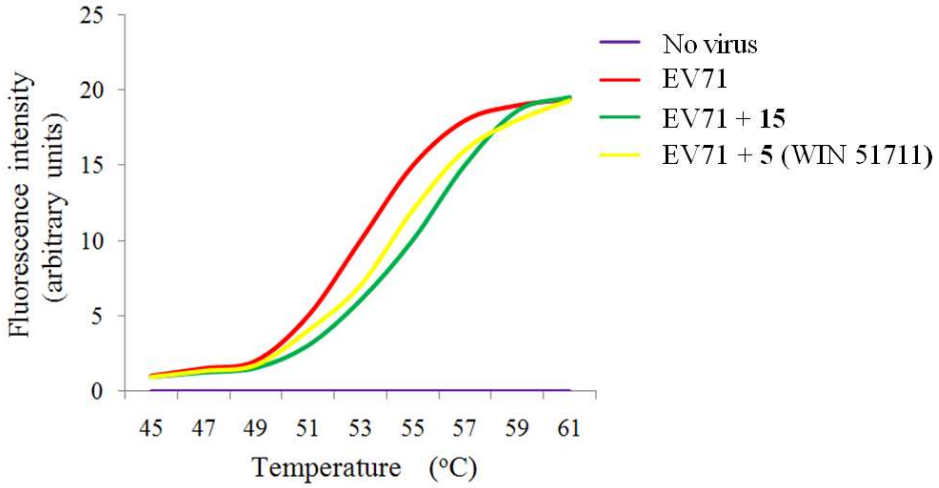


Figure 5. Impact of hydrazide on EV71 virion stability. SYBR green fluorescent assays to measure stability of EV71 particles. EV71 virions were mixed with SYBR green dyes I and II and heated to the indicated temperatures. The fluorescent signal increases as the dye binds to the RNA that is released from thermally destabilized particles. Red line, EV71 virions; Green line, EV71 with 5 μg/mL compound **15**; Yellow line, EV71 with 50 μg/mL compound **5** (WIN 51711); Purple line, control without virus.

To exclude the possibility that the antiviral activity was due to toxic side effects, we tested the effect of compound **15** on virus production over a range of concentrations during a single replication cycle.[37] For comparison, we chose the anti-EV71 agent **5** (WIN 51711) as a positive control in the virus titer assay (pink line, Figure 6). The results demonstrate that compound **15** is active against EV71 in a single replication cycle without apparent toxicity (green line, Figure 6).

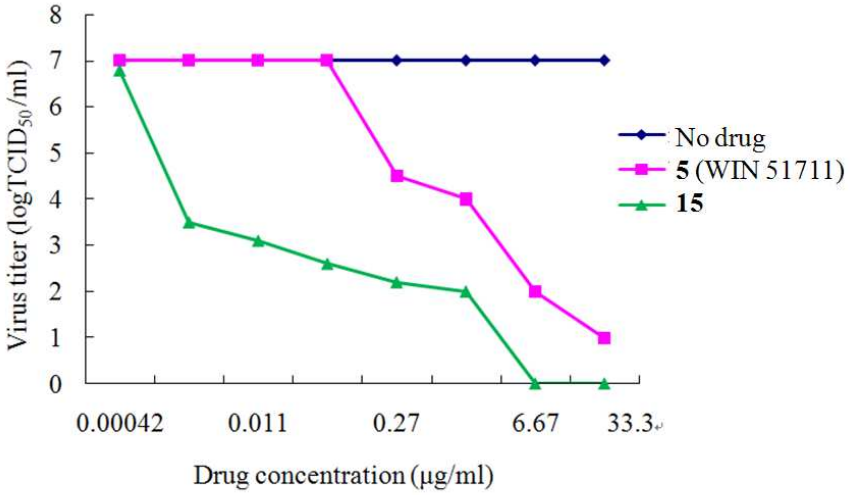


Figure 6. Virus titers in supernatants. RD cells were treated with compound **5** (WIN 51711) (pink line) or **15** (green line) and infected with EV71. The virus titers in the supernatants were determined at 72 hpi.

These observations are consistent with the molecular modeling result of compound **15** with EV71 (enterovirus 71) using the crystal complex of the latter with **5** (WIN 51711) as a template, with the aid of the software of AutoDock Vina. It is believed that **5** (WIN 51711) is an active enterovirus and rhinovirus inhibitor targeting the viral capsid protein VP1 to interfere with or block the combination of the virus and host cell.[36,49,50] A significant VP1 pocket (blue area) can be found in the co-crystal structure of the native EV71 virion and **5** (WIN 51711) (PDB: 3ZFF), as shown in Figure 7A. Similar to the orientation of **5** (WIN 51711) in the native EV71 virion, the typical hydrazide compound **15** could also bind into the hydrophobic pocket of subunit VP1 (Figure 7B). The crystal complex of EV71 with **5** clearly shows that the latter fits into the VP1 pocket as a slender shape with one aromatic ring at each end (Figure 7C). Moreover, a hydrogen bond formed between the NH of **5** and the residue ASP 112. Similarly, we found that compound **15** could enter into the hydrophobic VP1 pocket that was formed by a series of necessary residues, e.g., Ile 111, Asp 112, Ile 113, Phe 131, Phe 135, Val 192, Met 195 and Tyr 201, as an active pocket between the ligand and macromolecule (Figure 7D). However, a similar hydrogen bond was

not found between **15** and ASP 112 because the aromatic ring A of compound **15** binds farther away from the Trp 203 - Ile 113 - Asp 112 cave due to its shorter length than **5**, although this does not affect the inhibitory activity against enteroviruses. In addition to the hydrophobic interaction, several π–π interactions between the two aromatic rings A and B and the residues Tyr 201, Phe 135 and Phe 155 were also found to be significant contributors to the enhanced binding force and higher inhibitory activity of **15**.

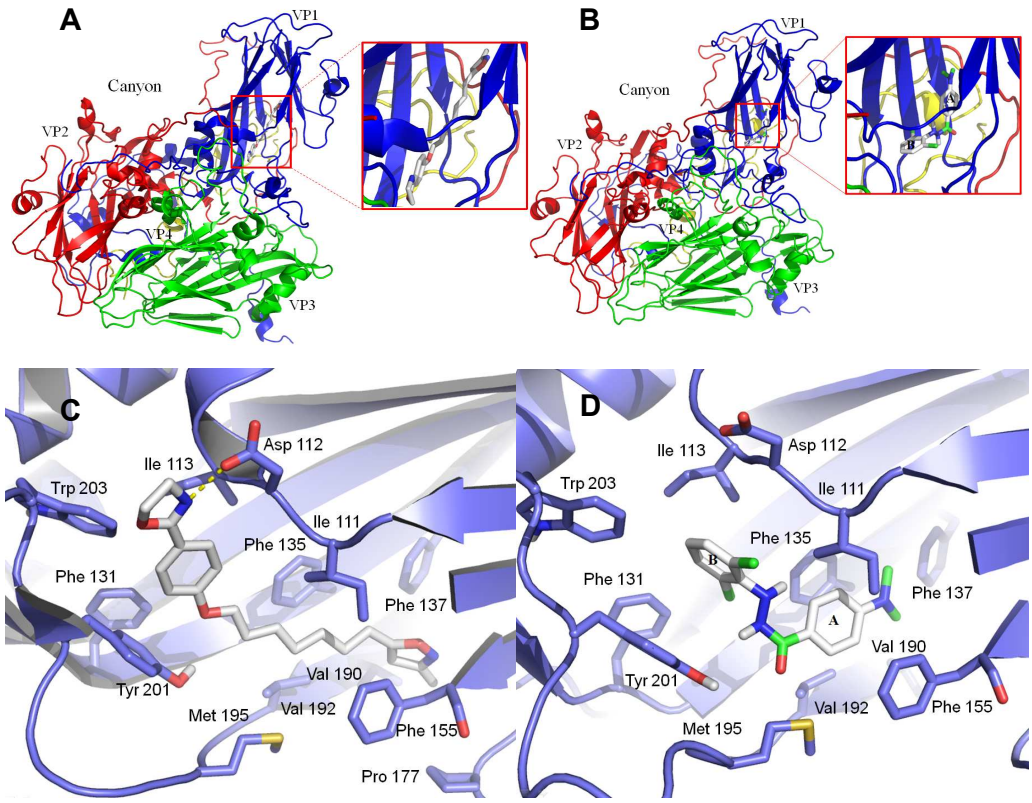


Figure 7. Model of diarylhydrazide **15** bound into the VP1 pocket of EV71 virion and comparisons with **5** (WIN 51711). **(A)** The co-crystal structure of EV71 and **5** (PDB: 3ZFF) is shown as an overview type with capsid protein subunits VP1 (blue), VP2 (red), VP3 (green), and VP4 (yellow) in a cartoon representation. **(B)** Computer modeling of the complex of **15** with EV71 at the same VP1 pocket (PDB: 3ZFF) for **5**. **(C)** VP1 is shown in cartoon representation in blue with side chains of residues forming the hydrophobic pocket shown as sticks and **5** (WIN 51711) binding deep into the pocket. **(D)** Compound **15** in the same hydrophobic VP1 pocket formed by side chains of residues in cartoon representation.

