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Structure-Activity Relationship Studies on Diversified Salicylamide Derivatives as Potent Inhibitors of Human Adenovirus Infection

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ABSTRACT:

The effective treatment of adenovirus (HAdV) infections in immunocompromised patients still poses great challenges. Herein, we reported our continued efforts to optimize a series of salicylamide derivatives as potent inhibitors of HAdV infection. Of these, nine compounds (**11**, **13**, **14**, **17**, **20**, **58**, **60**, **62** and **70**) showed significantly improved anti-HAdV activities with nanomolar to submicromolar IC_{50} values and high selectivity indexes (SI > 100), indicating better safety windows, compared to the lead compound niclosamide. Our mechanistic assays suggest that compounds **13**, **62** and **70** exert their activities in the HAdV entry pathway, while compounds **14** and **60** likely target the HAdV DNA replication, and **11, 17, 20** and **58** inhibit later steps after DNA replication. Given the broad antiviral activity profile of niclosamide, these derivatives may also offer therapeutic potential for other viral infections.

INTRODUCTION

Human adenoviruses (HAdVs) are non-enveloped viruses with an icosahedral capsid which contains a linear and double stranded DNA genome of approximately 36 kb in size that encodes about 35 genes.[1] Currently, HAdVs consist of at least 70 different serotypes divided into seven species (HAdV A-G) that belong to the *Mastadenovirus* genus of the family *Adenoviridae*; most of these species spread globally with predominant types differing geographically.[2-4] HAdVs can cause a wide variety of clinical diseases including acute upper respiratory disease, conjunctivitis, gastroenteritis, hepatitis, myocarditis and pneumonia. Primary infections occur in young children with virus transmitted via multiple routes (respiratory, fecal-oral, direct conjunctival inoculation, environmentally, etc.), and are typically self-limiting and rarely associated with severe clinical symptoms in immunocompetent individuals.[3, 5] However, HAdV infections can cause significant morbidity and mortality in immunocompromised patients, such as solid-organ transplant (SOT) or allogenic hematopoietic stem cell transplant (allo-HSCT) patients, AIDS patients and patients with genetic immunodeficiencies.[5-9] Pediatric allo-HSCT patients are particularly at risk of HAdV infections with frequencies ranging from 5% to 47%; the reported mortality rates are up to 26% for them with symptomatic infection and as high as 80% for them with disseminated disease.[4-6, 10]

Despite the significant clinical impact, there remains no specifically approved antiviral therapy for HAdV infection to date. Several broad-spectrum antiviral drugs are used off-label to treat HAdV infections in immunocompromised patients, being the most common the acyclic nucleoside phosphonates.[11-14] Cidofovir (**Figure 1**), an acyclic nucleoside phosphonate cytosine analogue, is the most frequently used drug to treat HAdV infections. Cidofovir was approved to

treat cytomegalovirus (CMV) retinitis and can inhibit viral DNA replication acting as a chain terminator.[15] Cidofovir displays broad antiviral activities against all HAdV species, but it has

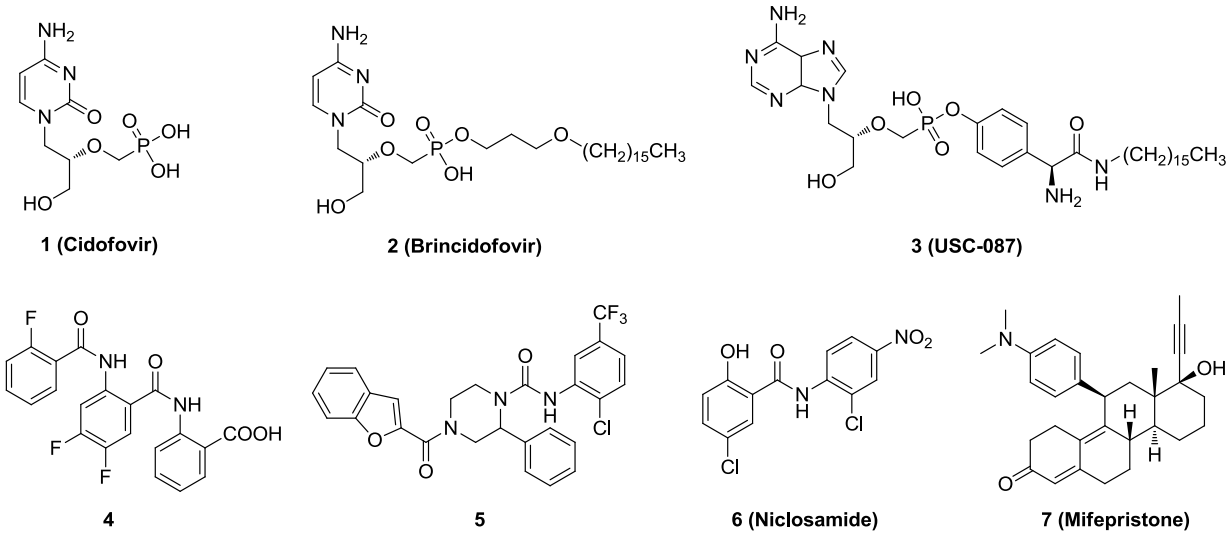


Figure 1. Representative nucleoside and non-nucleoside inhibitors of adenovirus infection.

low oral bioavailability, long plasma half-life, and significant nephrotoxicity.[13, 14, 16-19] Considering its proven efficacy, a lipid-linked derivative of cidofovir, named brincidofovir (BCV, previously named CMX001, 3-hexadecyloxy-1-propanol-cidofovir), was developed with improved antiviral potency, safety, bioavailability and pharmacokinetics.[20-24] BCV was proven to be effective in eliminating disseminated HAdV infection in the Syrian Hamster model which is permissive for HAdV-5 replication.[25] More excitingly, the good results from the phase II and III trials (NCT01231344 & NCT02087306) confirm the antiviral activity of BCV against adenoviruses and support the continued development of BCV as the first therapeutic option for HAdV infection.[26-29] However, BCV administration caused gastrointestinal disturbances, primarily diarrhea, in some patients and a new administration strategy is needed to avoid this adverse effect.[28] USC-087 (**3**), an *N*-alkyl tyrosinamide phosphonate ester prodrug of HPMPA, the adenine analog of cidofovir, was highly effective against multiple HAdV types in cell

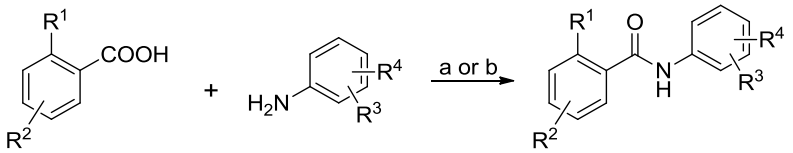
culture.[11] Promisingly, USC-087 protected Syrian hamsters against lethal challenge with HAdV-5 or -6 even when administered starting at 4 days post challenge. Besides the nucleoside analogues reported in recent years and described above, a few non-nucleoside analogues have also been identified as anti-HAdV agents.[30-40] Benzoic acid analogue (**4**) was discovered by high-throughput screening (HTS) and subsequent optimization.[31, 32] It inhibited HAdV-5 replication with an EC_{50} of 0.58 μM and exhibited low cell toxicity. Our research team has been devoted to anti-HAdV drug discovery for many years and reported a series of inhibitors of HAdV infection including compounds **5-7**. Piperazine derivative **5** significantly inhibited HAdV and CMV infections in different phases of their life cycle, with little or no cytotoxicity.[33, 34] Niclosamide (**6**) is an FDA-approved anthelminthic drug used in humans to treat tapeworm infections, involving uncoupling of oxidative phosphorylation. Accumulated studies indicated that niclosamide can modulate multiple signaling pathways and biological processes,[41-46] and has great antiviral potential against various viruses including Zika virus,[47, 48] coronavirus,[49] hepatitis C virus,[50] Ebola Virus,[51] HIV,[52] Japanese encephalitis virus,[53] human rhinoviruses or influenza virus,[54] etc. Our research team first found that niclosamide significantly inhibited HAdV infection at low micromolar concentration (IC_{50} = 0.60 μM). However, it showed moderate cytotoxicity (CC_{50} = 22.9 μM) and a relatively narrow safety window with a low selectivity index (SI = 38.2).[35] Mifepristone (**7**), a commercially available synthetic steroid drug, showed potent *in vitro* anti-HAdV activity by interfering with HAdV genome accessibility into the nucleus.[36] While significant progress has been made in anti-HAdV drug discovery, there is still an urgent need to develop highly effective antiviral agents lacking major adverse effects. As part of our ongoing antiviral drug discovery and development program,[52, 55-57] herein we reported our continued structure-activity relationship (SAR) optimization studies of niclosamide and its

salicylamide derivatives, aiming to acquire an alternative for the treatment of HAdV infections with increased activity and low toxicity.

RESULTS AND DISCUSSION

Chemistry. The general synthesis of salicylanilide derivatives is summarized in **Scheme 1**. Commercially available substituted 2-methoxybenzoic acids **8a-d** were coupled with various anilines **9a-i** in the presence of PCl_3, followed by demethylation with BBr_3, to afford the corresponding salicylanulides **10-20**. R^2-R^4 groups are defined in **Table 1**. Derivative **21** was accessed under the same coupling conditions by condensation of 3-chlorobenzoic acid **8e** with 3-fluoro-5-(trifluoromethyl)aniline **9d**.

Scheme 1. Synthesis of Salicylanilide Derivatives 10-21[a]

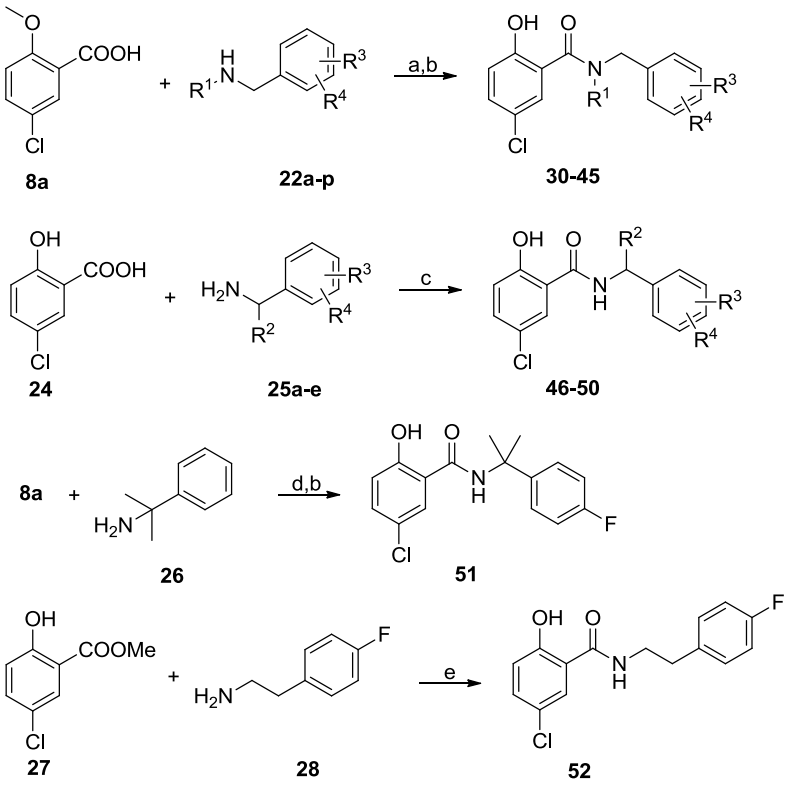


8a-d: R^1 = OMe; **8e**: R^1 = H; **9a-i** **10-20**: R^1 = OH; **21**: R^1 = H;

[a]Reagents and conditions: (a) PCl_3, toluene, 100 °C, 93%. (b) i. PCl_3, toluene, 100 °C; ii. BBr_3, CH_2Cl_2, -78 °C to 0 °C, 33-95% (two steps).

As described in **Scheme 2**, condensation of 5-chloro-2-methoxybenzoic acid **8a** with different substituted benzylamine **22a-p** was followed by demethylation using BBr_3 to give salicylamide derivatives **30-45**. Direct coupling of 5-chlorosalicylic acid **24** with substituted benzylamine **25a-e** afforded salicylamide derivatives **46-50**. R^1-R^4 groups are defined in **Table 2**. Derivative **51** was synthesized by EDCI-mediated condensation of acid **8a** and cumylamine **26** and subsequent demethylation with BBr_3. Substitution of methyl 5-chloro-2-hydroxybenzoate **27** with 4-fluorophenethylamine **28** in methanol directly provided derivative **52**.

Scheme 2. Synthesis of Salicylamide Derivatives 30-52[a]



[a]Reagents and conditions: (a) EDCI, DMAP, Et_3N, CH_2Cl_2, r.t. (b) BBr_3, CH_2Cl_2, -78 °C to 0 °C, 51-93% (two steps). (c) EDCI, DMAP, CH_2Cl_2, r.t., 39-64%. (d) EDCI, DMAP, CH_2Cl_2, 50 °C, 15%. (e) CH_3OH, r.t., 52%.

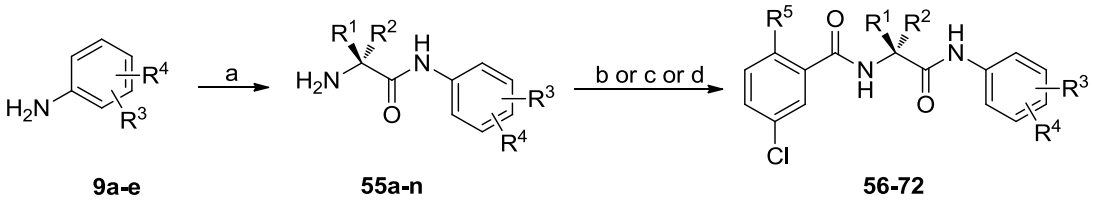
Scheme 3. Synthesis of 2-Phenylacetamide Derivatives 53-54[a]

[a]Reagents and conditions: (a) i. PCl_3, toluene, 100 °C; ii. BBr_3, CH_2Cl_2, -78 °C to 0 °C, 77-82% (two steps).