Conclusions

In summary, we have synthesized a series of diarylhydrazide analogues and evaluated their biological activities against EV71. These compounds possess high anti-EV71 activity and induce a very low cytotoxic effect in Vero cells and RD cells. The SAR results indicate that the electron-donating groups at the 4-position of phenyl ring A and 2,6-disubstituted phenyl ring B play key roles for their inhibitory activity, which is supported by the molecular modeling of the complex structure of the EV71 protease with **15**. Among all of the hydrazide compounds studied, **15** is the best one, with an EC_{50} value of 0.02 μM against EV71. Compounds **15**, **27**, **41** and **47** also exhibited excellent inhibitory activity for other human enteroviruses, including CVB1, CVB2, CVB3, CVB4, CVB5, and CVB6 (EC_{50} = 0.0005-6.52 μM). Therefore, this series of diarylhydrazide compounds could be considered as a novel class of enterovirus inhibitors with a simple chemical structure. A preliminary mechanistic study of hydrazides with enterovirus 71 indicated that the attachment site of VP1 might be the target of this type of compound. Further SAR studies and in-depth mechanistic research of hydrazides with enteroviruses are currently underway in our group and will be reported in due course.

Experimental Section

General Methods. Unless otherwise noted, reagents and materials were obtained from commercial suppliers and were used without further purification. The TEA and CH_2Cl_2 were dried over $CaCl_2$ and distilled prior to use, respectively. Reactions were monitored by thin-layer chromatography (TLC) and column chromatography purifications performed using a 230–400 mesh silica gel. NMR spectra were measured on a Bruker Biospin AV400 instrument at 400 MHz for ^1H NMR spectra, 100 MHz for ^{13}C NMR spectra, and 375 MHz for ^{19}F NMR spectra and calibrated from the residual solvent signal. High-resolution mass spectra were obtained from the Shanghai Mass Spectrometry Center. Melting points were determined by an X-4 Beijing Tech melting point apparatus and were uncorrected. All compounds assayed

were >95% pure, as determined by an HPLC analysis conducted on an Agilent Technologies 1220 Infinity system with UV detection at 254 nm using both a normal-phase column and a reversed-phase column. The normal-phase HPLC was recorded on a Thermo Scientific Hypersil Silica column using hexane/ethyl acetate (80:20) as the eluent, and the reversed-phase HPLC was recorded on an Agilent ZORBAX SB-C18 column using MeOH/H_2O (90:10) as the eluent (see Supporting Information for details).

Representative procedure for synthesis of 4-(*tert*-butyl)-*N'*-(2,6-dichlorophenyl)-benzohydrazide 10: To a solution of (2,6-dichlorophenyl)hydrazine hydrochloride (55.5 mg, 0.26 mmol) in 3 mL of CH_2Cl_2 was added 52.6 mg (2.6 eq.) of Et_3N. The mixture was stirred at room temperature for 0.5 h and then 4-(*tert*-butyl)benzoyl chloride (39.3 mg, 0.2 mmol) was added at 0 °C. After the reaction was completed, the solvents were removed, and the residue was purified by flash chromatography on silica gel, eluting with petroleum ether/ethyl acetate (8:1) to yield 62.1 mg (92% as a white solid) of *4-(tert-Butyl)-N'-(2,6-dichlorophenyl)benzohydrazide* **10**: mp 150-151 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.86 (d, J = 2.4 Hz, 1H), 7.91 – 7.87 (m, 2H), 7.52 (d, J = 8.4 Hz, 2H), 7.33 (d, J = 8.0 Hz, 2H), 7.17 (d, J = 3.2 Hz, 1H), 6.96 (t, J = 8.0 Hz, 1H), 1.33 (s, 9H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 167.00, 155.90, 142.98, 130.86, 130.40, 129.94, 128.26, 126.23, 125.60, 123.86, 35.50, 31.42. HRMS (ESI) calcd for $C_{17}H_{18}Cl_2N_2NaO$ $[M + Na]^+$ 359.0694, found 359.0687.

N'-(2,6-Dichlorophenyl)-4-ethylbenzohydrazide **11**: yield 93% as a white solid; mp 113-114 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.86 (d, J = 1.8 Hz, 1H), 7.87 (d, J = 8.2 Hz, 2H), 7.32 (dd, J = 8.0, 3.2 Hz, 4H), 7.17 (d, J = 3.0 Hz, 1H), 6.96 (t, J = 8.0 Hz, 1H), 2.71 – 2.65 (m, 2H), 1.22 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 167.08, 149.25, 142.98, 131.12, 129.94, 128.78, 128.53, 125.61, 123.87, 29.33, 15.80. HRMS (ESI) calcd for $C_{15}H_{14}Cl_2N_2NaO$ $[M + Na]^+$ 331.0381, found 331.0368.

N'-(2,6-Dichlorophenyl)-4-methylbenzohydrazide **12**: yield 91% as a white solid; mp 140-141 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.86 (s, 1H), 7.84 (d, J = 8.2 Hz, 2H), 7.30 (dd, J = 16.4, 8.0 Hz, 4H), 7.16 (d, J = 2.8 Hz, 1H), 6.95 (t, J = 8.0 Hz, 1H), 2.37 (s, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 167.10, 143.00, 142.97, 130.87, 129.94, 129.93, 128.42, 125.61, 123.91, 123.87, 21.45. HRMS calcd for $C_{14}H_{12}Cl_2N_2NaO$ [M + Na]$^+$ 317.0224, found 317.0215.

N'-(2,6-Dichlorophenyl)-4-ethoxybenzohydrazide **13**: yield 94% as a white solid; mp 154-155 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.77 (s, 1H), 7.93 – 7.89 (m, 2H), 7.33 (d, J = 8.0 Hz, 2H), 7.15 (d, J = 3.2 Hz, 1H), 7.01 – 6.95 (m, 3H), 4.10 (t, J = 7.0 Hz, 2H), 1.38 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 166.71, 162.83, 143.09, 130.22, 129.93, 125.59, 123.81, 114.95, 64.33, 14.98. HRMS (ESI) calcd for $C_{15}H_{15}Cl_2N_2O_2$ [M + H]$^+$ 325.0511, found 325.0504.

N'-(2,6-Dichlorophenyl)-4-propylbenzohydrazide **14**: yield 95% as a white solid; mp 125-126 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.86 (s, 1H), 7.87 (d, J = 8.2 Hz, 2H), 7.31 (t, J = 8.6 Hz, 4H), 7.16 (d, J = 3.0 Hz, 1H), 6.96 (t, J = 8.0 Hz, 1H), 2.69 – 2.59 (m, 2H), 1.69 – 1.59 (m, 2H), 0.92 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 167.10, 147.66, 142.98, 131.15, 129.94, 129.37, 128.44, 125.62, 123.87, 38.40, 25.10, 14.03. HRMS (ESI) calcd for $C_{16}H_{16}Cl_2N_2NaO$ [M + Na]$^+$ 345.0537, found 345.0531.

N'-(2,6-Dichlorophenyl)-4-(dimethylamino)benzohydrazide **15**: yield 83% as a white solid; mp 156-157 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.56 (s, 1H), 7.84 – 7.80 (m, 2H), 7.31 (d, J = 8.0 Hz, 2H), 7.11 (d, J = 3.2 Hz, 1H), 6.95 (t, J = 8.0 Hz, 1H), 6.76 – 6.72 (m, 2H), 3.02 (s, 6H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 167.14, 153.78, 143.39, 129.89, 129.75, 125.57, 123.66, 120.09, 111.79, 40.14. HRMS (ESI) calcd for $C_{15}H_{15}Cl_2N_3NaO$ [M + Na]$^+$ 346.0490, found 346.0482.

N'-(2,6-Dichlorophenyl)-4-methoxybenzohydrazide **16**: yield 88% as a white solid; mp 148-150 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, J = 4.6 Hz, 1H), 7.76 (d, J = 8.8 Hz, 2H), 7.28 – 7.25 (m, 2H), 7.05 (d, J = 4.8 Hz, 1H), 6.92 (m, 3H), 3.84 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.19, 162.75, 141.35, 129.03, 128.95, 126.11, 124.23, 124.04, 114.03, 55.44. HRMS (ESI) calcd for $C_{14}H_{12}Cl_2N_2NaO_2$ [M + Na]$^+$ 347.0330,

found 347.0332.

N'-(2,6-Dichlorophenyl)-2-methoxybenzohydrazide **17**: yield 88% as a white solid; mp 128-129 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 10.08 (d, J = 4.8 Hz, 1H), 8.04 (dd, J = 7.8, 1.8 Hz, 1H), 7.51 (ddd, J = 8.4, 7.4, 1.8 Hz, 1H), 7.40 (d, J = 5.2 Hz, 1H), 7.34 (d, J = 8.0 Hz, 2H), 7.17 (d, J = 8.4 Hz, 1H), 7.08 – 7.04 (m, 1H), 6.99 (t, J = 8.0 Hz, 1H), 4.06 (s, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 164.97, 158.75, 143.00, 134.31, 132.56, 129.91, 126.20, 124.66, 121.86, 120.68, 112.86, 56.72. HRMS (ESI) calcd for $C_{14}H_{12}Cl_2N_2NaO_2$ $[M + Na]^+$ 347.0330, found 347.0334.

N'-(2,6-Dichlorophenyl)benzohydrazide **18**: yield 90% as a white solid; mp 139-140 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.92 (s, 1H), 7.94 (dd, J = 5.2, 3.2 Hz, 2H), 7.56 (ddd, J = 6.6, 3.8, 1.2 Hz, 1H), 7.52 – 7.47 (m, 2H), 7.34 (d, J = 8.0 Hz, 2H), 7.19 (d, J = 2.8 Hz, 1H), 6.97 (t, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 167.11, 142.91, 133.73, 132.59, 129.95, 129.33, 128.38, 125.63, 123.91. HRMS (ESI) calcd for $C_{13}H_{10}Cl_2N_2NaO$ $[M + Na]^+$ 303.0068, found 303.0056.

4-Chloro-N'-(2,6-dichlorophenyl)benzohydrazide **19**: yield 92% as a white solid; mp 161-162 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 10.02 (s, 1H), 7.97 – 7.94 (m, 2H), 7.55 – 7.51 (m, 2H), 7.34 (d, J = 8.0 Hz, 2H), 7.20 (d, J = 2.8 Hz, 1H), 6.98 (t, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 166.23, 142.70, 138.21, 132.41, 130.21, 129.97, 129.53, 125.62, 124.00. HRMS (ESI) calcd for $C_{13}H_9Cl_3N_2NaO$ $[M + Na]^+$ 336.9678, found 336.9674.

N'-(2,6-Dichlorophenyl)picolinohydrazide **20**: yield 75% as a white solid; mp 143-144 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 10.09 (s, 1H), 8.66 (d, J = 4.8 Hz, 1H), 8.06 (d, J = 7.6 Hz, 1H), 8.00 (td, J = 7.6, 1.6 Hz, 1H), 7.62 (m, 1H), 7.36 (d, J = 8.0 Hz, 2H), 7.23 (d, J = 4.0 Hz, 1H), 7.01 (t, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 163.66, 149.98, 149.59, 142.58, 138.60, 129.92, 127.82, 125.99, 124.40, 123.00. HRMS (ESI) calcd for $C_{12}H_8Cl_2N_3O$ $[M - H]^-$ 280.0044, found 280.0040.

N'-(2,6-Dichlorophenyl)thiophene-2-carbohydrazide **21**: yield 85% as a white solid; mp 155-156 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 7.59 (d, J = 3.0 Hz, 1H), 7.52 (dd, J = 5.0, 0.8 Hz, 1H), 7.28 – 7.25 (m, 2H), 7.09 (dd, J = 4.8, 3.8 Hz, 1H), 6.99 – 6.89 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.38, 140.94, 135.40, 130.95,

129.19, 129.17, 128.96, 127.85, 126.13, 124.27. HRMS (ESI) calcd for $C_{11}H_8Cl_2N_2NaOS$ $[M + Na]^+$ 308.9632, found 308.9626.

4-Ethoxy-N'-(2,4,6-trichlorophenyl)benzohydrazide **22**: yield 90% as a white solid; mp 160-162 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.70 (s, 1H), 7.76 (d, J = 8.6 Hz, 2H), 7.26 (s, 2H), 7.03 (s, 1H), 6.84 (d, J = 8.4 Hz, 2H), 4.01 – 3.95 (m, 2H), 1.24 (t, J = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 166.75, 162.88, 142.36, 132.51, 130.25, 129.43, 127.09, 126.13, 125.40, 114.98, 64.34, 15.00. HRMS (ESI) calcd for $C_{15}H_{13}Cl_3N_2NaO_2$ $[M + Na]^+$ 380.9940, found 380.9944.

2,6-Dichloro-N'-(4-ethoxyphenyl)benzohydrazide **23**: yield 88% as a white solid; mp 163-164 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.58 (s, 1H), 7.71 (s, 2H), 7.52 – 7.44 (m, 4H), 6.94 (d, J = 9.0 Hz, 2H), 4.05 (d, J = 7.0 Hz, 2H), 1.36 (d, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 162.56, 156.75, 137.77, 132.80, 131.88, 129.02, 128.39, 122.10, 122.01, 115.44, 115.00, 64.20, 15.16. HRMS (ESI) calcd for $C_{15}H_{14}Cl_2N_2NaO_2$ $[M + Na]^+$ 347.0330, found 347.0325.

4-(tert-Butyl)-N'-(2,6-dimethoxyphenyl)benzohydrazide **24**: yield 87% as a white solid; mp 147-148 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.50 (d, J = 6.9 Hz, 1H), 7.82 (d, J = 8.6 Hz, 2H), 7.52 – 7.47 (m, 2H), 7.31 (d, J = 8.4 Hz, 1H), 6.89 (d, J = 8.4 Hz, 1H), 6.66 (d, J = 8.4 Hz, 2H), 3.90 (s, 6H), 1.33 (s, 9H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 155.38, 151.63, 131.51, 127.86, 127.12, 126.15, 125.93, 123.04, 105.97, 56.69, 35.44, 31.54, 31.45. HRMS (ESI) calcd for $C_{19}H_{24}N_2NaO_3$ $[M + Na]^+$ 351.1685, found 351.1689.

4-(tert-Butyl)-N'-(2,6-diethylphenyl)benzohydrazide **25**: yield 84% as a white solid; mp 152-154 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.99 (s, 1H), 8.01 (d, J = 8.4 Hz, 2H), 7.57 (d, J = 8.4 Hz, 2H), 7.27 – 7.10 (m, 4H), 2.66 – 2.61 (m, 4H), 1.37 (s, 9H), 1.15 (t, J = 7.6 Hz, 6H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 166.59, 155.49, 143.15, 133.10, 128.31, 128.24, 127.00, 126.23, 35.48, 31.48, 25.67, 15.00. HRMS (ESI) calcd for $C_{21}H_{28}N_2NaO$ $[M + Na]^+$ 347.2099, found 347.2093.

4-(tert-Butyl)-N'-(2,6-diisopropylphenyl)benzohydrazide **26**: yield 83% as a white solid; mp 169-171 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, J = 8.4 Hz, 2H), 7.80 (d, J = 8.4 Hz, 1H), 7.50 (d, J = 8.4 Hz, 2H), 7.43 (s, 1H), 7.32 (d, J = 7.4 Hz, 1H), 7.22

(d, J = 7.7 Hz, 2H), 3.15 (m, 2H), 1.36 (s, 9H), 1.21 (s, 6H), 1.19 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.81, 155.36, 146.47, 131.63, 131.32, 128.43, 127.12, 126.80, 125.75, 125.65, 123.53, 35.01, 31.21, 28.90, 23.68, 22.72. HRMS (ESI) calcd for $C_{23}H_{32}N_2NaO$ $[M + Na]^+$ 347.2099, found 347.2093.

4-(tert-Butyl)-N'-(2,6-dimethylphenyl)benzohydrazide **27**: yield 92% as a white solid; mp 155-156 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.54 (d, J = 2.4 Hz, 1H), 7.85 – 7.81 (m, 2H), 7.51 – 7.46 (m, 2H), 6.93 (d, J = 7.4 Hz, 2H), 6.79 (d, J = 7.4 Hz, 1H), 6.50 (d, J = 3.2 Hz, 1H), 2.48 (s, 6H), 1.31 (s, 9H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 167.64, 155.65, 146.10, 131.32, 129.64, 129.03, 128.11, 126.17, 123.10, 35.46, 31.44, 19.06. HRMS (ESI) calcd for $C_{19}H_{24}N_2NaO$ $[M + Na]^+$ 319.1786, found 319.1776.

N'-(2,6-Dimethylphenyl)-4-ethylbenzohydrazide **28**: yield 91% as a white solid; mp 127-128 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.54 (s, 1H), 7.82 (d, J = 8.2 Hz, 2H), 7.30 (d, J = 8.0 Hz, 2H), 6.94 (d, J = 7.5 Hz, 2H), 6.78 (t, J = 7.6 Hz, 1H), 6.50 (d, J = 3.2 Hz, 1H), 2.70 – 2.65 (m, 2H), 2.48 (s, 6H), 1.21 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 167.66, 149.00, 146.11, 131.57, 129.62, 129.03, 128.73, 128.35, 123.09, 19.02, 15.79. HRMS (ESI) calcd for $C_{17}H_{20}N_2NaO$ $[M + Na]^+$ 291.1473, found 291.1469.

N'-(2,6-Dimethylphenyl)-4-methylbenzohydrazide **29**: yield 93% as a white solid; mp 139-142 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.54 (s, 1H), 7.80 (d, J = 8.2 Hz, 2H), 7.27 (d, J = 8.0 Hz, 2H), 6.94 (d, J = 7.4 Hz, 2H), 6.78 (t, J = 7.6 Hz, 1H), 6.49 (d, J = 3.2 Hz, 1H), 2.48 (s, 6H), 2.36 (s, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 167.64, 146.11, 142.73, 131.31, 129.88, 129.62, 129.03, 128.23, 123.08, 21.39, 19.01. HRMS (ESI) calcd for $C_{16}H_{18}N_2NaO$ $[M + Na]^+$ 277.1317, found 277.1308.

N'-(2,6-Dimethylphenyl)-4-methoxybenzohydrazide **30**: yield 90% as a white solid; mp 180-181 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.48 (s, 1H), 7.90 – 7.86 (m, 2H), 7.00 – 6.97 (m, 2H), 6.93 (d, J = 7.4 Hz, 2H), 6.77 (t, J = 7.6 Hz, 1H), 6.48 (d, J = 3.2 Hz, 1H), 3.85 (s, 3H), 2.48 (s, 6H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 167.31, 163.32, 146.22, 130.03, 129.60, 128.97, 126.23, 123.02, 114.47, 55.78, 19.01. HRMS (ESI) calcd for $C_{16}H_{18}N_2NaO_2$ $[M + Na]^+$ 293.1266, found 293.1262.

N'-(2,6-Dimethylphenyl)-4-propylbenzohydrazide **31**: yield 93% as a white solid; mp 117-118 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.54 (s, 1H), 7.82 (d, J = 8.0 Hz, 2H), 7.28 (d, J = 8.2 Hz, 2H), 6.94 (d, J = 7.4 Hz, 2H), 6.78 (t, J = 7.6 Hz, 1H), 6.50 (d, J = 3.2 Hz, 1H), 2.64 – 2.59 (m, 2H), 2.48 (s, 6H), 1.63 (m, 2H), 0.91 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 167.70, 147.40, 146.11, 131.59, 129.63, 129.33, 129.04, 128.27, 123.10, 38.38, 25.11, 19.04, 14.03. HRMS (ESI) calcd for $C_{18}H_{22}N_2NaO$ $[M + Na]^+$ 305.1630, found 305.1619.