As shown in **Scheme 3**, 2-phenylacetamide derivatives **53-54** were prepared in a similar manner to that described above for compounds **10-20**. Condensation of (5-chloro-2-methoxyphenyl)acetic acid **29** with anilines **9a** or **9d** was followed by demethylation to afford compound **53** and **54**, respectively.

Compounds **56-72** were prepared according to the general synthesis outlined in **Scheme 4**. Various anilines **9a-e** were coupled with different Fmoc-protected amino acids in the presence of PCl_3, followed by piperidine deprotection, to give the amino intermediates **55a-n**. The subsequent condensation of amino intermediates **55a-h, j-n** with acid **8a** was followed by demethylation to afford salicylamide derivatives **56-63** and **65-69**. The amino intermediate **55i** with *tert*-butoxycarbonyl moiety was condensed with 5-chlorosalicylic acid **24** to provide compound **64**. Derivatives **70-72** were accessed by the coupling reaction between amino **55d** and 5-chloro-2-(methylsulfonamido)benzoic acid **23**. R^1-R^5 groups are defined in **Table 3**.

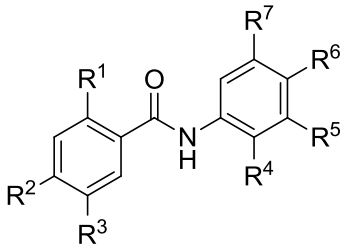
Scheme 4. Synthesis of Salicylamide Derivatives 56-72[a]



[a]Reagents and conditions: (a) i. Fmoc-amino acid, PCl_3, toluene, 100 °C; ii. piperidine, CH_3CN, r.t., 69-99% (two steps). (b) i. **8a**, EDCI, DMAP, CH_2Cl_2, r.t.; ii. BBr_3, CH_2Cl_2, -78 °C to 0 °C, 29-83% (two steps). (c) **24**, EDCI, DMAP, CH_2Cl_2, r.t., 18%. (d) 5-chloro-2-(methylsulfonamido)benzoic acid **23**, HBTU, DIEA, CH_2Cl_2, r.t., 60-77%.

***In Vitro* Evaluation of Human Adenovirus Inhibition.** All newly synthesized compounds were first screened in plaque assay at the concentration of 10 μM, quantifying their abilities to inhibit HAdV plaque formation. The active compounds screened out (inhibition > 90%) were further

Table 1. Inhibition of HAdV in Plaque Assay, Cytotoxicity and Selectivity Index for Compounds 10-21



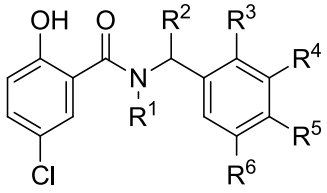
Compd	R^1	R^2	R^3	R^4	R^5	R^6	R^7	Plaque Assay[a] (%) Inhibition (10 μM)	Plaque Assay[a] IC_{50} (μM)	CC_{50} (μM)[b]	Selectivity index (SI)[c]
6	OH	H	Cl	Cl	H	NO_2	H	100 ± 0	0.6 ± 0.05	22.9 ± 9.8	38.2
10	OH	H	Cl	H	Cl	NO_2	H	100 ± 0	1.00 ± 0.37	15.1 ± 0.5	15.1
11	OH	H	Cl	F	H	NO_2	H	98.7 ± 1.9	0.05 ± 0.01	10.9 ± 0.5	218.2
12	OH	H	Cl	F	H	H	NO_2	100 ± 0	0.31 ± 0.10	8.1 ± 3.2	26.2
13	OH	H	Cl	H	H	NO_2	H	98.0 ± 2.8	0.11 ± 0.01	27.1 ± 0.1	246.6
14	OH	H	Cl	Cl	H	CF_3	H	100 ± 0	0.08 ± 0.00	35.0 ± 3.7	437.5
15	OH	H	Cl	H	F	F	H	100 ± 0	0.27 ± 0.02	8.0 ± 0.8	29.6
16	OH	H	Cl	H	CF_3	H	CF_3	100 ± 0	0.06 ± 0.00	4.1 ± 1.8	68.3
17	OH	H	Cl	H	F	H	CF_3	100 ± 0	0.18 ± 0.01	120.0 ± 33.6	666.7
18	OH	H	H	H	F	H	CF_3	98.7 ± 1.9	1.07 ± 0.09	16.3 ± 8.6	15.2
19	OH	H	Me	H	F	H	CF_3	98.0 ± 2.8	0.65 ± 0.43	15.2 ± 11.0	23.3
20	OH	Cl	H	H	F	H	CF_3	96.8 ± 1.9	0.11 ± 0.05	20.6 ± 1.0	186.8
21	H	H	Cl	H	F	H	CF_3	99.3 ± 0.9	5.61 ± 0.37	38.6 ± 1.7	6.9

[a]Percentage of control HAdV5-GFP inhibition at 10 μM and inhibitory concentration 50% (IC_{50}) at low MOI in a plaque assay using the 293β5 cell line. [b]Cytotoxic concentration 50% (CC_{50}). [c]Selectivity index value was determined as the ratio of CC_{50} to IC_{50} in a plaque assay for each compound. The results represent means ± SD of triplicate samples from three independent experiments.

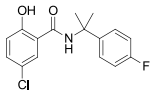
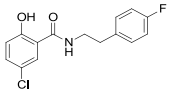
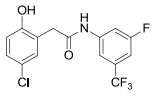
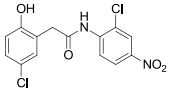
evaluated to characterize their antiviral activity (IC_{50}) in plaque assay and their cytotoxicity (CC_{50} values). Starting from niclosamide, we initially made a simple exploration on the effect generated in its antiviral activity by the replacement of the substituents on its two phenyl rings. As shown in **Table 1**, moving the 2′-Cl group on the aniline ring to the 3′-position (**10**) maintained the same level of potency (IC_{50} = 1.00 μM) and cytotoxicity (CC_{50} = 15.1 μM), in comparison with the parent compound (**6)**. Interestingly, substitution of 2′-Cl with 2′-F (**11**) resulted in a 11-fold increase in potency (IC_{50} = 0.05 μM) and an improved selectivity index (SI = 218.2), while it also displayed increased cytotoxicity (CC_{50} = 10.9 μM). In contrast to compound **11**, moving the 4′-NO_2 group to 5′-position (**12**) led to a slight loss of activity (IC_{50} = 0.31 μM). Nevertheless, it still showed the same level of potency compared to niclosamide. Removal of the 2′-Cl group of niclosamide yielded compound **13** with enhanced potency (IC_{50} = 0.11 μM) and selectivity index (SI = 246.6). Excitingly, compound **14** with 2′-Cl-4′-CF_3-aniline moiety showed significantly increased potency (IC_{50} = 0.08 μM) and decreased cytotoxicity (CC_{50} = 35.0 μM), resulting in a high selectivity index (SI = 437.5). Compounds **15** with 3′,4′-difluoro substitution and **16** with 3′,5′-bis(trifluoromethyl) substitution exhibited potent anti-HAdV activities with IC_{50} values of 0.27 μM and 0.06 μM, respectively. However, these two compounds also showed increased cytotoxicity (CC_{50} = 8.0 μM and 4.1 μM, respectively), and consequently no obvious improvement on their selectivity index was observed (SI = 29.6 and 68.3, respectively). Remarkably, compound **17** with 3′-F-5′-CF_3-aniline moiety possessed improved potency against HAdV (IC_{50} = 0.18 μM), meanwhile it showed significantly decreased cytotoxicity (CC_{50} = 120.0 μM), with a high selectivity index (SI = 666.7). These explorations described above indicated that the introduction of fluoro and trifluoromethyl groups at the proper positions on the aniline ring was beneficial for antiviral potency, consistent with the previous

results.[58] Considering its potent activity and low cytotoxicity, we next explored the effect of the substitution on the salicylic ring while retaining the 3′-F-5′-CF_3-aniline moiety of compound **17**. Removal of the 5-Cl group (**18**) or replacement of the 5-Cl group with 5-methyl group (**19**) led to a slight loss of potency (IC_{50} = 1.07 μM and 0.65 μM, respectively) and increased cytotoxicity (CC_{50} = 16.3 μM and 15.2 μM, respectively), compared to compound **17**. Moving the 5-Cl group to 4-position (**20**) maintained the same level of potency (IC_{50} = 0.11 μM), with increased cytotoxicity (CC_{50} = 20.6 μM) and decreased selectivity index (SI = 186.8). However, removal of the 2-OH group resulted in a significant loss of potency (IC_{50} = 5.6 μM). These results suggested the 2-phenolic hydroxyl group at the salicylic part was essential for salicylanilide derivatives to maintain their anti-HAdV activities.

Table 2. Inhibition of HAdV in Plaque Assay, Cytotoxicity and Selectivity Index for Compounds 30-54



Compd	R^1	R^2	R^3	R^4	R^5	R^6	Plaque Assay[a] (%) Inhibition (10 μM)	Plaque Assay[a] IC_{50} (μM)	CC_{50} (μM)[b]	Selectivity index (SI)[c]
30	H	H	Cl	H	NO_2	H	100 ± 0	1.40 ± 0.11	51.4 ± 3.7	5.5
31	H	H	Cl	H	F	H	97.1 ± 4.1	2.56 ± 0.06	30.9 ± 10.2	12.1
32	H	H	Cl	H	Cl	H	99.2 ± 0.4	1.73 ± 0.51	30.9 ± 13.6	17.9
33	H	H	F	H	F	H	74.7 ± 6.9	NT[d]	NT	NT
34	H	H	H	F	F	H	37.2 ± 23.4	NT	NT	NT
35	H	H	H	F	H	CF_3	98.1 ± 1.4	2.40 ± 0.05	36.3 ± 7.9	15.1

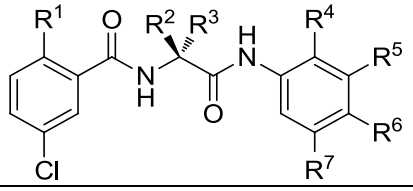
36	H	H	H	F	CF_3	H	99.0 ± 1.5	1.40 ± 0.02	22.3 ± 7.2	15.9
37	H	H	F	H	CF_3	H	98.8 ± 0.8	1.32 ± 0.04	45.6 ± 7.3	34.6
38	H	H	H	H	Cl	H	97.4 ± 3.7	3.57 ± 1.43	27.1 ± 1.1	7.6
39	H	H	H	Cl	H	H	72.0 ± 1.5	NT	NT	NT
40	H	H	H	H	F	H	72.0 ± 3.0	NT	NT	NT
41	Me	H	H	H	F	H	0.0 ± 0.0	NT	NT	NT
42	H	H	H	H	CF_3	H	99.6 ± 0.2	2.59 ± 0.42	23.9 ± 9.2	9.2
43	H	H	H	H	NO_2	H	99.5 ± 0.7	6.56 ± 0.47	175.0 ± 43.9	26.7
44	H	H	H	H	CH_3	H	96.6 ± 4.0	8.12 ± 2.16	87.7 ± 4.7	10.8
45	H	H	H	H	H	H	12.7 ± 2.9	NT	NT	NT
46	H	(*R*)-Me	H	H	H	H	100 ± 0	NT	84.6±20.5	NT
47	H	(*S*)-Me	H	H	H	H	100 ± 0	2.54 ± 0.1	199.3 ± 8.7	78.5
48	H	(*R*)-Me	H	H	Cl	H	100 ± 0	NT	31.1±3.2	NT
49	H	(*R*)-Me	H	H	F	H	100 ± 0	3.02 ± 0.07	>200	66.2
50	H	(*R*)-Me	H	H	OMe	H	33.5 ± 14.8	NT	NT	NT
51							100 ± 0	3.46 ± 0.88	>200	57.8
52							98.7 ± 1.8	3.94 ± 1.38	154.1 ± 13.1	39.1
53							91.0 ± 3.6	4.92 ± 0.01	23.7 ± 1.5	4.8
54							0.0 ± 0.0	NT	NT	NT

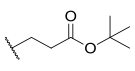
[a]Percentage of control HAdV5-GFP inhibition at 10 μM and inhibitory concentration 50% (IC_{50}) at low MOI in a plaque assay using the 293β5 cell line. [b]Cytotoxic concentration 50% (CC_{50}). [c]Selectivity index value was determined as the ratio of CC_{50} to IC_{50} in a plaque assay for each compound. [d]NT: not tested. The results represent means ± SD of triplicate samples from three independent experiments.

Next, we turned our attention to the amide linker region. In order to improve the flexibility of salicylanilide derivatives, we attempted to insert one or two additional carbon atoms between two aromatic rings, and compounds **30**-**54** were designed, synthesized, and evaluated (**Table 2**). We first replaced the aniline moiety with various substituted benzylamines to yield a series of *N*-benzylsalicylamide derivatives (**30**-**51**), and most of these compounds showed potency at the concentration of 10 μM, with IC_{50} values ranging from 1 to 10 μM. In general, disubstituted benzylamine derivatives (**30**-**32** and **35**-**37**) were more potent than monosubstituted benzylamine derivatives (**38**-**40** and **42**-**44**). Unexpectedly, difluoro substitution (**33** and **34**) reduced the potency, especially 3,4-difluoro substituted derivative **34** with no inhibition against HAdV at 10 μM, and inconsistent with the trend observed before. Methylation of amide (**41**) resulted in a complete loss of potency (0% at 10 μM) in contrast to **40**. Compound **45** with unsubstituted benzylamine moiety displayed no inhibitory activity against HAdV up to 10 μM. Introduction of one or two methyl groups at the benzylic site produced compounds **46**-**51**, and all these compounds except compound **50** with (*R*)-4-methoxy-α-methylbenzylamine moiety fully inhibited HAdV plaque formation at the concentration of 10 μM. Interestingly, compounds **47** with (*S*)-α-methylbenzylamine moiety, **49** with (*R*)-4-fluoro-α-methylbenzylamine moiety and **51** with α, α -dimethyl-4-fluorobenzylamine showed similar potency (IC_{50} = 2.54 μM, 3.02 μM and 3.46 μM, respectively) and very low cytotoxicity (CC_{50} = 199.3 μM, >200 μM and >200 μM, respectively), along with similar selectivity index as well (SI = 78.5, 57.8 and 39.1, respectively). Insertion of two carbon atoms between the nitrogen atom of the amide and the aromatic ring (**52**) also retained the potency with an IC_{50} of 3.94 μM, while it showed low cytotoxicity (CC_{50} = 154.1 μM) and a similar selectivity index (SI = 39.1) compared to niclosamide. Inserting one carbon between the amide carbonyl and the aromatic ring yielded two

derivatives **53** and **54** with entirely different inhibitory activities against HAdV. Intriguingly, compound **53** with 3´-F-5´-CF_3-aniline moiety exhibited potency with an IC_{50} of 4.92 μM, while **54** with 2´-Cl-4´-NO_2-aniline moiety showed no inhibitory activity against HAdV at 10 μM. Due to the weak potency, no more optimization efforts on this series of compounds were further pursued.

Table 3. Inhibition of HAdV in Plaque Assay, Cytotoxicity and Selectivity Index for Compounds 56-72



Compd	R^1	R^2	R^3	R^4	R^5	R^6	R^7	Plaque Assay[a] (%) Inhibition (10 μM)	Plaque Assay[a] IC_{50} (μM)	CC_{50} (μM)[b]	Selectivity index (SI)[c]
56	OH	H	H	Cl	H	NO_2	H	100 ± 0	2.43 ± 0.59	129.2 ± 34.7	53.2
57	OH	Me	H	Cl	H	NO_2	H	97.4 ± 2.7	3.85 ± 0.04	100.1 ± 13.3	26.0
58	OH	*iso*-Pr	H	Cl	H	NO_2	H	100 ± 0	0.45 ± 0.06	200.0 ± 1.9	444.4
59	OH	Bn	H	Cl	H	NO_2	H	100 ± 0	NT[d]	15.2 ± 2.1	NT
60	OH	H	Bn	Cl	H	NO_2	H	100 ± 0	0.30 ± 0.00	77.9 ± 10.2	259.7
61	OH	*iso*-Bu	H	Cl	H	NO_2	H	17.4 ± 12.3	NT	NT	NT
62	OH	*sec*-Bu	H	Cl	H	NO_2	H	99.0 ± 0.3	0.16± 0.00	20.2 ± 6.9	127.6
63	OH		H	Cl	H	NO_2	H	34.8 ± 16.9	NT	NT	NT
64	OH		H	Cl	H	NO_2	H	97.3 ± 0.3	0.67± 0.11	22.9 ± 5.2	34.2
65	OH	*iso*-Pr	H	H	CF_3	H	CF_3	99.5 ± 0.7	0.40 ± 0.09	30.9 ± 0.3	77.4
66	OH	*iso*-Pr	H	Cl	H	CF_3	H	24.8 ± 12.3	NT	NT	NT

67	OH	*iso*-Pr	H	H	F	H	CF_3	95.8 ± 5.9	0.66 ± 0.03	43.0 ± 14.4	65.2
68	OH	*iso*-Pr	H	H	H	F	F	98.3 ± 2.4	2.96 ± 0.50	95.0 ± 18.7	32.1
69	OH	Bn	H	H	CF_3	H	CF_3	99.0 ± 1.4	0.40 ± 0.02	17.9 ± 1.1	44.9
70		*iso*-Pr	H	Cl	H	NO_2	H	99.1 ± 1.2	0.68 ± 0.08	174.5 ± 12.1	256.0
71		Bn	H	Cl	H	NO_2	H	88.9 ± 15.7	NT	NT	NT
72		H	H	Cl	H	NO_2	H	99.6 ± 5.5	9.65 ± 0.01	141.1 ± 13.5	14.6

[a]Percentage of control HAdV5-GFP inhibition at 10 μM and inhibitory concentration 50% (IC_{50}) at low MOI in a plaque assay using the 293β5 cell line. [b]Cytotoxic concentration 50% (CC_{50}). [c]Selectivity index value was determined as the ratio of CC_{50} to IC_{50} in a plaque assay for each compound. [d]NT: not tested. The results represent means ± SD of triplicate samples from three independent experiments.

Amino acid linkers were widely used to tune the flexibility and improve the pharmacokinetic profiles for those compounds with two aromatic rings linked by a simple amide bond.[59] To explore the effect of introducing various α-amino acid linkers, compounds **56**-**72** were prepared and evaluated as shown in **Table 3**. Introducing different substitution on the linker moiety yielded compounds **56**-**64** while retaining the salicylic and aniline moieties of niclosamide. Use of an unsubstituted glycine linker (**56**) reduced the potency (IC_{50} = 2.43 μM) as well as cytotoxicity (CC_{50} = 129.2 μM) compared to niclosamide. The linker with (*S*)-methyl group (**57**) was similar in potency (IC_{50} = 3.85 μM) and cytotoxicity (CC_{50} = 100.1 μM) to compound **56**. Encouragingly, compound **58** with (*S*)-isopropyl substitution displayed potent activity (IC_{50} = 0.45 μM) similar to niclosamide, with decreased cytotoxicity (CC_{50} = 200 μM) and significantly improved selectivity index (SI = 444.4). Compound **60** with (*R*)-benzyl substitution also showed improved potency (IC_{50} = 0.30 μM) and diminished cytotoxicity (CC_{50} = 77.9 μM), with a high SI value of 259.7. However, its enantiomer **59** with (*S*)-benzyl

substitution exhibited increased cytotoxicity (CC_{50} = 15.2 μM). Linkers with (*S*)-isobutyl (**61**) and (*S*)-2-(methylthio)ethyl (**63**) displayed no significant inhibitory activity at the concentration of 10 μM, while compounds **62** with (*S*)-*sec*-butyl and **64** with (*S*)-3-(*tert*-butoxy)-3-oxopropyl maintained the same level of activity (IC_{50} = 0.16 μM and 0.67 μM, respectively) and cytotoxicity (CC_{50} = 20.2 μM and 22.9 μM, respectively) compared to niclosamide, with SI values of 127.6 and 34.2, respectively. Considering the potent activity and low cytotoxicity of compound **58**, we next investigated the effect of the substitution on the aniline ring, using *L*-valine as a linker. Compounds **65** with 3′,5′-bis(trifluoromethyl) substitution and **67** with 3′-fluoro-5′-trifluoromethyl substitution retained similar potency (IC_{50} = 0.40 μM and 0.66 μM, respectively) to compound **58**, with increased cytotoxicity (CC_{50} = 30.9 μM and 43.0 μM, respectively). Interestingly, 2′-chloro-4′-trifluoromethyl substitution (**66**) led to a complete loss of potency at 10 μM, while compounds **68** with 3′,4′-difluoro substitution retained some activity (IC_{50} = 2.96 μM). Compared to compound **65**, use of *L*-phenylalanine linker (**69**) maintained the same level of potency (IC_{50} = 0.40 μM), with a slight increase in cytotoxicity (CC_{50} = 17.9 μM). Substitution of 2-phenolic hydroxyl at the salicylic part with 2-methylsulfonamido yielded compounds **70**-**72** with different amino acid linkers. Of these, compound **70** with a *L*-valine linker displayed similar potency (IC_{50} = 0.68 μM) and lower cytotoxicity (CC_{50} = 174.5 μM) to compound **58**, with a high selectivity index (SI = 256.0). For the comparison using the same *in vitro* assay protocols, the antiviral activity and safety of these niclosamide derivatives demonstrated to be significantly better than those of cidofovir (IC_{50} = 24.06 ± 5.9 μM; CC_{50} = 50.06 ± 9.8 μM; SI = 7.5), the drug of current choice for the treatment of HAdV infections.

Those compounds with high selectivity index (SI > 100) were selected for further evaluation in entry assay, using human A549 epithelial cells infected with HAdV-GFP in

presence of the candidate compound and incubated for 48 h. As shown in **Table 4**, most of the selected molecules inhibited expression of the HAdV-GFP transgene in a significant way (> 90%) like niclosamide (**6**). Compounds **58** and **60** showed lower percentage of inhibition, 81.8% and 68.6%, respectively, but they were selected based on their low IC_{50} in the plaque assay (0.45 μM and 0.30 μM, respectively). As for their IC_{50} in entry assay, we observed that derivatives whose percentage of inhibition was higher than 90% also showed a low IC_{50} value, ranging between 0.2 μM and 2.8 μM. Compounds **58** and **60** were the only ones whose IC_{50} values were higher than this range (11.20 μM and 9.70 μM, respectively). These results provided us with preliminary information regarding their potential mechanisms of inhibition since the effect of these compounds in a step between HAdV attachment to its cellular receptors and HAdV DNA import into the cell nucleus would result in decreased GFP expression.

Table 4. Inhibition of HAdV in Entry Assay for Selected Compounds with High Selectivity Index

Compound	(%) Inhibition Entry Assay (50 μM)[a]	IC_{50} Entry Assay (μM)	CC_{50} (μM)
6	100.0 ± 0.0	1.22 ± 0.44	22.9 ± 9.8
11	92.4 ± 0.2	1.57 ± 0.97	10.9 ± 0.5
13	100.0 ± 0.0	2.71 ± 0.07	27.1 ± 0.1
14	100.0 ± 0.0	1.67 ± 0.25	35.0 ± 3.7
17	100.0 ± 0.0	2.23 ± 0.58	120.0 ± 30.6
20	100.0 ± 0.0	0.26 ± 0.09	20.6 ± 1.0
58	81.8 ± 8.6	11.20 ± 3.60	200.0 ± 1.9
60	68.6 ± 10.7	9.70 ± 3.80	77.9 ± 10.2
62	98.2 ± 0.5	2.50 ± 0.22	20.2 ± 6.9
70	99.1 ± 1.2	1.38 ± 0.10	174.5 ± 12.1

[a]Percentage of control HAdV5-GFP inhibition at 50 μM and inhibitory concentration 50% (IC_{50}) at high MOI in an entry assay using the A549 cell line. [b]Cell cytotoxic concentration 50% (CC_{50}) using A549 cell line.

The next step was to evaluate the effect of these selected compounds on virus replication using a virus burst assay which measures the production of virus particles. In this assay, A549 cells were infected with the HAdV-5 wild-type virus, and the $TCID_{50}$ values of an infection in the presence and absence of the selected compounds were calculated. As summarized in **Table 5**, the overall reductions in virus yield varied from as low as 1.8-fold for compound **11** to as high as 989-fold for compound **17**.

Table 5. Virus Yield Reduction for Selected Compounds with High Selectivity Index

Compound	Virus Yield Reduction (fold)[a]
6	82±35
11	1.8±0.3
13	137±71
14	175±33
17	989±361
20	385±273
58	213 ± 78
60	528 ± 100
62	10.0 ± 3.8
70	1.0 ± 0.4

[a]Fold-reduction in virus yield as the ratio of particles produced in the presence of DMSO divided by the yield in the presence of each selected compound at the concentration based on CC_{50} (**11** was tested at 2 μM, **6**, **13**, **20** and **62** at 5 μM, **14** at 10 μM, **60** at 20 μM and **17**, **58** and **70** at 50 μM). Virus Yield Reduction assay used A549 cell line and the MOI of HAdV was 100 vp/cell. The results represent means ± SD of triplicate samples from three independent experiments.

To gain further mechanistic understanding for inhibition we then measured the time dependence of these analogues addition on their ability to inhibit HAdV infection as an alternative step toward identifying the specific stage of HAdV replicative cycle that was inhibited by these compounds. HAdV has been shown to be internalized within 5 min after binding, to escape the endosome after 15 min, and to attach to the nuclear pore complex after 35-45 min.[60] We observed that all compounds exhibited a time-dependent decrease in inhibitory activity although with different patterns (**Figure 2**). Compounds **13**, **20**, **62** and **70** inhibited infection in more than a 50% when they were added either at the beginning of the 60 min incubation at 4 °C (-60 min) or at 120 min post-infection (p.i.). Compounds **11** and **14** showed abrupt decreases in their antiviral activity at early time points. Compound **14** lost 20% of inhibition when added between 5 and 10 min p.i. while compound **11** lost 34% of inhibition when added between 20 and 40 p.i. In contrast, compound **60** showed a constant decrease from the beginning of the 60 min incubation at 4 °C losing the 50% inhibition when added after 20 min p.i. and compounds **17** and **58** showed a little bit more but also constant decrease in inhibition from the beginning of the 60 min incubation at 4 °C showing less than 50% inhibition after the addition at the moment of the infection (0 min) (**Figure 2**). Collectively, the results indicated that these analogues were inhibiting an early step in virus entry occurring after cell attachment and also that the observed inhibition of HAdV infection were due to an effect on virus entry and not to an indirect effect on GFP expression.

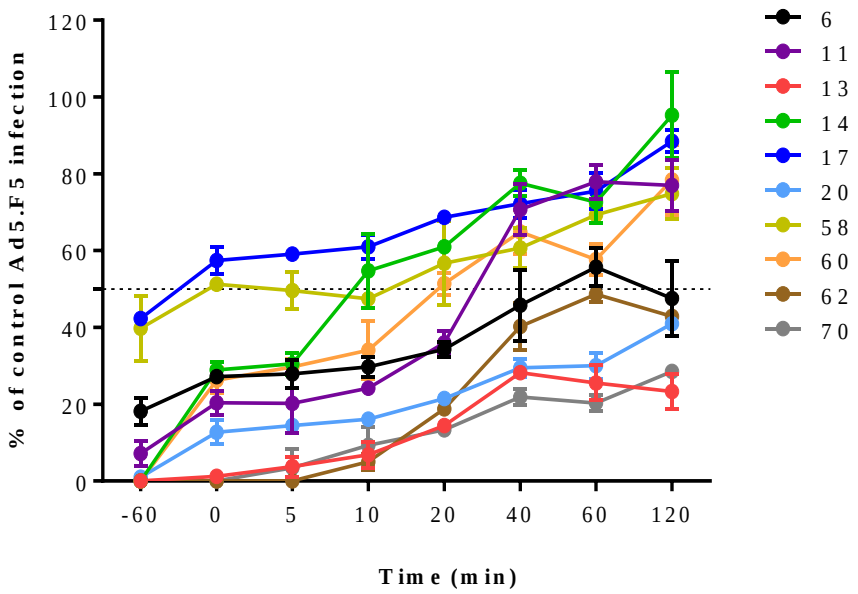


Figure 2. Effect of selected compounds on HAdV infection at different time points. The concentrations selected were depending on their CC_{50}: 10 μM for derivatives **14, 17, 58, 60** and **70**; 5 μM for derivatives **11**, **13, 20** and **62**; and 1 μM for **6**. Line chart represent means ± SD of duplicate samples.