4-(Dimethylamino)-N'-(2,6-dimethylphenyl)benzohydrazide **32**: yield 81% as a white solid; mp 140-141 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.29 (s, 1H), 7.79 (d, J = 8.8 Hz, 2H), 6.92 (d, J = 7.4 Hz, 2H), 6.74 (m, 3H), 6.46 (s, 1H), 2.99 (s, 6H), 2.48 (s, 6H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 167.89, 153.64, 146.54, 132.14, 129.60, 128.82, 122.85, 120.58, 117.85, 111.80, 111.60, 40.15, 19.08. HRMS (ESI) calcd for $C_{17}H_{21}N_3NaO$ $[M + Na]^+$ 306.1582, found 306.1572.

4-(tert-Butyl)-N'-(2-chloro-6-methylphenyl)benzohydrazide **33**: yield 93% as a white solid; mp 112-113 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.69 (s, 1H), 7.88 – 7.84 (m, 2H), 7.52 – 7.49 (m, 2H), 7.18 (d, J = 8.0 Hz, 1H), 7.05 (d, J = 7.4 Hz, 1H), 6.86 (m, 2H), 2.50 (s, 3H), 1.32 (s, 9H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 167.31, 155.82, 144.24, 130.99, 130.88, 130.77, 128.43, 128.35, 128.17, 126.23, 124.87, 123.55, 123.52, 35.49, 31.48, 31.44, 19.47. HRMS (ESI) calcd for $C_{18}H_{21}ClN_2NaO$ $[M + Na]^+$ 339.1240, found 339.1233.

N'-(2-Chloro-6-methylphenyl)-4-ethylbenzohydrazide **34**: yield 91% as a white solid; mp 140-141 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, J = 4.4 Hz, 1H), 7.68 (d, J = 8.2 Hz, 2H), 7.24 (d, J = 8.2 Hz, 2H), 7.20 (d, J = 8.0 Hz, 1H), 7.03 (d, J = 7.3 Hz, 1H), 6.89 (t, J = 7.8 Hz, 1H), 6.65 (d, J = 4.6 Hz, 1H), 2.68 (q, J = 7.6 Hz, 2H), 2.52 (s, 3H), 1.24 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.50, 148.57, 142.09, 130.13, 129.54, 129.28, 127.94, 127.63, 126.87, 125.28, 123.74, 28.52, 18.25, 14.94. HRMS (ESI) calcd for $C_{16}H_{17}ClN_2NaO$ $[M + Na]^+$ 311.0927, found 311.0931.

N'-(2-Chloro-6-methylphenyl)-4-methylbenzohydrazide **35**: yield 90% as a white solid; mp 125-126 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, J = 8.0 Hz, 2H), 7.62 (s, 1H), 7.30 (d, J = 7.8 Hz, 3H), 7.18 (m, 2H), 2.44 (s, 3H), 2.33 (s, 3H). ^{13}C NMR (100 MHz,

$CDCl_3$) δ 165.71, 142.66, 138.17, 132.79, 131.17, 129.46, 129.39, 127.78, 127.46, 127.01, 21.57, 19.15. HRMS (ESI) calcd for $C_{15}H_{15}ClN_2NaO$ $[M + Na]^+$ 297.0771, found 297.0766.

N'-(2-Chloro-6-methylphenyl)-4-methoxybenzohydrazide **36**: yield 92% as a white solid; mp 145-147 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, J = 4.2 Hz, 1H), 7.74 (d, J = 8.8 Hz, 2H), 7.21 (d, J = 8.0 Hz, 1H), 7.05 (d, J = 7.4 Hz, 1H), 6.96 – 6.89 (m, 3H), 6.66 (d, J = 4.8 Hz, 1H), 3.85 (s, 3H), 2.55 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.68, 142.49, 130.44, 129.82, 128.92, 128.01, 125.70, 124.41, 124.13, 114.03, 55.46, 18.53. HRMS (ESI) calcd for $C_{15}H_{15}ClN_2NaO_2$ $[M + Na]^+$ 313.0720, found 313.0713.

N'-(2-Chloro-6-methylphenyl)-4-ethoxybenzohydrazide **37**: yield 94% as a white solid; mp 133-134 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.61 (s, 1H), 7.89 (d, J = 8.8 Hz, 2H), 7.18 (d, J = 8.0 Hz, 1H), 7.05 (d, J = 7.4 Hz, 1H), 6.97 (d, J = 8.8 Hz, 2H), 6.85 (t, J = 7.6 Hz, 2H), 4.13 – 4.07 (m, 2H), 2.50 (s, 3H), 1.38 (d, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 167.06, 162.77, 144.43, 130.87, 130.74, 130.11, 128.33, 125.77, 124.84, 123.46, 114.95, 64.32, 19.48, 15.00. HRMS (ESI) calcd for $C_{16}H_{17}ClN_2NaO_2$ $[M + Na]^+$ 327.0876, found 327.0871.

N'-(2-Chloro-6-methylphenyl)-4-propylbenzohydrazide **38**: yield 92% as a white solid; mp 126-128 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.67 (s, 1H), 7.84 (d, J = 8.2 Hz, 2H), 7.30 (d, J = 8.2 Hz, 2H), 7.19 (t, J = 6.4 Hz, 1H), 7.06 (d, J = 7.4 Hz, 1H), 6.86 (m, 2H), 2.65 (d, J = 7.4 Hz, 2H), 2.51 (s, 3H), 1.67 – 1.61 (m, 2H), 0.92 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 167.42, 147.58, 144.26, 131.31, 130.88, 130.79, 130.56, 129.38, 128.34, 128.32, 124.89, 123.56, 123.53, 38.39, 25.10, 19.47, 14.02. HRMS (ESI) calcd for $C_{17}H_{19}ClN_2NaO$ $[M + Na]^+$ 325.1084, found 325.1073.

N'-(2-Chloro-6-methylphenyl)-4-(dimethylamino)benzohydrazide **39**: yield 78% as a white solid; mp 133-134 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, J = 4.4 Hz, 1H), 7.69 – 7.65 (m, 2H), 7.20 (d, J = 8.0 Hz, 1H), 7.03 (d, J = 7.0 Hz, 1H), 6.88 (t, J = 7.8 Hz, 1H), 6.69 – 6.64 (m, 3H), 3.02 (s, 6H), 2.55 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.86, 152.82, 142.83, 130.37, 129.79, 128.58, 127.93, 125.60, 123.84, 118.64, 111.12, 40.07, 18.60. HRMS (ESI) calcd for $C_{16}H_{18}ClN_3NaO$ $[M + Na]^+$ 326.1036,

found 326.1031.

N'-(2-Chloro-6-methylphenyl)-4-(methylthio)benzohydrazide **40**: yield 88% as a white solid; mp 125-127 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, *J* = 8.0 Hz, 2H), 7.60 (s, 1H), 7.31 (dd, *J* = 8.0, 4.8 Hz, 3H), 7.18 (m, 2H), 2.54 (s, 3H), 2.33 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.62, 144.38, 138.14, 132.70, 131.15, 129.98, 129.41, 127.84, 127.04, 125.49, 19.15, 15.03. HRMS (ESI) calcd for $C_{15}H_{15}ClN_2NaOS$ $[M + Na]^+$ 329.0491, found 329.0485.

4-(tert-Butyl)-N'-(2,6-dibromophenyl)benzohydrazide **41**: yield 93% as a white solid; mp 168-169 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.83 (s, 1H), 7.91 (d, *J* = 8.4 Hz, 2H), 7.54 (dd, *J* = 11.6, 8.4 Hz, 4H), 7.05 (d, *J* = 2.8 Hz, 1H), 6.83 (t, *J* = 8.0 Hz, 1H), 1.34 (s, 9H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 166.90, 155.91, 144.70, 133.96, 130.83, 128.32, 126.22, 125.05, 114.82, 110.90, 35.50, 31.41. HRMS (ESI) calcd for $C_{17}H_{18}Br_2N_2NaO$ $[M + Na]^+$ 446.9684, found 446.9689.

N'-(2,6-Dibromophenyl)-4-ethylbenzohydrazide **42:** yield 94% as a white solid; mp 146-148 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.82 (s, 1H), 7.89 (d, *J* = 8.4 Hz, 2H), 7.55 (d, *J* = 8.0 Hz, 2H), 7.32 (d, *J* = 8.4 Hz, 2H), 7.04 (d, *J* = 2.8 Hz, 1H), 6.83 (t, *J* = 8.0 Hz, 1H), 2.72 – 2.66 (m, 2H), 1.22 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 166.99, 149.26, 144.69, 133.97, 131.08, 128.78, 128.59, 125.07, 114.84, 29.31, 15.79. HRMS (ESI) calcd for $C_{15}H_{14}Br_2N_2NaO$ $[M + Na]^+$ 418.9371, found 418.9365.

N'-(2,6-Dibromophenyl)-4-methylbenzohydrazide **43**: yield 91% as a white solid; mp 123-124 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.82 (s, 1H), 7.87 (d, *J* = 8.2 Hz, 2H), 7.56 (d, *J* = 8.0 Hz, 2H), 7.29 (d, *J* = 8.0 Hz, 2H), 7.04 (d, *J* = 2.8 Hz, 1H), 6.83 (t, *J* = 8.0 Hz, 1H), 2.38 (s, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 166.95, 144.70, 143.01, 133.96, 130.84, 129.91, 128.47, 125.06, 114.83, 21.43. HRMS (ESI) calcd for $C_{14}H_{12}Br_2N_2NaO$ $[M + Na]^+$ 404.9214, found 404.9218.