Impact on HAdV Entry. The HAdV cell entry pathway is a coordinated multi-stage process in which following attachment and internalization of the HAdV particle, the exposure of protein VI provokes endosome lysis and subsequent endosomal escape of virions into cytoplasm. Then, the partially uncoated HAdV capsid is translocated along microtubules towards the nuclear pore complex where further disassembly occurs and the HAdV genome is finally delivered into the cell nucleus. If a compound blocks any step of the HAdV entry, this inhibitory effect will be reflected in the number of HAdV genomes that reach the host nucleus after a synchronized infection. To further validate whether these compounds could inhibit any of the steps in the entry pathway, we performed an assay to quantitatively measure the HAdV genome accessibility to the nucleus. As shown in **Figure 3**, cells treated with compounds **13**, **62** and **70** showed significant ($p \leq 0.0001$) reductions in the amount of HAdV genomes isolated from the nucleus versus those

treated with DMSO at 45 min post infection, consistent with the corresponding IC_{50} values obtained from the entry assay. Compounds **11**, **14**, **20** and **60** partially inhibited the accessibility of HAdV genomes to the nucleus while compounds **17** and **58** showed no significant differences in the amount of nuclear-associated HAdV genomes versus the control group. These results indicated that compounds **13**, **62** and **70** exerted their main antiviral activity in the early steps of the HAdV replication cycle while compounds **17** and **58** inhibited later steps after the HAdV genome is imported into the nucleus.

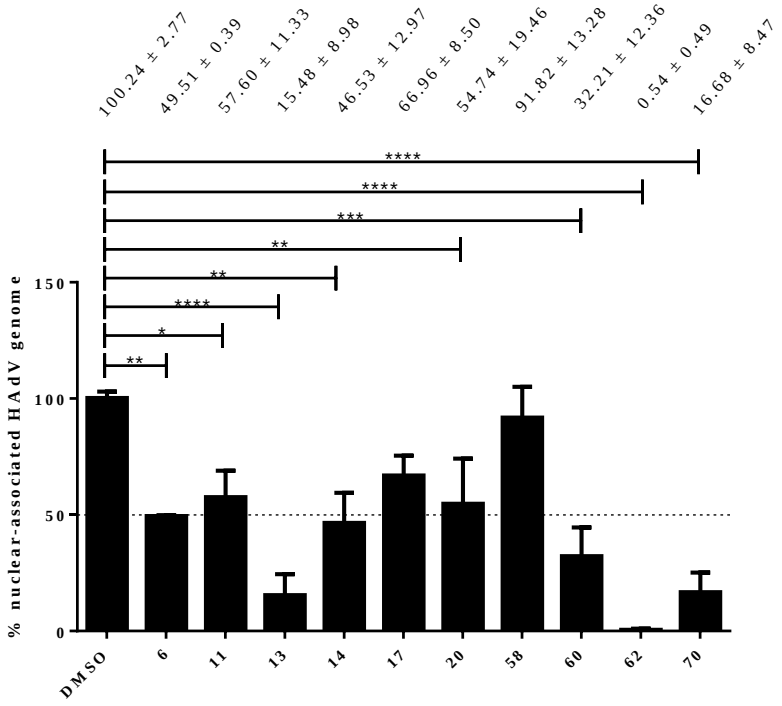


Figure 3. Percentage of nuclear-associated HAdV genome of selected compounds. Concentration of these compounds for the assay was selected based on CC_{50}: **11** was tested at 2 μM, **6**, **13**, **20** and **62** at 5 μM, **14** at 10 μM, **60** at 20 μM and **17**, **58** and **70** at 50 μM. Bars represent means ± SD of triplicate samples. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$.

Impact on HAdV Replication. To evaluate HAdV DNA replication efficiency we next carried out quantitative real-time PCR (qPCR) in the presence of these selected compounds in a 24 h

assay to avoid the influence of subsequent rounds of infection occurring 32-36 h post infection. We used qPCR to quantify the newly synthesized HAdV DNA copies in a single round of infection as a measure of DNA replication efficiency. As shown in **Figure 4**, compounds **13**, **62** and **70** significantly inhibited HAdV-5 DNA replication by more than 90% in the same way as that of niclosamide, while compounds **11**, **17, 20** and **58** showed low inhibition on DNA replication when compared to a control treated with the same concentration of DMSO. Compounds **14** and **60** which did not show a significant inhibition in the nuclear-associate genomes assay exhibited a significant ($p \leq 0.0001$) inhibitory activity, indicating that they may be targeting the HAdV DNA replication process.

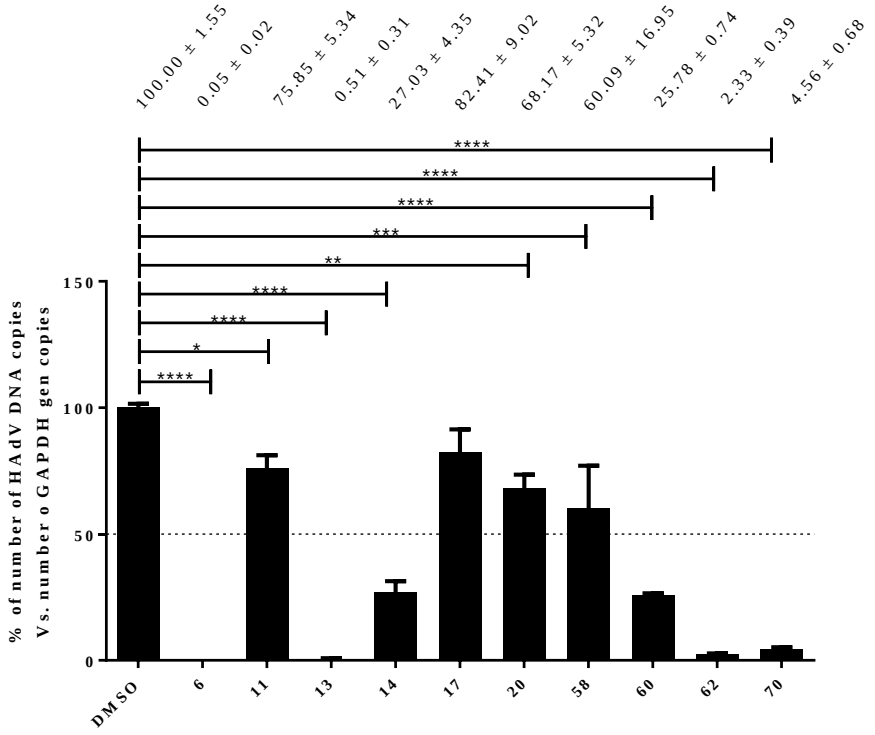


Figure 4. Effect of selected compounds on HAdV DNA replication. Concentration of these compounds for the assay was selected based on CC_{50}: compound **11** was tested at 2 μM, compounds **6**, **13**, **20** and **62** at 5 μM, **14** at 10 μM, **60** at 20 μM and **17**, **58** and **70** at 50 μM. Bars represent means ± SD of triplicate samples. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$.

In summary, compounds **13**, **62** and **70** caused a significant decrease in HAdV DNA replication, and these activities were possibly attributed to their potent inhibitory effects during the HAdV entry pathway. Compounds **17** and **58** did not show obvious inhibition against either during HAdV entry or HAdV DNA replication, indicating that their mechanism of HAdV inhibition may be related to later steps of the HAdV infection cycle, such as the viral protein maturation, viral particles assembly, or release processes. Compounds **14** and **60** showed moderate potency against HAdV-5 DNA replication, meanwhile partially blocking the accessibility of HAdV genomes to the nucleus. Thus, it is hard to conclude whether these two compounds had direct impacts on HAdV replication by interfering with a protein involved in this process or impacting transcription of the immediate early genes, which is essential for subsequent DNA replication. Despite their partial inhibition on the accessibility of HAdV genomes to the nucleus, compounds **11** and **20** did not display significant effects on HAdV-5 DNA replication. Based on the current data, it is still unable to determine at what stage compounds **11** and **20** were acting, and more studies are needed to elucidate their exact mechanisms of action.

CONCLUSION

In conclusion, a series of salicylamide derivatives was optimized to identify potent inhibitors of HAdV infection. Of these, nine compounds (**11**, **13**, **14**, **17**, **20**, **58**, **60**, **62** and **70**) showed improved anti-HAdV activities with nanomolar to submicromolar IC_{50} values and high selectivity indexes (SI > 100), indicating better safety windows, compared to niclosamide. Moreover, our preliminary mechanistic assays demonstrate that compounds **13**, **62** and **70** exert

their activities in the HAdV entry pathway, while compounds **11, 17, 20** and **58** possibly inhibit later steps after DNA replication. With respect to compounds **14** and **60**, they seem to be targeting the HAdV DNA replication process although more studies are imperative to elucidate their specific mechanisms of action. Given the broad antiviral activities of niclosamide, and once their antiviral properties are validated *in vivo*, these derivatives may also offer great therapeutic potential to be developed for other viral infections. The next step in the development of these new antiviral drugs will be the further extensive evaluation of their efficacy and safety in the animal model of viral infections as well as exact modes of actions to advance them into potential clinical applications.

EXPERIMENTAL SECTION

General Chemistry Information. All commercially available starting materials and solvents were reagent grade and used without further purification. Reactions were performed under a nitrogen atmosphere in dry glassware with magnetic stirring. Preparative column chromatography was performed using silica gel 60, particle size 0.063-0.200 mm (70-230 mesh, flash). Analytical TLC was carried out employing silica gel 60 F254 plates (Merck, Darmstadt). Visualization of the developed chromatograms was performed with detection by UV (254 nm). NMR spectra were recorded on a Brucker-300 (^{1}H, 600 and 300 MHz; ^{13}C, 150 and 75 MHz) spectrometer. ^{1}H and ^{13}C NMR spectra were recorded with TMS as an internal reference. Chemical shifts were expressed in ppm, and *J* values were given in Hz. High-resolution mass spectra (HRMS) were obtained from Thermo Fisher LTQ Orbitrap Elite mass spectrometer. Parameters include the following: Nano ESI spray voltage was 1.8 kV; Capillary temperature was 275 °C and the resolution was 60,000; Ionization was achieved by positive mode. Melting points were measured on a Thermo Scientific Electrothermal Digital Melting Point Apparatus

and uncorrected. Purities of final compounds were established by analytical HPLC, which was carried out on a Shimadzu HPLC system (model: CBM-20A LC-20AD SPD-20A UV/VIS). HPLC analysis conditions: Waters μBondapak C18 (300 × 3.9 mm); flow rate 0.5 mL/min; UV detection at 270 and 254 nm; linear gradient from 10% acetonitrile in water to 100% acetonitrile in water in 20 min followed by 30 min of the last-named solvent (0.1% TFA was added into both acetonitrile and water). All biologically evaluated compounds are > 95% pure.

5-Chloro-*N*-(3-chloro-4-nitrophenyl)-2-hydroxybenzamide (10). To a solution of 5-chloro-2-methoxybenzoic acid (281 mg, 1.5 mmol) and 3-chloro-4-nitroaniline (200 mg, 1.2 mmol) in 20 mL of toluene was added PCl_3 (286 mg, 2.1 mmol) at r.t. The resulting mixture was stirred at 100 °C for 6 h and concentrated. Then MeOH (20 mL) and H_2O (5 mL) was added successively, and the mixture was stirred at r.t. for 20 min. The amide intermediate was isolated by filtration as a pale yellow solid. The amide intermediate was dissolved in 40 mL of DCM, and BBr_3 (5.0 mL, 5.0 mmol, 1 M in DCM) was added dropwise at 0 °C. The mixture was stirred at 0 °C for 2 h until the reaction was completed monitored by TLC. Then the mixture was diluted with DCM, washed with H_2O, and concentrated. The residue was purified by recrystallization ($MeOH/H_2O$) to afford compound **10** as a pale solid (310 mg, 95% in two steps). HPLC purity 99.1% (t_R = 19.10 min). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.39 (s, 1H), 10.82 (s, 1H), 8.20 – 8.14 (m, 2H), 7.86 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.80 (d, *J* = 2.7 Hz, 1H), 7.48 (dd, *J* = 9.0, 2.7 Hz, 1H), 7.04 (d, *J* = 8.7 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.1, 155.8, 143.3, 142.0, 133.1, 128.8, 127.4, 126.6, 122.8, 121.6, 120.8, 118.9 (2C). HRMS (ESI) calcd for $C_{13}H_9Cl_2N_2O_4$, 326.9939 $(M + H)^+$; found, 326.9931.

5-**Chloro-*N*-(2-fluoro-4-nitrophenyl)-2-hydroxybenzamide (11).** Compound **11** (318 mg, 80% in two steps) was prepared by a procedure similar to that used to prepare compound **10**.

The title compound was obtained as a grey solid. HPLC purity 99.3% (t_R = 18.54 min). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.34 (s, 1H), 11.1 (d, J = 2.1 Hz, 1H), 8.66 (t, J = 8.4 Hz, 1H), 8.28 – 8.13 (m, 2H), 7.92 (d, J = 2.7 Hz, 1H), 7.51 (dd, J = 8.7, 2.7 Hz, 1H), 7.06 (d, J = 8.7 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.7, 155.2, 151.1 (d, J = 245.6 Hz), 142.4 (d, J = 8.8 Hz), 133.9, 133.0 (d, J = 10.3 Hz), 129.8, 123.7, 121.1 (d, J = 2.9 Hz), 120.8 (d, J = 0.8 Hz), 119.5, 119.2, 111.3 (d, J = 24.5 Hz). HRMS (ESI) calcd for $C_{13}H_9ClFN_2O_4$, 311.0235 $(M + H)^+$; found, 311.0226.

5-Chloro-*N*-(2-fluoro-5-nitrophenyl)-2-hydroxybenzamide (12). Compound **12** (370 mg, 93% in two steps) was prepared by a procedure similar to that used to prepare compound **10**. The title compound was obtained as a pale solid. HPLC purity 99.7% (t_R = 18.27 min). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.25 (s, 1H), 10.94 (s, 1H), 9.24 (dd, J = 6.6, 3.0 Hz, 1H), 8.11 – 8.03 (m, 1H), 7.93 (d, J = 3.0 Hz, 1H), 7.62 (t, J = 9.6 Hz, 1H), 7.50 (dd, J = 8.7, 2.7 Hz, 1H), 7.05 (d, J = 8.7 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.9, 155.9 (d, J = 252.6 Hz), 155.3, 143.8 (d, J = 2.5 Hz), 133.7, 129.7, 127.2 (d, J = 12.4 Hz), 123.6, 120.4 (d, J = 9.7 Hz), 119.4, 119.1, 116.9 (d, J = 3.5 Hz), 116.3 (d, J = 22.1 Hz). HRMS (ESI) calcd for $C_{13}H_9ClFN_2O_4$, 311.0235 $(M + H)^+$; found, 311.0229.

5-Chloro-2-hydroxy-*N*-(4-nitrophenyl)benzamide (13). Compound **13** (400 mg, 63% in two steps) was prepared by a procedure similar to that used to prepare compound **10**. The title compound was obtained as a yellow solid. HPLC purity 99.4% (t_R = 18.19 min). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.45 (s, 1H), 10.82 (s, 1H), 8.31 – 8.24 (m, 2H), 8.03 – 7.95 (m, 2H), 7.83 (d, J = 2.7 Hz, 1H), 7.48 (dd, J = 8.7, 2.7 Hz, 1H), 7.04 (d, J = 9.0 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.0, 155.8, 144.5, 142.7, 133.0, 128.8, 124.9 (2C), 122.8, 120.9, 120.0 (2C), 118.9. HRMS (ESI) calcd for $C_{13}H_{10}ClN_2O_4$, 293.0329 $(M + H)^+$; found, 293.0318.

5-Chloro-*N*-(2-chloro-4-(trifluoromethyl)phenyl)-2-hydroxybenzamide (14). Compound **14** (120 mg, 42% in two steps) was prepared by a procedure similar to that used to prepare compound **10**. The title compound was obtained as a white solid. HPLC purity 98.7% (t_R = 21.02 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.42 (s, 1H), 8.59 (d, *J* = 8.4 Hz, 2H), 7.74 (d, *J* = 1.8 Hz, 1H), 7.62 (dd, *J* = 8.7, 1.8 Hz, 1H), 7.53 (d, *J* = 2.4 Hz, 1H), 7.45 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.02 (d, *J* = 9.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.3, 160.6, 136.7, 135.5, 127.8 (q, *J* = 33.6 Hz), 126.6 (q, *J* = 3.9 Hz), 125.3 (q, *J* = 3.8 Hz), 125.3, 124.4, 123.9, 123.3 (q, *J* = 270.5 Hz), 122.0, 120.9, 115.4. HRMS (ESI) calcd for $C_{14}H_9Cl_2F_3NO_2$, 349.9962 $(M + H)^+$; found, 349.9950.

5-Chloro-*N*-(3,4-difluorophenyl)-2-hydroxybenzamide (15). Compound **15** (120 mg, 50% in two steps) was prepared by a procedure similar to that used to prepare compound **10**. The title compound was obtained as a grey solid. HPLC purity 99.9% (t_R = 19.46 min). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.60 (s, 1H), 10.50 (s, 1H), 7.94 – 7.82 (m, 2H), 7.51 – 7.37 (m, 3H), 7.02 (d, *J* = 8.7 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 164.9, 156.4, 148.9 (dd, *J* = 242.0, 13.1 Hz), 145.9 (dd, *J* = 241.2, 12.7 Hz), 135.1 (dd, *J* = 9.0, 3.0 Hz), 133.0, 128.4, 122.7, 119.9, 119.0, 117.4 (d, *J* = 17.3Hz), 117.1 (dd, *J* = 6.0, 3.4 Hz), 109.8 (d, *J* = 21.4 Hz). HRMS (ESI) calcd for $C_{13}H_9ClF_2NO_2$, 284.0290 $(M + H)^+$; found, 284.0282.

***N*-(3,5-Bis(trifluoromethyl)phenyl)-5-chloro-2-hydroxybenzamide (16).** Compound **16** (105 mg, 55% in two steps) was prepared by a procedure similar to that used to prepare compound **10**. The title compound was obtained as a light yellow solid. HPLC purity 99.5% (t_R = 21.53 min). ^{1}H NMR (300 MHz, CD_3OD) δ 8.26 (s, 2H), 7.89 (d, *J* = 2.7 Hz, 1H), 7.62 (s, 1H), 7.29 (dd, *J* = 9.0, 2.7 Hz, 1H), 6.87 (d, *J* = 8.7 Hz, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ 167.4, 158.7, 141.2, 135.0, 133.2 (q, *J* = 33.2 Hz, 2C), 129.8, 125.5, 124.6 (q, *J* = 270.3 Hz, 2C), 121.5

(q, J = 3.2 Hz, 2C), 120.0, 119.1, 118.2 (hept, J = 3.8 Hz). HRMS (ESI) calcd for $C_{15}H_9ClF_6NO_2$, 384.0226 $(M + H)^+$; found, 384.0219.

5-Chloro-*N*-(3-fluoro-5-(trifluoromethyl)phenyl)-2-hydroxybenzamide (17). Compound **17** (100 mg, 33% in two steps) was prepared by a procedure similar to that used to prepare compound **10**. The title compound was obtained as a white solid. HPLC purity 96.7% (t_R = 20.86 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.42 (s, 1H), 8.04 (s, 1H), 7.79 (dt, J = 10.2, 2.1 Hz, 1H), 7.58 (s, 1H), 7.52 (d, J = 2.4 Hz, 1H), 7.43 (dd, J = 8.7, 2.4 Hz, 1H), 7.18 (d, J = 7.8 Hz, 1H), 7.02 (d, J = 8.7 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.2, 162.0 (d, J = 242.8 Hz), 156.2, 141.0 (d, J = 11.3 Hz), 133.1, 131.0 (qd, J = 32.4, 9.8 Hz), 128.6, 123.3 (q, J = 270.6 Hz), 122.7, 120.4, 119.0, 112.9 (quint, J = 3.4 Hz), 110.9 (d, J = 26.1 Hz), 107.8 (dq, J = 28.7, 3.8 Hz). HRMS (ESI) calcd for $C_{14}H_9ClF_4NO_2$, 334.0258 $(M + H)^+$; found, 334.0259.

***N*-(3-Fluoro-5-(trifluoromethyl)phenyl)-2-hydroxybenzamide (18).** Compound **18** (165 mg, 82% in two steps) was prepared by a procedure similar to that used to prepare compound **10**. The title compound was obtained as a pale yellow solid. HPLC purity 99.3% (t_R = 18.99 min). ^{1}H NMR (300 MHz, CD_3OD+ $CDCl_3$) δ 7.96 – 7.89 (m, 1H), 7.84 – 7.70 (m, 2H), 7.40 – 7.31 (m, 1H), 7.05 (d, J = 8.4 Hz, 1H), 6.96 – 6.87 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD+ $CDCl_3$) δ 168.2, 163.4 (d, J = 244.5 Hz), 159.5, 141.4 (d, J = 11.1 Hz), 135.0, 133.1 (qd, J = 33.0, 9.3 Hz), 129.7, 124.0 (qd, J = 270.5, 3.5 Hz), 120.3, 118.0, 113.8 (m), 117.2, 111.8 (d, J = 26.0 Hz), 108.5 (dq, J = 24.9, 3.8 Hz). HRMS (ESI) calcd for $C_{14}H_{10}F_4NO_2$, 300.0648 $(M + H)^+$; found, 300.0640.

***N*-(3-Fluoro-5-(trifluoromethyl)phenyl)-2-hydroxy-5-methylbenzamide (19).** Compound **19** (210 mg, 92% in two steps) was prepared by a procedure similar to that used to

prepare compound **10**. The title compound was obtained as a white solid. HPLC purity 98.4% (t_R = 19.72 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.28 (s, 1H), 8.09 (s, 1H), 7.82 (dt, *J* = 10.2, 2.1 Hz, 1H), 7.56 (s, 1H), 7.33 – 7.27 (m, 2H), 7.15 (d, *J* = 7.8 Hz, 1H), 6.98 – 6.93 (m, 1H), 2.35 (s, 3H). ^{13}C NMR (75 MHz, CD_3OD+ $CDCl_3$) δ 168.1, 163.3 (d, *J* = 244.4 Hz), 157.1, 141.3 (d, *J* = 11.0 Hz), 135.7, 133.0 (qd, *J* = 33.2, 9.3 Hz), 129.6, 129.6, 124.0 (qd, *J* = 270.4, 3.5 Hz), 117.7, 116.7, 113.7 (quint, *J* = 3.7 Hz), 111.7 (d, *J* = 26.5 Hz), 108.3 (dq, *J* = 25.0, 3.8 Hz), 20.5. HRMS (ESI) calcd for $C_{15}H_{12}F_4NO_2$, 314.0804 $(M + H)^+$; found, 314.0797.

4-Chloro-*N*-(3-fluoro-5-(trifluoromethyl)phenyl)-2-hydroxybenzamide (20). Compound **20** (320 mg, 89% in two steps) was prepared by a procedure similar to that used to prepare compound **10**. The title compound was obtained as a yellow solid. HPLC purity 99.9% (t_R = 20.02 min). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.70 (s, 1H), 10.67 (s, 1H), 7.99 (s, 1H), 7.93 (dt, *J* = 11.1, 1.8 Hz, 1H), 7.88 – 7.81 (m, 1H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.07 – 7.01 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.6, 162.0 (d, *J* = 242.7 Hz), 158.1, 141.0 (d, *J* = 11.3 Hz), 137.5, 131.1, 130.0 (qd, *J* = 25.0, 9.9 Hz),123.3 (qd, *J* = 270.9, 3.5 Hz), 119.4, 118.0, 116.7, 112.8 (m), 110.8 (d, *J* = 26.0 Hz), 107.7 (dq, *J* = 25.0, 3.6 Hz). HRMS (ESI) calcd for $C_{14}H_9ClF_4NO_2$, 334.0258 $(M + H)^+$; found, 334.0251.

3-Chloro-*N*-(3-fluoro-5-(trifluoromethyl)phenyl)benzamide (21). To a solution of 3-chlorobenzoic acid (100 mg, 0.64 mmol) and 3-fluoro-5-(trifluoromethyl)aniline (137 mg, 0.77 mmol) in toluene (15 mL) was added PCl_3 (132 mg, 0.96 mmol). The resulting mixture was stirred at 100 °C for 24 h, and then concentrated. The residue was purified by column chromatography (Hex/EtOAc = 5/1 to 3/1) to afford compound **21** as a white solid (190 mg, 93%). HPLC purity 99.9% (t_R = 19.73 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.83 (s, 1H), 7.80 – 7.70 (m, 2H), 7.66 (dt, *J* = 7.8, 1.2 Hz, 1H), 7.59 (s, 1H), 7.50 – 7.40 (m, 1H), 7.31 (t, *J* = 7.8 Hz,

1H), 7.12 – 7.02 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.6, 162.7 (d, *J* = 246.4 Hz), 139.9 (d, *J* = 11.0 Hz), 135.7, 135.1, 132.8 (qd, *J* = 33.4, 9.3 Hz), 132.5, 130.2, 127.5, 125.4. 123.1 (qd, *J* = 270.8, 3.3 Hz), 113.0 (quint, *J* = 3.7 Hz), 111.2 (d, *J* = 26.1 Hz), 109.0 (dq, *J* = 24.6, 3.6 Hz). HRMS (ESI) calcd for $C_{14}H_9ClF_4NO$, 318.0309 $(M + H)^+$; found, 318.0305.

5-Chloro-*N*-(2-chloro-4-nitrobenzyl)-2-hydroxybenzamide (30). To a solution of 5-chloro-2-methoxybenzoic acid (378 mg, 2.0 mmol), (2-chloro-4-nitrophenyl)methanamine (270 mg, 1.5 mmol) and DMAP (35 mg, 0.29 mmol) in DCM (20 mL) was added Et_3N (202 mg, 2.0 mmol) and EDCI (556 mg, 2.9 mmol) successively at 0 °C. The resulting mixture was stirred at r.t. for 12 h and concentrated. The residue was purified by column chromatography (Hex/EtOAc = 6/1 to 3/1) to afford the amide intermediate as a yellow solid. The amide intermediate was dissolved in DCM (30 mL), and then BBr_3 (4.4 mL, 4.4 mmol, 1 M in DCM) was added dropwise at 0 °C. The mixture was stirred at 0 °C for 4 h until the reaction was completed monitored by TLC. The mixture was diluted with DCM, washed with H_2O and concentrated. The residue was purified by recrystallization ($MeOH/H_2O$) to afford compound **30** as a brown solid (285 mg, 57% in two steps). HPLC purity 99.7% (t_R = 18.66 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.85 (s, 1H), 8.28 (d, *J* = 2.3 Hz, 1H), 8.12 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.63 (d, *J* = 8.5 Hz, 1H), 7.41 – 7.31 (m, 2H), 7.00 – 6.90 (m, 1H), 6.85 (s, 1H), 4.79 (d, *J* = 6.1 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.3, 160.3, 148.0, 141.9, 134.8, 134.5, 130.7, 125.2, 125.0, 123.8, 122.3, 120.5, 114.7, 41.6. HRMS (ESI) calcd for $C_{14}H_{11}Cl_2N_2O_4$, 341.0096 $(M + H)^+$; found, 341.0091.