N'-(2,6-Dibromophenyl)-4-methoxybenzohydrazide **44**: yield 93% as a white solid; mp 150-151 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 10.18 (s, 1H), 8.05 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.57 (d, *J* = 8.0 Hz, 2H), 7.55 – 7.49 (m, 1H), 7.30 (d, *J* = 5.2 Hz, 1H), 7.21 (d, *J* = 8.4 Hz, 1H), 7.10 – 7.05 (m, 1H), 6.88 (t, *J* = 8.0 Hz, 1H), 4.10 (s, 3H). ^{13}C

NMR (100 MHz, Acetone-d_6) δ 164.84, 158.77, 144.88, 134.36, 133.89, 132.59, 125.94, 121.87, 120.56, 115.74, 112.88, 56.78. HRMS (ESI) calcd for $C_{14}H_{12}Br_2N_2NaO_2$ $[M + Na]^+$ 420.9163, found 420.9159.

N'-(2,6-Dibromophenyl)-4-ethoxybenzohydrazide **45**: yield 95% as a white solid; mp 161-163 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.74 (s, 1H), 7.95 – 7.91 (m, 2H), 7.55 (d, J = 8.0 Hz, 2H), 7.02 (d, J = 2.8 Hz, 1H), 7.00 – 6.97 (m, 2H), 6.82 (t, J = 8.0 Hz, 1H), 4.11 (q, J = 7.0 Hz, 2H), 1.38 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 166.63, 162.84, 144.80, 133.95, 130.30, 125.57, 125.01, 114.95, 114.82, 64.33, 15.00. HRMS (ESI) calcd for $C_{15}H_{14}Br_2N_2NaO_2$ $[M + Na]^+$ 434.9320, found 434.9325.

N'-(2,6-Dibromophenyl)-4-propylbenzohydrazide **46**: yield 92% as a white solid; mp 117-118 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.83 (s, 1H), 7.89 (d, J = 8.2 Hz, 2H), 7.55 (d, J = 8.0 Hz, 2H), 7.31 (d, J = 8.0 Hz, 2H), 7.04 (d, J = 2.8 Hz, 1H), 6.83 (t, J = 8.0 Hz, 1H), 2.66 – 2.61 (m, 2H), 1.65 (m, 2H), 0.92 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 167.01, 147.67, 144.70, 133.96, 131.11, 129.38, 128.51, 125.08, 114.86, 38.41, 25.10, 14.04. HRMS (ESI) calcd for $C_{16}H_{16}Br_2N_2NaO$ $[M + Na]^+$ 432.9527, found 432.9524.

N'-(2,6-Dibromophenyl)-4-(dimethylamino)benzohydrazide **47**: yield 81% as a white solid; mp 156-158 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.53 (s, 1H), 7.87 – 7.82 (m, 2H), 7.54 (d, J = 8.0 Hz, 2H), 7.00 (d, J = 3.0 Hz, 1H), 6.81 (t, J = 8.0 Hz, 1H), 6.73 (d, J = 9.0 Hz, 2H), 3.02 (s, 6H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 167.04, 153.78, 145.10, 133.92, 129.82, 124.86, 120.04, 114.79, 111.79, 40.14. HRMS (ESI) calcd for $C_{15}H_{15}Br_2N_3NaO$ $[M + Na]^+$ 433.9480, found 433.9486.

N'-(2,6-Dibromophenyl)benzohydrazide **48**: yield 90% as a white solid; mp 142-143 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (d, J = 4.4 Hz, 1H), 7.83 (d, J = 7.2 Hz, 2H), 7.49 (m, 5H), 7.01 (d, J = 4.8 Hz, 1H), 6.80 (t, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.43, 143.05, 132.92, 132.29, 132.01, 128.83, 127.22, 125.38, 115.69. HRMS (ESI) calcd for $C_{13}H_{10}Br_2N_2NaO$ $[M + Na]^+$ 390.9058, found 390.9063.

N'-(2,6-Dibromophenyl)-2-methoxybenzohydrazide **49**: yield 88% as a white solid; mp

147-148 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 10.18 (s, 1H), 8.05 (dd, J = 7.8, 1.8 Hz, 1H), 7.57 (d, J = 8.0 Hz, 2H), 7.30 (d, J = 5.1 Hz, 1H), 7.21 (d, J = 8.3 Hz, 1H), 7.10 – 7.05 (m, 1H), 6.88 (t, J = 8.0 Hz, 1H), 4.10 (s, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 164.84, 158.77, 144.88, 134.36, 133.89, 132.59, 125.94, 121.87, 120.56, 115.74, 112.88, 56.78. HRMS (ESI) calcd for $C_{14}H_{12}Br_2N_2NaO_2$ $[M + Na]^+$ 420.9163, found 420.9158.

4-(tert-Butyl)-N'-(2,6-difluorophenyl)benzohydrazide **50**: yield 94% as a white solid; mp 112-113 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.90 (d, J = 3.0 Hz, 1H), 7.88 (d, J = 8.6 Hz, 2H), 7.52 (d, J = 8.4 Hz, 2H), 7.05 (d, J = 3.2 Hz, 1H), 6.95 – 6.89 (m, 3H), 1.33 (s, 9H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 167.51, 154.72 (d, $^{1}J_{C\text{-}F}$ = 241.00 Hz), 154.67 (d, $^{2}J_{C\text{-}F}$ = 245.00 Hz), 131.02, 128.15, 127.44 (t, $^{3}J_{C\text{-}F}$ = 13.00 Hz; $^{4}J_{C\text{-}F}$ = 12.00 Hz), 126.23, 121.73 (t, $^{5}J_{C\text{-}F}$ = 9.00 Hz; $^{6}J_{C\text{-}F}$ = 10.00 Hz), 112.48 (d, $^{7}J_{C\text{-}F}$ = 16.00 Hz), 112.41 (d, $^{8}J_{C\text{-}F}$ = 16.00 Hz), 35.49, 31.44. ^{19}F NMR (375 MHz, Acetone-d_6) δ -129.06 (m, 2F), -164.90 (C_6F_6). HRMS (ESI) calcd for $C_{17}H_{18}F_2N_2NaO$ $[M + Na]^+$ 327.1285, found 327.1289.

N'-(2,6-Difluorophenyl)-4-methoxybenzohydrazide **51**: yield 93% as a white solid; mp 117-118 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.86 (d, J = 4.2 Hz, 1H), 8.02 (dd, J = 7.8, 1.8 Hz, 1H), 7.51 (td, J = 7.2, 2.0 Hz, 1H), 7.17 (d, J = 8.0 Hz, 2H), 7.06 (td, J = 7.8, 0.8 Hz, 1H), 6.94 (m, 3H), 4.04 (s, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 165.75, 158.76, 154.78 (d, $^{1}J_{C\text{-}F}$ = 242.00 Hz), 154.72 (d, $^{2}J_{C\text{-}F}$ = 242.00 Hz), 134.20, 132.43, 127.32 (t, $^{3}J_{C\text{-}F}$ = 13.00 Hz; $^{4}J_{C\text{-}F}$ = 13.00 Hz), 122.22 (t, $^{5}J_{C\text{-}F}$ = 10.00 Hz; $^{6}J_{C\text{-}F}$ = 9.00 Hz), 121.79, 121.03, 112.84, 112.51 (d, $^{7}J_{C\text{-}F}$ = 16.00 Hz), 112.44 (d, $^{8}J_{C\text{-}F}$ = 16.00 Hz), 56.62. ^{19}F NMR (375 MHz, Acetone-d_6) δ -129.04 (s, 2F), -164.90 (C_6F_6). HRMS (ESI) calcd for $C_{14}H_{12}F_2N_2NaO_2$ $[M + Na]^+$ 301.0765, found 301.0760.

N'-(2,6-Difluorophenyl)-4-ethoxybenzohydrazide **52**: yield 95% as a white solid; mp 125-126 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.80 (s, 1H), 7.89 (d, J = 8.8 Hz, 2H), 6.98 (d, J = 8.8 Hz, 3H), 6.96 – 6.86 (m, 3H), 4.11 (q, J = 6.8 Hz, 2H), 1.38 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 167.20, 162.82, 154.74 (d, $^{1}J_{C\text{-}F}$ = 241.00 Hz), 154.68 (d, $^{2}J_{C\text{-}F}$ = 241.00 Hz), 130.09, 127.56 (t, $^{3}J_{C\text{-}F}$ = 12.00 Hz; $^{4}J_{C\text{-}F}$ = 13.00

Hz), 125.79, 121.71 (t, $^{5}J_{C\text{-}F}$ = 10.00 Hz; $^{6}J_{C\text{-}F}$ = 9.00 Hz), 114.96, 112.46 (d, $^{7}J_{C\text{-}F}$ = 16.00 Hz), 112.39 (d, $^{8}J_{C\text{-}F}$ = 16.00 Hz), 64.33, 14.99. ^{19}F NMR (375 MHz, Acetone-d_6) δ -129.06 (s, 2F), -164.90 (C_6F_6). HRMS (ESI) calcd for $C_{15}H_{14}F_2N_2NaO_2$ $[M + Na]^+$ 315.0921, found 315.0925.