5-Chloro-*N*-(2-chloro-4-fluorobenzyl)-2-hydroxybenzamide (31). Compound **31** (257 mg, 73% in two steps) was prepared by a procedure similar to that used to prepare compound **30**. The title compound was obtained as a yellow solid. HPLC purity 99.9% (t_R = 18.82 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.05 (s, 1H), 7.44 (dd, *J* = 8.4, 6.0 Hz, 1H), 7.37 – 7.28 (m, 2H),

7.17 (dd, *J* = 8.4, 2.4 Hz, 1H), 7.04 – 6.90 (m, 2H), 6.66 (s, 1H), 4.68 (d, *J* = 5.7 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$+CD_3OD) δ 168.3, 161.8 (d, *J* = 247.7 Hz), 158.2, 134.0 (d, *J* = 10.4 Hz), 133.5 (2C), 131.2 (d, *J* = 3.1 Hz), 130.6 (d, *J* = 8.8 Hz), 127.3, 123.9, 119.0, 116.8 (d, *J* = 24.8 Hz), 114.0 (d, *J* = 20.9 Hz), 40.7. HRMS (ESI) calcd for $C_{14}H_{11}Cl_2FNO_2$, 314.0151 $(M + H)^+$; found, 314.0143.

5-Chloro-*N*-(2,4-dichlorobenzyl)-2-hydroxybenzamide (32). Compound **32** (310 mg, 72% in two steps) was prepared by a procedure similar to that used to prepare compound **30**. The title compound was obtained as a yellow solid. HPLC purity 99.4% (t_R = 19.63 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.01 (s, 1H), 7.45 – 7.21(m, 5H), 6.93 (d, *J* = 8.7 Hz, 1H), 6.69 (s, 1H), 4.67 (d, *J* = 5.7 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.0, 160.3, 134.8, 134.6, 134.5, 133.4, 131.5, 129.8, 127.7, 125.1, 123.6, 120.4, 115.0, 41.4. HRMS (ESI) calcd for $C_{14}H_{11}Cl_3NO_2$, 329.9855 $(M + H)^+$; found, 329.9844.

5-Chloro-*N*-(2,4-difluorobenzyl)-2-hydroxybenzamide (33). Compound **33** (207 mg, 53% in two steps) was prepared by a procedure similar to that used to prepare compound **30**. The title compound was obtained as a yellow solid. HPLC purity 99.1% (t_R = 18.27 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.06 (s, 1H), 7.46 – 7.29 (m, 3H), 6.99 – 6.79 (m, 3H), 6.58 (s, 1H), 4.63 (d, *J* = 6.0 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.0, 162.9 (dd, *J* = 248.2, 11.9 Hz), 161.3 (dd, *J* = 247.4, 12.0 Hz), 160.4, 134.5, 131.7 (dd, *J* = 9.7, 5.7 Hz), 125.1, 123.6, 120.4, 120.4 (dd, *J* = 14.7, 4.1 Hz), 115.1, 111.8 (dd, *J* = 21.0, 3.7 Hz), 104.3 (t, *J* = 25.3 Hz), 37.5 (d, *J* = 3.2 Hz). HRMS (ESI) calcd for $C_{14}H_{11}ClF_2NO_2$, 298.0446 $(M + H)^+$; found, 298.0439.

5-Chloro-*N*-(3,4-difluorobenzyl)-2-hydroxybenzamide (34). Compound **34** (288 mg, 80% in two steps) was prepared by a procedure similar to that used to prepare compound **30**. The

title compound was obtained as a grey solid. HPLC purity 99.2% (t_R = 18.33 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.03 (s, 1H), 7.40 – 7.30 (m, 2H), 7.22 – 7.04 (m, 3H), 6.96 (d, *J* = 8.7 Hz, 1H), 6.55 (s, 1H), 4.58 (d, *J* = 5.7 Hz, 2H). ^{13}C NMR (75 MHz, CD_3OD+ $CDCl_3$) δ 168.7, 158.6, 150.7 (dd, *J* = 246.3, 12.7 Hz), 150.3 (dd, *J* = 245.5, 12.6 Hz), 136.0 (dd, *J* = 5.1, 4.0 Hz), 133.9, 128.0, 124.4, 124.0 (dd, *J* = 6.3, 3.7 Hz), 119.4, 117.6 (d, *J* = 17.3 Hz), 117.3, 116.9 (d, *J* = 17.6 Hz), 42.7. HRMS (ESI) calcd for $C_{14}H_{11}ClF_2NO_2$, 298.0446 $(M + H)^+$; found, 298.0437.

5-Chloro-*N*-(3-fluoro-5-(trifluoromethyl)benzyl)-2-hydroxybenzamide (35). Compound **35** (198 mg, 66% in two steps) was prepared by a procedure similar to that used to prepare compound **30**. The title compound was obtained as a grey solid. HPLC purity 99.5% (t_R = 19.04 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.97 (s, 1H), 7.45 – 7.29 (m, 3H), 7.23 (d, *J* = 8.4 Hz, 2H), 7.04 (s, 1H), 6.91 (d, *J* = 9.0 Hz, 1H), 4.65 (d, *J* = 5.4 Hz, 2H) ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.2, 162.8 (d, *J* = 248.5 Hz), 160.0, 141.4 (d, *J* = 7.2 Hz), 134.7, 133.2 (qd, *J* = 33.2, 8.2 Hz), 125.4, 123.9, 123.2 (qd, *J* = 270.8, 2.9 Hz), 120.3 (m), 120.3, 118.4 (d, *J* = 22.3 Hz), 114.9, 112.4 (dq, *J* = 24.4, 3.7 Hz), 43.0. HRMS (ESI) calcd for $C_{15}H_{11}ClF_4NO_2$, 348.0414 $(M + H)^+$; found, 348.0407.

5-Chloro-*N*-(3-fluoro-4-(trifluoromethyl)benzyl)-2-hydroxybenzamide (36). Compound **36** (215 mg, 51% in two steps) was prepared by a procedure similar to that used to prepare compound **30**. The title compound was obtained as an off-white solid. HPLC purity 97.6% (t_R = 19.17 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.93 (s, 1H), 7.61 (t, *J* = 7.5 Hz, 1H), 7.41 – 7.32 (m, 2H), 7.25 – 7.16 (m, 2H), 6.97 (d, *J* = 8.7 Hz, 1H), 6.64 (s, 1H), 4.68 (d, *J* = 6.0 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$+CD_3OD) δ 168.3, 159.7 (qd, *J* = 254.3, 2.3 Hz), 158.1, 145.6 (d, *J* = 7.4 Hz), 133.5, 127.4, 127.1 (dq, *J* = 4.6, 1.7 Hz), 123.9, 122.8 (d, *J* = 3.5 Hz),

122.5 (q, *J* = 270.2 Hz), 118.9, 116.9 (dq, *J* = 32.8, 12.5 Hz), 116.5, 115.5 (q, *J* = 21.1 Hz), 42.3. HRMS (ESI) calcd for $C_{15}H_{11}ClF_4NO_2$, 348.0414 $(M + H)^+$; found, 348.0406.

5-Chloro-*N*-(2-fluoro-4-(trifluoromethyl)benzyl)-2-hydroxybenzamide (37). Compound **37** (153 mg, 50% in two steps) was prepared by a procedure similar to that used to prepare compound **30**. The title compound was obtained as a yellow solid. HPLC purity 98.7% (t_R = 19.23 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.97 (s, 1H), 7.54 (t, *J* = 7.5 Hz, 1H), 7.45 – 7.30 (m, 4H), 6.93 (d, *J* = 9.6 Hz, 1H), 6.77 (s, 1H), 4.71 (d, *J* = 5.7 Hz, 2H). ^{13}C NMR (75 MHz, CD_3OD+ $CDCl_3$) δ 168.9, 161.0 (d, *J* = 247.1 Hz), 158.6, 134.1, 131.9 (qd, *J* = 33.2, 8.0 Hz), 130.8 (d, *J* = 4.5 Hz), 130.3 (d, *J* = 14.9 Hz), 128.5, 124.6, 123.9 (qd, *J* = 270.0, 2.6 Hz), 121.6 (quint, *J* = 3.8 Hz), 119.4, 117.6, 113.1 (dq, *J* = 24.9, 3.8 Hz), 37.5 (d, *J* = 4.4 Hz). HRMS (ESI) calcd for $C_{15}H_{11}ClF_4NO_2$, 348.0414 $(M + H)^+$; found, 348.0408.

5-Chloro-*N*-(4-chlorobenzyl)-2-hydroxybenzamide (38). Compound **38** (300 mg, 82% in two steps) was prepared by a procedure similar to that used to prepare compound **30**. The title compound was obtained as a grey solid. HPLC purity 96.1% (t_R = 18.79 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.11 (s, 1H), 7.37 – 7.23 (m, 6H), 6.94 (d, *J* = 9.6 Hz, 1H), 6.60 (s, 1H), 4.58 (d, *J* = 5.7 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.0, 160.3, 135.8, 134.5, 134.0, 129.4 (2C), 129.2 (2C), 125.1, 123.6, 120.4, 115.1, 43.3. HRMS (ESI) calcd for $C_{14}H_{12}Cl_2NO_2$, 296.0245 $(M + H)^+$; found, 296.0239.

5-Chloro-*N*-(3-chlorobenzyl)-2-hydroxybenzamide (39). Compound **39** (290 mg, 68% in two steps) was prepared by a procedure similar to that used to prepare compound **30**. The title compound was obtained as a yellow solid. HPLC purity 98.8% (t_R = 18.79 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.10 (s, 1H), 7.38 – 7.19 (m, 6H), 6.95 (d, *J* = 9.0 Hz, 1H), 6.57 (s, 1H), 4.60

(d, J = 5.7 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.0, 160.4, 139.3, 135.0, 134.5, 130.4, 128.3, 128.2, 126.2, 125.1, 123.6, 120.4, 115.0, 43.4. HRMS (ESI) calcd for $C_{14}H_{12}Cl_2NO_2$, 296.0245 $(M + H)^+$; found, 296.0237.

5-Chloro-*N*-(4-fluorobenzyl)-2-hydroxybenzamide (40). Compound **40** (260 mg, 51% in two steps) was prepared by a procedure similar to that used to prepare compound **30**. The title compound was obtained as an off-white solid. HPLC purity 99.9% (t_R = 18.00 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.15 (s, 1H), 7.38 – 7.27 (m, 4H), 7.05 (t, J = 8.7 Hz, 2H), 6.94 (d, J = 8.7 Hz, 1H), 6.55 (s, 1H), 4.59 (d, J = 5.4 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.9, 162.6 (d, J = 245.1 Hz), 160.3, 134.4, 133.1 (d, J = 3.2 Hz), 129.9 (d, J = 8.2 Hz, 2C), 125.1, 123.6, 120.4, 116.0 (d, J = 21.5 Hz, 2C), 115.1, 43.3. HRMS (ESI) calcd for $C_{14}H_{12}ClFNO_2$, 280.0541 $(M + H)^+$; found, 280.0533.

5-Chloro-*N*-(4-fluorobenzyl)-2-hydroxy-*N*-methylbenzamide (41). Compound **41** (340 mg, 93% in two steps) was prepared by a procedure similar to that used to prepare compound **30**. The title compound was obtained as a yellow solid. HPLC purity 95.0% (t_R = 16.29 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.71 (s, 1H), 7.32 – 7.22 (m, 4H), 7.07 (t, J = 8.7 Hz, 2H), 6.96 (d, J = 8.4 Hz, 1H), 4.69 (s, 2H), 3.06 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9, 162.6 (d, J = 245.1 Hz), 157.8, 132.8, 131.9 (d, J = 3.2 Hz), 129.6 (d, J = 7.5 Hz, 2C), 127.8, 123.5, 119.7, 118.5, 116.0 (d, J = 21.5 Hz, 2C), 52.6, 36.7. HRMS (ESI) calcd for $C_{15}H_{14}ClFNO_2$, 294.0697 $(M + H)^+$; found, 294.0689.

5-Chloro-2-hydroxy-*N*-(4-(trifluoromethyl)benzyl)benzamide (42). Compound **42** (220 mg, 65% in two steps) was prepared by a procedure similar to that used to prepare compound **30**. The title compound was obtained as an off-white solid. HPLC purity 99.8% (t_R =

19.04 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.05 (s, 1H), 7.61 (d, *J* = 7.8 Hz, 2H), 7.44 (d, *J* = 7.9 Hz, 2H), 7.39 – 7.30 (m, 2H), 6.94 (d, *J* = 9.0 Hz, 1H), 6.73 (s, 1H), 4.67 (d, *J* = 5.4 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.1, 160.3, 141.4, 134.6, 130.4 (q, *J* = 32.3 Hz), 128.2 (2C), 126.0 (q, *J* = 3.8 Hz, 2C), 125.2, 124.1 (q, *J* = 270.4 Hz), 123.7, 120.4, 115.0, 43.4. HRMS (ESI) calcd for $C_{15}H_{12}ClF_3NO_2$, 330.0509 $(M + H)^+$; found, 330.0501.

5-Chloro-2-hydroxy-*N*-(4-nitrobenzyl)benzamide (43). Compound **43** (260 mg, 69% in two steps) was prepared by a procedure similar to that used to prepare compound **30**. The title compound was obtained as a yellow solid. HPLC purity 99.8% (t_R = 17.81 min). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.25 (s, 1H), 9.46 (s, 1H), 8.21 (d, *J* = 8.7 Hz, 2H), 7.96 (d, *J* = 2.4 Hz, 1H), 7.60 (d, *J* = 8.7 Hz, 2H), 7.46 (dd, *J* = 9.0, 2.7 Hz, 1H), 6.97 (d, *J* = 8.7 Hz, 1H), 4.64 (d, *J* = 6.0 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.4, 158.2, 146.8, 146.5, 133.3, 128.3 (2C), 127.6, 123.5 (2C), 122.5, 119.3, 117.0, 42.1. HRMS (ESI) calcd for $C_{14}H_{12}ClN_2O_4$, 307.0486 $(M + H)^+$; found, 307.0478.