N'-(2,6-Difluorophenyl)-4-propylbenzohydrazide **53**: yield 92% as a white solid; mp 92-93 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.87 (s, 1H), 7.85 (d, J = 8.2 Hz, 2H), 7.31 (d, J = 8.2 Hz, 2H), 7.04 (d, J = 3.0 Hz, 1H), 6.98 – 6.89 (m, 3H), 2.67 – 2.61 (m, 2H), 1.65 (m, 2H), 0.92 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 167.52, 154.72 (d, $^{1}J_{C\text{-}F}$ = 242.00 Hz), 154.66 (d, $^{2}J_{C\text{-}F}$ = 241.00 Hz), 147.62, 131.33, 129.37, 128.28, 127.45 (t, $^{3}J_{C\text{-}F}$ = 12.00 Hz; $^{4}J_{C\text{-}F}$ = 12.00 Hz), 121.73 (t, $^{5}J_{C\text{-}F}$ = 9.00 Hz; $^{6}J_{C\text{-}F}$ = 10.00 Hz), 112.47 (d, $^{7}J_{C\text{-}F}$ = 17.00 Hz), 112.40 (d, $^{8}J_{C\text{-}F}$ = 16.00 Hz), 38.39, 25.10, 13.99. ^{19}F NMR (375 MHz, Acetone-d_6) δ -129.05 (s, 2F), -164.90 (C_6F_6). HRMS (ESI) calcd for $C_{16}H_{16}F_2N_2NaO$ $[M + Na]^+$ 313.1128, found 313.1122.

N'-(2,6-Difluorophenyl)-4-(dimethylamino)benzohydrazide **54**: yield 80% as a white solid; mp 150-151 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.61 (s, 1H), 7.82 (d, J = 8.8 Hz, 2H), 6.97 – 6.86 (m, 4H), 6.72 (d, J = 9.0 Hz, 2H), 3.00 (s, 6H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 167.64, 154.77 (d, $^{1}J_{C\text{-}F}$ = 242.00 Hz), 154.71 (d, $^{2}J_{C\text{-}F}$ = 242.00 Hz), 153.77, 129.64, 127.88 (t, $^{3}J_{C\text{-}F}$ = 12.00 Hz; $^{4}J_{C\text{-}F}$ = 12.00 Hz), 121.58 (t, $^{5}J_{C\text{-}F}$ = 10.00 Hz; $^{6}J_{C\text{-}F}$ = 9.00 Hz), 120.29, 112.42 (d, $^{7}J_{C\text{-}F}$ = 16.00 Hz), 112.35 (d, $^{8}J_{C\text{-}F}$ = 16.00 Hz), 111.79, 40.15. ^{19}F NMR (375 MHz, Acetone-d_6) δ -129.03 (s, 2F), -164.90 (C_6F_6). HRMS (ESI) calcd for $C_{15}H_{15}F_2N_3NaO$ $[M + Na]^+$ 314.1081, found 314.1089.

N'-(2,6-Dichlorophenyl)-4-fluorobenzohydrazide **55**: yield 81% as a white solid; mp 154-156 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.98 (s, 1H), 8.05 – 7.99 (m, 2H), 7.34 (d, J = 8.0 Hz, 2H), 7.25 (m, 2H), 7.19 (d, J = 3.2 Hz, 1H), 6.97 (t, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 166.10, 165.71 (d, $^{1}J_{C\text{-}F}$ = 248.00 Hz), 142.75, 133.30, 133.21, 131.06 (d, $^{2}J_{C\text{-}F}$ = 9.00 Hz), 130.07, 129.96, 125.63, 124.00, 116.25 (d, $^{3}J_{C\text{-}F}$ = 22.00 Hz). ^{19}F NMR (375 MHz, Acetone-d_6) δ -110.17 (s, F), -164.90 (C_6F_6). HRMS (ESI) calcd for $C_{13}H_9BrCl_2N_2NaO$ $[M + Na]^+$ 380.9173, found 380.9170.

4-Bromo-N'-(2,6-dichlorophenyl)benzohydrazide **56**: yield 84% as a white solid; mp 164-165 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 10.04 (s, 1H), 7.91 – 7.86 (m, 2H), 7.72 – 7.66 (m, 2H), 7.34 (d, J = 8.0 Hz, 2H), 7.20 (d, J = 2.8 Hz, 1H), 6.98 (t, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 166.29, 142.70, 132.83, 132.55, 130.37, 129.97, 126.71, 125.61, 124.00. HRMS (ESI) calcd for $C_{13}H_9Cl_2FN_2NaO$ $[M + Na]^+$ 320.9974, found 320.9971.

4-Cyano-N'-(2,6-dichlorophenyl)benzohydrazide **57**: yield 89% as a white solid; mp 142-144 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 10.21 (s, 1H), 8.13 – 8.08 (m, 2H), 7.96 – 7.91 (m, 2H), 7.35 (d, J = 8.0 Hz, 2H), 7.25 (s, 1H), 6.99 (t, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 165.84, 142.49, 137.69, 133.34, 130.00, 129.28, 125.66, 124.14, 118.75, 115.95. HRMS (ESI) calcd for $C_{14}H_9Cl_2N_3NaO$ $[M + Na]^+$ 328.0020, found 328.0022.

N'-(2,6-Dichlorophenyl)-4-nitrobenzohydrazide **58**: yield 85% as a white solid; mp 167-168 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 10.30 (s, 1H), 8.38 – 8.33 (m, 2H), 8.20 – 8.16 (m, 2H), 7.36 (d, J = 8.0 Hz, 2H), 7.27 (s, 1H), 7.00 (t, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 165.65, 150.76, 142.43, 139.38, 130.02, 129.86, 125.66, 124.51, 124.17. HRMS (ESI) calcd for $C_{13}H_9Cl_2N_3NaO_3$ $[M + Na]^+$ 347.9919, found 347.9915.

3-Chloro-N'-(2,6-dichlorophenyl)benzohydrazide **59**: yield 91% as a white solid; mp 146-148 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, J = 4.2 Hz, 1H), 7.70 (d, J = 7.6 Hz, 1H), 7.40 (q, J = 7.8 Hz, 2H), 7.29 (m, 3H), 7.11 (d, J = 5.0 Hz, 1H), 6.96 (t, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.34, 140.91, 132.11, 131.34, 130.65, 130.52, 128.95, 127.14, 126.35, 124.47. HRMS (ESI) calcd for $C_{13}H_9Cl_3N_2NaO$ $[M + Na]^+$ 336.9678, found 336.9672.

Antiviral Activity. The antiviral activity of the compounds was calculated by an MTS-based CPE reduction assay that compares the optical density of infected compound-treated cells with uninfected compound-free cells.[23] Briefly, 5×10^5 Vero cells were grown in 96-well plates, and then serial dilutions of the compounds and 100 $TCID_{50}$ of EV71 virus were added. After incubation at 37 °C for 24-36 h (until complete CPE was observed in the virus-infected and compound-free control (VC)),

the cell viability was measured using the MTS/PMS method (Promega, Leiden, The Netherlands). The absorbance at 498 nm was recorded using a Tecan GENios microplate reader (Tecan, Switzerland). CPE values were calculated, and the 50% effective concentration (EC_{50}) was defined as the concentration of compound that inhibited the virus-induced cytopathic effect formation by 50%, as calculated using the software Calcusyn.[51] Each experiment was repeated at least three times.

Thermal Stability Assay Method.[36,47,52] Virions of EV71 (final concentration 0.15 mg/mL) were mixed with compound **15** (final concentration 5 μg/mL) or **5** (WIN 51711) (final concentration 50 μg/mL) in NaCl-Tris-EDTA buffer (20 mM Tris, pH 8.0, 120 mM NaCl, and 1 mM EDTA) and incubated for 1 h at 37 °C. SYBR green I and SYBR green II dyes (Life Technologies) were added at 3 × concentration (according to the manufacturer's instructions) together with ANTI-RNasin (Promega) at a 1 U/μL final concentration. The SYBR green fluorescence of the mixture was then analyzed in a real-time PCR machine (Applied Biosystems 7300). The mixture was heated to temperatures in the range of 37 to 70 °C in 1-°C steps. After heating to a given temperature for 2 min, the mixture was cooled to 36 °C for 2 min, during which the fluorescence could be measured.

Method for virus titer assay.[37,53] The levels of reduction of the virus titers in the supernatants were determined as follows. Briefly, RD cells (5×10^4 per well) were cultured in a 48-well plate and incubated at 37 °C under 5% CO_2. On the following days, the RD cells were treated with serially diluted compounds at concentrations ranging from 0.00042 to 33.3 μg/mL in the presence of 10% FBS and 0.5% DMSO. After 2 h, EV71 at a multiplicity of infection (MOI) of 1 was used to infect the cells. The viruses in the supernatants of the wells containing each concentration were added to a new 96-well plate and diluted from 10^{-1} to 10^{-8}. The cytopathic effect was observed in every well after 72 hpi. The $TCID_{50}$ values were calculated by use of the Reed and Muench calculator.

Molecular modeling. The protein structure used in the docking simulations was based on the X-ray crystallographic structure of EV71 (enterovirus 71) bound to **5** (WIN 51711) (PDB code: 3ZFF).[36,49,54] Compound **15** was docked into the VP1

function pocket of the EV71-WIN 51711 complex with AutoDock software (version 4.2).[50] The crystallographic coordinates of **15** were created by Biochemoffice, and all water molecules were removed. The preparations of all ligands and the protein were performed with AutoDock Tools (ADT). A docking cube with edges of 108 Å, 90 Å, and 90 Å in the X, Y, and Z dimensions (a grid spacing of 0.375 Å), which encompassed the whole active site, was used throughout docking. On the basis of the Lamarckian genetic algorithm (LGA), 80 runs were performed for each ligand with 500 individuals in the population. The figures were prepared using PyMOL.

Supporting Information

Characterization data for compounds **10-59**: ^{1}H NMR and ^{13}C NMR spectral information and HPLC results and HPLC traces; ^{19}F NMR spectral information for compounds **50-55**.

AUTHOR INFORMATION

Corresponding Authors

Notes *(H.-B.Z.) Tel.: 86 27 68759586; E-mail: zhouhb@whu.edu.cn.

*(S.-W.W.) E-mail: shuwenwu@hotmail.com;

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We are grateful to the NSFC (81172935, 81373255), the Key Scientific Research Projects of the Ministry of Education of China (313040), Hubei Province's Outstanding Medical Academic Leader Program, the Fundamental Research Funds for the Central Universities of China (2014306020201, 2042014kf0204), and the Hunan Province Cooperative Innovation Center for Molecular Target New Drug Study for their support of this research.

ABBREVIATIONS USED

EV71, Enterovirus 71; SI, selectivity index; HFMD, Hand-foot-mouth disease; ORF, open reading frame; SCARB2, scavenger receptor B2; PSGL1, P-selectin

glycoprotein ligand-1; OSBP, oxy-sterol-binding protein; ORP4, OSBP-related protein 4; CB1, Coxsackie virus B1; CB2, Coxsackie virus B2; CB3, Coxsackie virus B3; CB4, Coxsackie virus B4; CB5, Coxsackie virus B5; CB6, Coxsackie virus B6; ND, Not determined; VPs, viral proteins; TLC, thin layer chromatography; ADT, AutoDockTools; LGA, Lamarckian genetic algorithm.

REFERENCES

(1) Kuo, C. J.; Shie, J. J.; Fang, J. M.; Yen, G. R.; Hsu, J. T.; Liu, H. G.; Tseng, S. N.; Chang, S. C.; Lee, C. Y.; Shih, S. R.; Liang, P. H. Design, synthesis, and evaluation of 3C protease inhibitors as anti-enterovirus 71 agents. *Bioorg. Med. Chem.* **2008**, *16*, 7388-7398.

(2) Shia, K. S.; Li, W. T.; Chang, C. M.; Hsu, M. C.; Chern, J. H.; Leong, M. K.; Tseng, S. N.; Lee, C. C.; Lee, Y. C.; Chen, S. J.; Peng, K. C.; Tseng, H. Y.; Chang, Y. L.; Tai, C. L.; Shih, S. R. Design, synthesis, and structure-activity relationship of pyridyl imidazolidinones: a novel class of potent and selective human enterovirus 71 inhibitors. *J. Med. Chem.* **2002**, *45*, 1644-1655.

(3) Chang, C. S.; Lin, Y. T.; Shih, S. R.; Lee, C. C.; Lee, Y. C.; Tai, C. L.; Tseng, S. N.; Chern, J. H. Design, synthesis, and antipicornavirus activity of 1-[5-(4-arylphenoxy)alkyl]-3-pyridin-4-ylimidazolidin-2-one derivatives. *J. Med. Chem.* **2005**, *48*, 3522-3535.

(4) Schmidt, N. J.; Lennette, E. H.; Ho, H. H. An apparently new enterovirus isolated from patients with disease of the central nervous system. *J. Infect. Dis.* **1974**, *129*, 304-309.

(5) Hsu, C. H.; Lu, C. Y.; Shao, P. L.; Lee, P. I.; Kao, C. L.; Chung, M. Y.; Chang, L. Y.; Huang, L. M. Epidemiologic and clinical features of non-polio enteroviral infections in northern Taiwan in 2008. *J. Microbiol. Immunol. Infect.* **2011**, *44*, 265-273.

(6) Jin, M.; Shan, J.; Chen, Z.; Guo, X.; Shen, Z.; Qiu, Z.; Xue, B.; Wang, Y.; Zhu, D.; Wang, X.; Li, J. Chlorine dioxide inactivation of enterovirus 71 in water and its impact on genomic targets. *Environ. Sci. Technol.* **2013**, *47*, 4590-4597.

(7) Ma, E.; Chan, K. C.; Cheng, P.; Wong, C.; Chuang, S. K. The enterovirus 71 epidemic in 2008--public health implications for Hong Kong. *Int. J. Infect. Dis.* **2010**, *14*, e775-780.

(8) Wu, Y.; Yeo, A.; Phoon, M. C.; Tan, E. L.; Poh, C. L.; Quak, S. H.; Chow, V. T. The largest outbreak of hand; foot and mouth disease in Singapore in 2008: the role of enterovirus 71 and coxsackievirus A strains. *Int. J. Infect. Dis.* **2010**, *14*, e1076-1081.

(9) Sun, L.; Meijer, A.; Froeyen, M.; Zhang, L.; Thibaut, H. J.; Baggen, J.; George, S.; Vernachio, J.; Van Kuppeveld, F. J.; Leyssen, P.; Hilgenfeld, R.; Neyts, J.; Delang, L. Antiviral activity of broad-spectrum and enterovirus-specific inhibitors against clinical solates of enterovirus D68. *Antimicrob. Agents Chemother.* **2015**, DOI: 10.1128/AAC.01375-15.

(10) Ulferts, R.; Van der Linden, L.; Thibaut, H. J.; Lanke, K. H.; Leyssen, P.; Coutard, B.; De Palma, A. M.; Canard, B.; Neyts, J.; Van Kuppeveld, F. J. Selective serotonin reuptake inhibitor fluoxetine inhibits replication of human enteroviruses B and D by targeting viral protein 2C. *Antimicrob. Agents Chemother.* **2013**, *57*, 1952-1956.

(11) Van der Schaar, H. M.; Leyssen, P.; Thibaut, H. J.; De Palma, A.; Van der Linden, L.; Lanke, K. H.; Lacroix, C.; Verbeken, E.; Conrath, K.; Macleod, A. M.; Mitchell, D. R.; Palmer, N. J.; Van de Poel, H.; Andrews, M.; Neyts, J.; Van Kuppeveld, F. J. A novel, broad-spectrum inhibitor of enterovirus replication that targets host cell factor phosphatidylinositol 4-kinase IIIβ. *Antimicrob. Agents Chemother.* **2013**, *57*, 4971-4981.

(12) Lin, J. Y.; Chen, T. C.; Weng, K. F.; Chang, S. C.; Chen, L. L.; Shih, S. R. Viral and host proteins involved in picornavirus life cycle. *J. Biomed. Sci.* **2009**, *16*, 103-116.

(13) Lin, Y. W.; Wang, S. W.; Tung, Y. Y.; Chen, S. H. Enterovirus 71 infection of human dendritic cells. *Exp. Biol. Med.* **2009**, *234*, 1166-1173.

(14) Shang, L.; Xu, M.; Yin, Z. Antiviral drug discovery for the treatment of enterovirus 71 infections. *Antiviral Res.* **2013**, *97*, 183-194.

(15) Aguado, L.; Canela, M. D.; Thibaut, H. J.; Priego, E. M.; Camarasa, M. J.; Leyssen, P.; Neyts, J.; Perez-Perez, M. J. Efficient synthesis and anti-enteroviral activity of 9-arylpurines. *Eur. J. Med. Chem.* **2012**, *49*, 279-288.

(16) Aguado, L.; Thibaut, H. J.; Priego, E. M.; Jimeno, M. L.; Camarasa, M. J.; Neyts, J.; Perez-Perez, M. J. 9-Arylpurines as a novel class of enterovirus inhibitors. *J. Med. Chem.* **2010**, *53*, 316-324.

(17) De Palma, A. M.; Thibaut, H. J.; van der Linden, L.; Lanke, K.; Heggermont, W.; Ireland, S.; Andrews, R.; Arimilli, M.; Al-Tel, T. H.; De Clercq, E.; van Kuppeveld, F.; Neyts, J. Mutations in the nonstructural protein 3A confer resistance to the novel enterovirus replication inhibitor TTP-8307. *Antimicrob. Agents. Chemother.* **2009**, *53*, 1850-1857.

(18) Ji, X. Y.; Zhong, Z. J.; Xue, S. T.; Meng, S.; He, W. Y.; Gao, R. M.; Li, Y. H.; Li, Z. R. Synthesis and antiviral activities of synthetic glutarimide derivatives. *Chem. Pharm. Bull.* **2010**, *58*, 1436-1441.

(19) Xue, F.; Luo, X.; Ye, C.; Ye, W.; Wang, Y. Inhibitory properties of 2-substituent-1H-benzimidazole-4-carboxamide derivatives against enteroviruses. *Bioorg. Med. Chem.* **2011**, *19*, 2641-2649.

(20) Victor, F.; Brown, T. J.; Campanale, K.; Heinz, B. A.; Shipley, L. A.; Su, K. S.; Tang, J.; Vance, L. M.; Spitzer, W. A. Synthesis, antiviral activity, and biological properties of vinylacetylene analogs of enviroxime. *J. Med. Chem.* **1997**, *40*, 1511-1518.

(21) Victor, F.; Loncharich, R.; Tang, J.; Spitzer, W. A. Synthesis and antiviral activity of C2 analogs of enviroxime: an exploration of the role of critical functionality. *J. Med. Chem.* **1997**, *40*, 3478-3483.

(22) Hamdouchi, C.; Sanchez-Martinez, C.; Gruber, J.; del Prado, M.; Lopez, J.; Rubio, A.; Heinz, B. A. Imidazo[1,2-b]pyridazines, novel nucleus with potent and broad spectrum activity against human picornaviruses: Design, synthesis, and biological evaluation. *J. Med. Chem.* **2003**, *46*, 4333-4341.

(23) Chern, J. H.; Shia, K. S.; Hsu, T. A.; Tai, C. L.; Lee, C. C.; Lee, Y. C.; Chang, C. S.; Tseng, S. N.; Shih, S. R. Design, synthesis, and structure-activity relationships

of pyrazolo[3,4-d]pyrimidines: a novel class of potent enterovirus inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2519-2525.

(24) MacLeod, A. M.; Mitchell, D. R.; Palmer, N. J.; de Poel, H. V.; Conrath, K.; Andrews, M.; Leyssen, P.; Neyts, J. Identification of a Series of Compounds with Potent Antiviral Activity for the Treatment of Enterovirus Infections. *ACS Med. Chem. Lett.* **2013**, *4*, 585-589.