5-Chloro-2-hydroxy-*N*-(4-methylbenzyl)benzamide (44). Compound **44** (330 mg, 85% in two steps) was prepared by a procedure similar to that used to prepare compound **30**. The title compound was obtained as an off-white solid. HPLC purity 99.3% (t_R = 18.65 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.24 (s, 1H), 7.38 – 7.16 (m, 6H), 6.95 (d, *J* = 8.7 Hz, 1H), 6.47 (s, 1H), 4.58 (d, *J* = 5.4 Hz, 2H), 2.37 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.8, 160.4, 138.0, 134.3, 134.1, 129.8 (2C), 128.2 (2C), 125.1, 123.5, 120.3, 115.3, 43.8, 21.3. HRMS (ESI) calcd for $C_{15}H_{15}ClNO_2$, 276.0791 $(M + H)^+$; found, 276.0784.

***N*-Benzyl-5-chloro-2-hydroxybenzamide (45).** Compound **45** (35 mg, 55% in two steps) was prepared by a procedure similar to that used to prepare compound **30**. The title

compound was obtained as an off-white solid. HPLC purity 99.9% (t_R = 17.91 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.23 (s, 1H), 7.45 – 7.29 (m, 7H), 6.94 (d, *J* = 9.3 Hz, 1H), 6.56 (s, 1H), 4.62 (d, *J* = 5.7 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.9, 160.3, 137.2, 134.3, 129.1 (2C), 128.2, 128.1 (2C), 125.1, 123.5, 120.3, 115.2, 44.0. HRMS (ESI) calcd for $C_{14}H_{13}ClNO_2$, 262.0635 $(M + H)^+$; found, 262.0628.

(*R*)-5-Chloro-2-hydroxy-*N*-(1-phenylethyl)benzamide (46). To a solution of (*R*)-1-phenylethanamine (150 mg, 1.2 mmol), 5-chlorosalicylic acid (214 mg, 1.2 mmol) and DMAP (30 mg, 0.25 mmol) in 30 mL of DCM was added EDCI (475 mg, 2.5 mmol) at 0 °C. The resulting mixture was stirred at r.t. for 24 h and then concentrated. The residue was purified by column chromatography (Hex/EtOAc = 10/1) to afford compound **46** as an off-white solid (200 mg, 55%). HPLC purity 99.1% (t_R = 18.46 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.25 (s, 1H), 7.43 – 7.26 (m, 7H), 6.90 (d, *J* = 9.0 Hz, 1H), 6.74 (d, *J* = 7.2 Hz, 1H), 5.29 (p, *J* = 6.9 Hz, 1H), 1.60 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.2, 160.0, 142.3, 134.1, 128.9 (2C), 127.8, 126.2 (2C), 125.3, 123.4, 120.1, 115.4, 49.4, 21.6. HRMS (ESI) calcd for $C_{15}H_{15}ClNO_2$, 276.0791 $(M + H)^+$; found, 276.0790.

(*S*)-5-Chloro-2-hydroxy-*N*-(1-phenylethyl)benzamide (47). Compound **47** (108 mg, 39%) was prepared by a procedure similar to that used to prepare compound **46**. The title compound was obtained as a white solid. HPLC purity 97.1% (t_R = 19.75 min). ^{1}H NMR (600 MHz, $CDCl_3$) δ 12.22 (s, 1H), 7.28-7.48 (m, 7H), 6.93 (d, 1H, *J* = 9.0 Hz), 6.55 (d, 1H, *J* = 6.6 Hz), 5.29-5.34 (m, 1H), 1.64 (d, 3H, *J* = 7.2 Hz). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.2, 160.2, 142.3, 134.2, 129.0, 129.0, 127.9, 126.3, 126.3, 125.2, 123.4, 120.2, 115.4, 49.5, 21.8. HRMS (ESI) calcd for $C_{15}H_{15}ClNO_2$, 276.0791 $(M + H)^+$; found, 276.0790.

(*R*)-5-Chloro-*N*-(1-(4-chlorophenyl)ethyl)-2-hydroxybenzamide (48). Compound **48** (140 mg, 40%) was prepared by a procedure similar to that used to prepare compound **46**. The title compound was obtained as a pale yellow solid. HPLC purity 96.4% (t_R = 19.30 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.11 (s, 1H), 7.40 (d, *J* = 2.4 Hz, 1H), 7.34 – 7.24 (m, 5H), 6.89 (d, *J* = 9.0 Hz, 1H), 6.75 (d, *J* = 7.2 Hz, 1H), 5.22 (p, *J* = 7.2 Hz, 1H), 1.56 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.2, 160.0, 140.9, 134.2, 133.5, 129.0 (2C), 127.6 (2C), 125.4, 123.5, 120.1, 115.2, 48.9, 21.6. HRMS (ESI) calcd for $C_{15}H_{14}Cl_2NO_2$, 310.0402 $(M + H)^+$; found, 310.0399.

(*R*)-5-Chloro-*N*-(1-(4-fluorophenyl)ethyl)-2-hydroxybenzamide (49). Compound **49** (270 mg, 64%) was prepared by a procedure similar to that used to prepare compound **46**. The title compound was obtained as a yellow solid. HPLC purity 99.1% (t_R = 18.62 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.15 (s, 1H), 7.48 – 7.42 (m, 1H), 7.36 – 7.26 (m, 3H), 7.06 – 6.92 (m, 3H), 6.87 (d, *J* = 9.0 Hz, 1H), 5.24 (p, *J* = 7.2 Hz, 1H), 1.56 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1, 162.6 (d, *J* = 169.5 Hz), 159.7, 138.2 (d, *J* = 2.9 Hz), 134.1, 127.9 (d, *J* = 8.1 Hz, 2C), 125.6, 123.5, 119.9, 115.6 (d, *J* = 21.3 Hz, 2C), 115.4, 48.7, 21.6. HRMS (ESI) calcd for $C_{15}H_{14}ClFNO_2$, 294.0697 $(M + H)^+$; found, 294.0693.

(*R*)-5-Chloro-2-hydroxy-*N*-(1-(4-methoxyphenyl)ethyl)benzamide (50). Compound **50** (220 mg, 54%) was prepared by a procedure similar to that used to prepare compound **46**. The title compound was obtained as a pale yellow solid. HPLC purity 95.2% (t_R = 18.42 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.30 (s, 1H), 7.38 (d, *J* = 2.1 Hz, 1H), 7.32 – 7.26(m, 3H), 6.92 – 6.84 (m, 3H), 6.73 (d, *J* = 7.2 Hz, 1H), 5.23 (p, *J* = 6.9 Hz, 1H), 3.78 (s, 3H), 1.58 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.1, 160.0, 159.1, 134.3, 134.0, 127.5 (2C), 125.3, 123.3,

120.0, 115.4, 114.2 (2C), 55.3, 48.8, 21.4. HRMS (ESI) calcd for $C_{16}H_{17}ClNO_3$, 306.0897 $(M + H)^+$; found, 306.0892.

5-Chloro-*N*-(2-(4-fluorophenyl)propan-2-yl)-2-hydroxybenzamide (51). To a solution of 1-(4-fluorophenyl)-1-methylethylamine (180 mg, 1.2 mmol), 5-chloro-2-methoxybenzoic acid (219 mg, 1.2 mmol) and DMAP (28 mg, 0.23 mmol) in 20 mL of DCM was added EDCI (336 mg, 1.8 mmol). The resulting mixture was stirred at 50 °C for 48 h and then concentrated. The residue was purified by column chromatography (Hex/EtOAc = 10/1) to afford the amide intermediate 5-chloro-*N*-(2-(4-fluorophenyl)propan-2-yl)-2-methoxybenzamide (57 mg, 15%) as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.32 – 8.18 (m, 1H), 8.09 (d, *J* = 3.0 Hz, 1H), 7.45 – 7.33 (m, 3H), 7.05 – 6.95 (m, 2H), 6.91 (d, *J* = 8.7 Hz, 1H), 3.97 (s, 3H), 1.77 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.6, 161.5 (d, *J* = 243.0 Hz), 155.9, 142.9 (d, *J* = 3.2 Hz), 132.3, 132.0, 126.9, 126.6 (d, *J* = 7.9 Hz, 2C), 123.8, 115.1 (d, *J* = 21.1 Hz, 2C), 112.9, 56.5, 55.6, 29.5 (2C).

To a solution of the amide intermediate 5-chloro-*N*-(2-(4-fluorophenyl)propan-2-yl)-2-methoxybenzamide (57 mg, 0.18 mmol) in 15 mL of DCM was added BBr_3 (0.35 mL, 0.35 mmol, 1M in DCM) dropwise at 0 °C. The resulting mixture was stirred at r.t. for 1 h, and then the mixture was poured into 20 mL of ice water. The organic phase was separated and the aqueous phase was extracted with DCM (60 mL). The organic phases were combined, dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (Hex/EtOAc = 10/1) to afford compound **51** (50 mg, 92%) as an off-white solid. HPLC purity 99.0% (t_R = 18.91 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.03 (s, 1H), 7.45 – 7.29 (m, 4H), 7.10 – 6.99 (m, 2H), 6.91 (d, *J* = 8.7 Hz, 1H), 6.49 (s, 1H), 1.80 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.4, 161.8 (d, *J* = 244.1 Hz), 160.4, 141.7 (d, *J* = 3.2 Hz), 134.2, 126.4 (d, *J* = 8.0 Hz, 2C), 125.1, 123.3,

120.5, 115.7, 115.5 (d, J = 21.2 Hz, 2C), 56.5, 29.6 (2C). HRMS (ESI) calcd for $C_{16}H_{16}ClFNO_2$, 308.0854 $(M + H)^+$; found, 308.0852.

5-Chloro-*N*-(4-fluorophenethyl)-2-hydroxybenzamide (52). Methyl 5-chloro-2-hydroxybenzoate (100 mg, 0.54 mmol) was dissolved in 10 mL of methanol followed by addition of 2-(4-fluorophenyl)ethanamine (224 mg, 1.6 mmol). The resulting mixture was stirred at r.t. for 48 h, and then concentrated. The residue was purified by preparative TLC to afford compound **52** as a yellow solid (123 mg, 52%). HPLC purity 99.6% (t_R = 18.46 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.20 (s, 1H), 7.32 (dd, J = 8.7, 2.1 Hz, 1H), 7.23 – 7.14 (m, 3H), 7.02 (t, J = 8.7 Hz, 2H), 6.92 (d, J = 8.7 Hz, 1H), 6.35 (s, 1H), 3.67 (q, J = 6.6 Hz, 2H), 2.91 (t, J = 6.9 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.1, 162.0 (d, J = 243.5 Hz), 160.2, 134.3, 134.1 (d, J = 3.2 Hz), 130.3 (d, J = 7.8 Hz, 2C), 125.0, 123.5, 120.3, 115.8 (d, J = 21.2 Hz, 2C), 115.3, 41.2, 34.8. HRMS (ESI) calcd for $C_{15}H_{14}ClFNO_2$, 294.0697 $(M + H)^+$; found, 294.0691.

2-(5-Chloro-2-hydroxyphenyl)-*N*-(3-fluoro-5-(trifluoromethyl)phenyl)acetamide (53). Compound **53** (300 mg, 82% in two steps) was prepared by a procedure similar to that used to prepare compound **10** starting from 2-(5-chloro-2-methoxyphenyl)acetic acid and 3-fluoro-5-(trifluoromethyl)aniline. The title compound was obtained as a yellow solid. HPLC purity 99.5% (t_R = 18.13 min). ^{1}H NMR (300 MHz, CD_3OD) δ 7.77 – 7.65 (m, 2H), 7.16 (d, J = 2.4 Hz, 1H), 7.12 – 7.02 (m, 2H), 6.76 (d, J = 8.4 Hz, 1H), 3.67 (s, 2H). ^{13}C NMR (75 MHz, CD_3OD) δ 172.6, 164.1 (d, J = 243.8 Hz), 155.6, 142.7 (d, J = 11.0 Hz), 133.6 (qd, J = 33.1, 9.4 Hz), 131.9, 129.1, 125.0, 124.8, 124.7 (qd, J = 270.0, 3.5 Hz), 117.2, 113.1 (d, J = 3.7 Hz), 111.0 (d, J = 26.2 Hz), 108.3 (dq, J = 25.1, 3.8 Hz), 39.4. HRMS (ESI) calcd for $C_{15}H_{11}ClF_4NO_2$, 348.0414 $(M + H)^+$; found, 348.0408.

2-(5-Chloro-2-hydroxyphenyl)-*N*-(2-chloro-4-nitrophenyl)acetamide (54). Compound **54** (277 mg, 77% in two steps) was prepared by a procedure similar to that used to prepare compound **10** starting from 2-(5-chloro-2-methoxyphenyl)acetic acid and 2-chloro-4-nitroaniline. The title compound was obtained as a yellow solid. HPLC purity 98.4% (t_R = 17.79 min). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.05 (s, 1H), 9.78 (s, 1H), 8.338 – 8.28 (m, 2H), 8.21 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.27 (d, *J* = 2.7 Hz, 1H), 7.15 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.85 (d, *J* = 8.7 Hz, 1H), 3.80 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$+CD_3OD) δ 170.4, 153.1, 142.8, 140.9, 130.7, 128.9, 124.8, 124.7, 123.3, 122.5, 122.2, 120.4, 116.3, 40.3. HRMS (ESI) calcd for $C_{14}H_{11}Cl_2N_2O_4$, 341.0096 $(M + H)^+$; found, 341.0086.

5-Chloro-*N*-(2-((2-chloro-4-nitrophenyl)amino)-2-oxoethyl)-2-hydroxybenzamide (56). To a solution of 2-chloro-4-nitroanilin (1.0 g, 5.8 mmol) and Fmoc-Gly-OH (2.24 g, 7.5 mmol) in 100 mL of toluene was added PCl_3 (1.0 g, 7.5 mmol). The mixture was stirred at 80 °C for 1 h. The mixture was diluted with 200 mL of EtOAc, washed with water (70 mL), dried (Na_2SO_4) and concentrated to give the intermediate. The intermediate was dissolved in 200 mL of CH_3CN, and piperidine (1.3 g, 15.1 mmol) was added. The mixture was stirred at r.t. for 24 h and then concentrated. The residue was purified by column chromatography (DCM/MeOH) to give 2-amino-*N*-(2-chloro-4-nitrophenyl)acetamide **55a** as a yellow solid (800 mg, 69% in two steps). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.67 (d, *J* = 9.3 Hz, 1H), 8.42 (d, *J* = 2.7 Hz, 1H), 8.27 (dd, *J* = 9.0, 2.7 Hz, 1H), 5.33 (s, 2H), 3.37 (s, 2H).