(25) Seehafer, K.; Rominger, F.; Helmchen, G.; Langhans, M.; Robinson, D. G.; Ozata, B.; Brugger, B.; Strating, J. R.; van Kuppeveld, F. J.; Klein, C. D. Synthesis and biological properties of novel brefeldin A analogues. *J. Med. Chem.* **2013**, *56*, 5872-5884.

(26) Wang, Y.; Qing, J.; Sun, Y.; Rao, Z. Suramin inhibits EV71 infection. *Antiviral Res.* **2014**, *103*, 1-6.

(27) Zhang, H.; Tao, L.; Fu, W. W.; Liang, S.; Yang, Y. F.; Yuan, Q. H.; Yang, D. J.; Lu, A. P.; Xu, H. X. Prenylated benzoylphloroglucinols and xanthones from the leaves of Garcinia oblongifolia with antienteroviral activity. *J. Nat. Prod.* **2014**, *77*, 1037-1046.

(28) Nishimura, Y.; Shimojima, M.; Tano, Y.; Miyamura, T.; Wakita, T.; Shimizu, H. Human P-selectin glycoprotein ligand-1 is a functional receptor for enterovirus 71. *Nat. Med.* **2009**, *15*, 794-797.

(29) Yamayoshi, S.; Yamashita, Y.; Li, J.; Hanagata, N.; Minowa, T.; Takemura, T.; Koike, S. Scavenger receptor B2 is a cellular receptor for enterovirus 71. *Nat. Med.* **2009**, *15*, 798-801.

(30) Patick, A. K. Rhinovirus chemotherapy. *Antiviral. Res.* **2006**, *71*, 391-396.

(31) Wu, Z. Q.; Yang, F.; Zhao, R.; Zhao, L. N.; Guo, D. Y.; Jin, Q. Identification of small interfering RNAs which inhibit the replication of several Enterovirus 71 strains in China. *J. Virol. Methods* **2009**, *159*, 233-238.

(32) Huang, S. W.; Hsu, Y. W.; Smith, D. J.; Kiang, D.; Tsai, H. P.; Lin, K. H.; Wang, S. M.; Liu, C. C.; Su, I. J.; Wang, J. R. Reemergence of enterovirus 71 in 2008 in taiwan: dynamics of genetic and antigenic evolution from 1998 to 2008. *J. Clin. Microbiol.* **2009**, *47*, 3653-3662.

(33) Ni, H.; Yi, B.; Yin, J.; Fang, T.; He, T.; Du, Y.; Wang, J.; Zhang, H.; Xie, L.; Ding, Y.; Gu, W.; Zhang, S.; Han, Y.; Dong, H.; Su, T.; Xu, G.; Cao, G. Epidemiological and etiological characteristics of hand, foot, and mouth disease in Ningbo, China, 2008-2011. *J. Clin. Microbiol.* **2012**, *54*, 342-348.

(34) Wang, J.; Su, H.; Zhang, T.; Du, J.; Cui, S.; Yang, F.; Jin, Q. Inhibition of Enterovirus 71 replication by 7-hydroxyflavone and diisopropyl-flavon7-yl Phosphate. *PloS one* **2014**, *9*, e92565.

(35) Phillpotts, R. J.; Wallace, J.; Tyrrell, D. A.; Tagart, V. B. Therapeutic activity of enviroxime against rhinovirus infection in volunteers. *Antimicrob. Agents. Chemother.* **1983**, *23*, 671-675.

(36) Plevka, P.; Perera, Perera.; Yap, M. L.; Cardosa, J.; Kuhn, R. J.; Rossmann, R. Structure of human enterovirus 71 in complex with a capsid-binding inhibitor. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 5463-5467.

(37) Strating, J. R.; Van der Linden, L.; Albulescu, L.; Bigay, J.; Arita, M.; Delang, L.; Leyssen, P.; Van der Schaar, H. M.; Lanke, K. H.; Thibaut, H. J.; Ulferts, R.; Drin, G.; Schlinck, N.; Wubbolts, R. W.; Sever, N.; Head, S. A.; Liu, J. O.; Beachy, P. A.; De Matteis, M. A.; Shair, M. D.; Olkkonen, V. M.; Neyts, J.; Van Kuppeveld, F. J. Itraconazole inhibits enterovirus replication by targeting the oxysterol-binding protein. *Cell Rep.* **2015**, *10*, 600-615.

(38) Pan, J. W.; Han, X.; Sun, N. Y.; Wu, H. M.; Lin, D. D.; Tien, P.; Zhou, H. B.; Wu, S. W. Synthesis of N-benzyl-N-phenylthiophene-2-carboxamide analogues as a novel class of enterovirus 71 inhibitors. *RSC Adv.* **2015**, *5*, 55100-55108.

(39) Han, X.; Ouyang, W. J.; Liu, B.; Wang, W.; Tien, P.; Wu, S. W.; Zhou, H. B. Enantioselective inhibition of reverse transcriptase (RT) of HIV-1 by non-racemic indole-based trifluoropropanoates developed by asymmetric catalysis using recyclable organocatalysts. *Org. Biomol. Chem.* **2013**, *11*, 8463-8475.

(40) Han, X.; Wu, H. M.; Wang, W.; Dong, C.; Tien, P.; Wu, S. W.; Zhou, H. B. Synthesis and SARs of indole-based alpha-amino acids as potent HIV-1

non-nucleoside reverse transcriptase inhibitors. *Org. Biomol. Chem.* **2014**, *12*, 8308-8317.

(41) Han, X.; Wu, H. M.; Dong, C.; Tien, P.; Xie, W.; Wu, S. W.; Zhou, H. B. Halolactones are potent HIV-1 non-nucleoside reverse transcriptase inhibitors. *RSC Adv.* **2015**, *5*, 10005-10013.

(42) Laliberte, R.; Campbell, D. J.; Bruderlein, F. Anthelmintic activities of chalcones and related compounds. *Can. J. Phar. Sci.* **1967**, *2*, 37-43.

(43) Sui, Z. H.; Guan, J. H.; Ferro, M. P.; McCoy, K.; Wachter, M. P.; Murray, W. V.; Singer, M.; Steber, M.; Ritchie, D. M.; Argentieri, D. C. 1,3-Diarylcycloalkanopyrazoles and diphenyl hydrazides as selective inhibitors of cyclooxygenase-2. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 601-604.

(44) Yan, T.; Y, S. J.; Liu, P. F.; Liu, Z.; Wang, B. L.; Xiong, L. X.; Li, Z. M. Design, Synthesis and Biological Activities of Novel Benzoyl Hydrazines Containing Pyrazole. *Chin. J. Chem.* **2012**, *30*, 919-923.

(45) Fang, T.; Tan, Q. T.; Ding, Z. W.; Liu, B. X.; Xu, B. Pd-Catalyzed Oxidative Annulation of Hydrazides with Isocyanides: Synthesis of 2-Amino-1,3,4-oxadiazoles. *Org. Lett.* **2014**, *16*, 2342-2345.

(46) Belov, G. A.; Fogg, M. H.; Ehrenfeld, E. Poliovirus proteins induce membrane association of GTPase ADP-ribosylation factor. *J. Virol.* 2005, 79, 7207-7216.

(47) Liu, Y.; Sheng, J.; Fokine, A.; Meng, G.; Shin, W. H.; Long, F.; Kuhn, R. J.; Kihara, D.; Rossmann, M. G. Structure and inhibition of EV-D68, a virus that causes respiratory illness in children. *Science* **2015**, *347*, 71-74.

(48) Trott, O.; Olson, A. J. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* **2010**, *31*, 455-461.

(49) De Colibus, L. G.; Wang, X. X.; Spyrou, J. A. B.; Kelly, J.; Ren, J. S.; Grimes, J.; Puerstinger, G.; Stonehouse, N.; Walter, T. S.; Hu, Z. Y.; Wang, J. Z.; Li, X. M.; Peng, W.; Rowlands, D. J.; Fry, E. E.; Rao, Z. H.; Stuart, D. I. More-powerful virus inhibitors from structure-based analysis of HEV71 capsid-binding molecules. *Nat. Struct. Mol. Biol.* **2014**, *21*, 282-290.

(50) Plevka, P.; Perera, R.; Cardosa, J.; Kuhn, R. J.; Rossmann, M. G. Crystal structure of human enterovirus 71. *Science* **2012**, *336*, 1274-1276.

(51) An, T.; Ouyang, W.; Pan, W.; Guo, D.; Li, J.; Li, L.; Chen, G.; Yang, J.; Wu, S.; Tien, P. Amino acid derivatives of the (-) enantiomer of gossypol are effective fusion inhibitors of human immunodeficiency virus type 1. *Antiviral Res.* **2012**, *94*, 276-287.

(52) Plevka, P.; Lim, P. Y.; Perera, R.; Cardosa, J.; Suksatu, A.; Kuhn, R. J.; Rossmann, M. G. Neutralizing antibodies can initiate genome release from human enterovirus 71. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 2134-2139.

(53) Wang, Y.; Yang, B.; Zhai, Y.; Yin, Z.; Sun, Y.; Rao, Z. Peptidyl aldehyde NK-1.8k suppresses enterovirus 71 and enterovirus 68 infection by targeting protease 3C. *Antimicrob. Agents Chemother.* **2015**, *59*, 2636-2646.

(54) Zhang, Y.; Simpson, A. A.; Ledford, R. M.; Bator, C. M.; Chakravarty, S.; Skochko, G. A.; Demenczuk, T. M.; Watanyar, A.; Pevear, D. C.; Rossmann, M. G. Structural and virological studies of the stages of virus replication that are affected by antirhinovirus compounds. *J. Virol.* **2004**, *78*, 11061-11069.

Table of Contents Graphic