To a solution of 2-amino-*N*-(2-chloro-4-nitrophenyl)acetamide **55a** (173 mg, 0.75 mmol), 5-chloro-2-methoxybenzoic acid (168 mg, 0.90 mmol) and DMAP (22 mg, 0.18 mmol) in 30 mL of DCM was added EDCI (345 mg, 1.8 mmol) at 0 °C. The resulting mixture was stirred at r.t. for 12 h. The solvent was evaporated and 30 mL of MeOH was added. The resulting mixture

was stirred at r.t. for 20 min. The intermediate 5-chloro-*N*-(2-((2-chloro-4-nitrophenyl)amino)-2-oxoethyl)-2-methoxybenzamide (120 mg, 40%) was isolated by filtration as an off-white solid. ^{1}H NMR (300 MHz, DMSO-*d*6) δ 9.93 (s, 1H), 8.79 (t, *J* = 5.7 Hz, 1H), 8.38 (d, *J* = 2.4 Hz, 1H), 8.32 (d, *J* = 9.0 Hz, 1H), 8.23 (dd, *J* = 9.3, 2.4 Hz, 1H), 7.81 (d, *J* = 2.7 Hz, 1H), 7.58 (dd, *J* = 9.0, 2.7 Hz, 1H), 7.24 (d, *J* = 9.0 Hz, 1H), 4.29 (d, *J* = 5.7 Hz, 2H), 3.32 (s, 3H). ^{13}C NMR (75 MHz, DMSO-*d*6) δ 168.8, 163.9, 156.2, 143.2, 140.8, 132.3, 130.0, 125.0, 124.5, 123.9, 123.3, 123.2, 123.0, 114.4, 109.1, 56.5, 44.0.

To a solution of the intermediate 5-chloro-*N*-(2-((2-chloro-4-nitrophenyl)amino)-2-oxoethyl)-2-methoxybenzamide (100 mg, 0.25 mmol) in 20 mL of DCM was added BBr_3 (1.3 mL, 1.3 mmol, 1M in DCM) dropwise at 0 °C. The resulting mixture was stirred at 0 °C for 12 h until the reaction was completed monitored by TLC. Then the mixture was poured into 20 mL of ice water, extracted with EtOAc (2×60 mL), washed with water (30 mL) and brine (30 mL), dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography (Hex/EtOAc = 3/1 to 1/1) to afford compound **56** (70 mg, 72%) as a white solid. HPLC purity 99.8% (t_R = 18.39 min). ^{1}H NMR (300 MHz, DMSO-*d*6) δ 12.08 (s, 1H), 10.01 (s, 1H), 9.24 (t, *J* = 5.4 Hz, 1H), 8.38 (d, *J* = 2.4 Hz, 1H), 8.28 (d, *J* = 9.0 Hz, 1H), 8.22 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.95 (d, *J* = 2.7 Hz, 1H), 7.46 (dd, *J* = 8.7, 2.7 Hz, 1H), 6.99 (d, *J* = 8.7 Hz, 1H), 4.31 (d, *J* = 5.4 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-*d*6) δ 168.5, 166.9, 157.5, 143.3, 140.8, 133.3, 128.2, 125.0, 124.3, 123.4, 123.2, 122.6, 119.2, 117.5, 43.5. HRMS (ESI) calcd for $C_{15}H_{12}Cl_2N_3O_5$, 384.0154 $(M + H)^+$; found, 384.0148.

(*S*)-5-Chloro-*N*-(1-((2-chloro-4-nitrophenyl)amino)-1-oxopropan-2-yl)-2-hydroxybenzamide (57). Compound **57** was prepared by a procedure similar to that used to prepare compound **56** starting from 2-chloro-4-nitroanilin, Fmoc-*L*-Ala-OH and 5-chloro-2-

methoxybenzoic acid. The corresponding intermediate (*S*)-2-amino-*N*-(2-chloro-4-nitrophenyl)propanamide **55b** was obtained as a yellow solid (1.0 g, 71% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.70 (s, 1H), 8.73 (d, *J* = 9.0 Hz, 1H), 8.26 (d, *J* = 2.4 Hz, 1H), 8.13 (dd, *J* = 9.0, 2.4 Hz, 1H), 3.70 (q, *J* = 6.9 Hz, 1H), 1.72 (s, 2H), 1.47 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.7, 142.9, 140.7, 124.9, 123.6, 123.0, 119.6, 51.7, 21.5.

Compound **57** was obtained as a yellow solid (160 mg, 82% in two steps). HPLC purity 95.2% (t_R = 18.20 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.70 (s, 1H), 8.89 (s, 1H), 8.62 (d, *J* = 9.3 Hz, 1H), 8.29 (d, *J* = 1.5 Hz, 1H), 8.16 (d, *J* = 9.01 Hz, 1H), 7.45 – 7.32 (m, 2H), 6.99 – 6.85 (m, 2H), 4.97 – 4.85 (m, 1H), 1.65 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 169.4, 159.8, 143.3, 140.1, 134.8, 125.9, 124.9, 123.9, 123.4, 123.3, 120.8, 120.1, 114.4, 50.4, 16.8. HRMS (ESI) calcd for $C_{16}H_{14}Cl_2N_3O_5$, 398.0311 $(M + H)^+$; found, 398.0303.

(*S*)-5-Chloro-*N*-(1-((2-chloro-4-nitrophenyl)amino)-3-methyl-1-oxobutan-2-yl)-2-hydroxybenzamide (58). Compound **58** was prepared by a procedure similar to that used to prepare compound **56** starting from 2-chloro-4-nitroanilin, Fmoc-*L*-Val-OH and 5-chloro-2-methoxybenzoic acid. The corresponding intermediate (*S*)-2-amino-*N*-(2-chloro-4-nitrophenyl)-3-methylbutanamide **55c** was obtained as a yellow solid (1.45 g, 92% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.72 (s, 1H), 8.71 (d, *J* = 9.1 Hz, 1H), 8.23 (d, *J* = 2.4 Hz, 1H), 8.10 (dd, *J* = 9.1, 2.4 Hz, 1H), 3.45 (d, *J* = 3.6 Hz, 1H), 2.52 – 2.35 (m, 1H), 1.62 (s, 2H), 1.04 (d, *J* = 6.9 Hz, 3H), 0.86 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7, 142.7, 140.5, 124.8, 123.5, 122.9, 119.5, 60.9, 30.8, 19.7, 16.0.

Compound **58** was obtained as a pale yellow solid (170 mg, 74% in two steps). HPLC purity 98.9% (t_R = 19.85 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.76 (s, 1H), 8.65 (d, *J* = 9.3 Hz, 1H), 8.45 (s, 1H), 8.33 (d, *J* = 2.4 Hz, 1H), 8.19 (dd, *J* = 9.3, 2.4 Hz, 1H), 7.44 (d, *J* = 2.4 Hz,

1H), 7.39 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.97 (d, *J* = 8.7 Hz, 1H), 6.90 (d, *J* = 7.5 Hz, 1H), 4.61 (dd, *J* = 8.1, 7.2 Hz, 1H), 2.46 – 2.32 (m, 1H), 1.16 – 1.10 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.5, 169.4, 160.4, 143.7, 139.7, 135.1, 125.3, 125.0, 124.0, 123.7, 123.1, 120.8, 120.6, 114.6, 60.0, 30.8, 19.5, 18.6. HRMS (ESI) calcd for $C_{18}H_{18}Cl_2N_3O_5$, 426.0624 $(M + H)^+$; found, 426.0619.

(*S*)-5-chloro-*N*-(1-((2-chloro-4-nitrophenyl)amino)-1-oxo-3-phenylpropan-2-yl)-2-hydroxybenzamide (59). Compound **59** was prepared by a procedure similar to that used to prepare compound **56** starting from 2-chloro-4-nitroanilin, Fmoc-*L*-Phe-OH and 5-chloro-2-methoxybenzoic acid. The corresponding intermediate (*S*)-2-amino-*N*-(2-chloro-4-nitrophenyl)-3-phenylpropanamide **55d** was afforded as a yellow solid (1.75 g, 94% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.66 (s, 1H), 8.79 (d, *J* = 9.0 Hz, 1H), 8.29 (d, *J* = 2.4 Hz, 1H), 8.17 (dd, *J* = 9.3, 2.7 Hz, 1H), 7.39 – 7.20 (m, 5H), 3.83 (dd, *J* = 9.0, 2.7 Hz, 1H), 3.40 (dd, *J* = 13.8, 3.9 Hz, 1H), 2.83 (dd, *J* = 13.8, 9.6 Hz, 1H), 1.61 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.5, 143.0, 140.5, 137.2, 129.4 (2C), 129.1 (2C), 127.3, 124.9, 123.7, 123.1, 119.7, 57.3, 40.6.

Compound **59** was obtained as a brwon solid (100 mg, 83% in two steps). HPLC purity 99.2% (t_R = 20.48 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.71 (s, 1H), 8.62 (d, *J* = 9.0 Hz, 1H), 8.24 (d, *J* = 2.4 Hz, 2H), 8.16 (dd, *J* = 9.0, 2.45 Hz, 1H), 7.41 – 7.27 (m, 7H), 7.00 – 6.92 (m, 2H), 5.09 – 4.93 (m, 1H), 3.40 (dd, *J* = 13.8, 6.0 Hz, 1H), 3.23 (dd, *J* = 13.8, 8.4 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.5, 169.2, 160.2, 143.6, 139.6, 135.5, 135.0, 129.4 (2C), 129.2 (2C), 128.0, 125.5, 123.9, 123.6, 123.0, 120.6, 120.4, 118.2, 114.5, 56.0, 37.9. HRMS (ESI) calcd for $C_{22}H_{18}Cl_2N_3O_5$, 474.0624 $(M + H)^+$; found, 474.0623.

(*R*)-5-Chloro-*N*-(1-((2-chloro-4-nitrophenyl)amino)-1-oxo-3-phenylpropan-2-yl)-2-hydroxybenzamide (60). Compound **60** was prepared by a procedure similar to that used to

prepare compound **56** starting from 2-chloro-4-nitroanilin, Fmoc-*D*-Phe-OH and 5-chloro-2-methoxybenzoic acid. The corresponding intermediate (*R*)-2-amino-*N*-(2-chloro-4-nitrophenyl)-3-phenylpropanamide **55e** was afforded as a yellow solid (869 mg, 94% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.66 (s, 1H), 8.77 (d, *J* = 9.3 Hz, 1H), 8.25 (d, *J* = 2.4 Hz, 1H), 8.14 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.39 – 7.23 (m, 5H), 3.82 (dd, *J* = 9.6, 3.6 Hz, 1H), 3.39 (dd, *J* = 14.1, 3.6 Hz, 1H), 2.83 (dd, *J* = 14.1, 9.6 Hz, 1H), 1.63 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.4, 142.9, 140.5, 137.2, 129.3 (2C), 129.0 (2C), 127.3, 124.8, 123.6, 122.9, 119.6, 57.2, 40.5.

Compound **60** was obtained as a yellow solid (300 mg, 72% in two steps). HPLC purity 96.7% (t_R = 20.47 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.71 (s, 1H), 8.61 (d, *J* = 9.3 Hz, 1H), 8.31 (s, 1H), 8.23 (d, *J* = 2.4 Hz, 1H), 8.15 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.41 – 7.26 (m, 7H), 7.13 (d, *J* = 7.2 Hz, 1H), 6.98 – 6.88 (m, 1H), 5.08 – 4.97 (m, 1H), 3.38 (dd, *J* = 13.8, 6.3 Hz, 1H), 3.24 (dd, *J* = 13.8, 8.4 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.4, 169.2, 160.3, 143.6, 139.6, 135.4, 135.1, 129.5 (2C), 129.3 (2C), 128.0, 125.5, 124.9, 124.0, 123.6, 123.0, 120.6, 120.5, 114.4, 56.0, 38.0. HRMS (ESI) calcd for $C_{22}H_{18}Cl_2N_3O_5$, 474.0624 $(M + H)^+$; found, 474.0618.

(*S*)-5-Chloro-*N*-(1-((2-chloro-4-nitrophenyl)amino)-4-methyl-1-oxopentan-2-yl)-2-hydroxybenzamide (61). Compound **61** was prepared by a procedure similar to that used to prepare compound **56** starting from 2-chloro-4-nitroanilin, Fmoc-*L*-Leu-OH and 5-chloro-2-methoxybenzoic acid. The corresponding intermediate (*S*)-2-amino-*N*-(2-chloro-4-nitrophenyl)-4-methylpentanamide **55f** was afforded as a yellow solid (1.5 g, 90% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.75 (s, 1H), 8.69 (d, *J* = 9.3 Hz, 1H), 8.22 (d, *J* = 2.4 Hz, 1H), 8.09 (dd, *J* = 9.3, 2.4 Hz, 1H), 3.64 – 3.48 (m, 1H), 1.87 – 1.75 (m, 2H), 1.68 (s, 2H), 1.54 – 1.37 (m, 1H), 0.97 (t, *J* = 6.3 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.7, 142.7, 140.7, 124.8, 123.6, 122.8, 119.5, 54.4, 43.8, 25.1, 23.4, 21.3.

Compound **61** was obtained as a pale yellow solid (145 mg, 74% in two steps). HPLC purity 99.6% (t_R = 19.88 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.70 (s, 1H), 8.81 (s, 1H), 8.61 (d, *J* = 9.0 Hz, 1H), 8.29 (d, *J* = 2.4 Hz, 1H), 8.15 (dd, *J* = 9.3, 2.4 Hz, 1H), 7.45 – 7.32 (m, 2H), 6.93 (d, *J* = 8.7 Hz, 1H), 6.86 (d, *J* = 7.2 Hz, 1H), 4.90 – 4.78 (m, 1H), 2.03 – 1.74 (m, 3H), 1.08 – 0.98 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3, 169.6, 160.3, 143.6, 140.1, 135.1, 125.4, 125.0, 124.0, 123.6, 123.2, 120.8, 120.5, 114.3, 53.1, 40.1, 25.1, 23.0, 22.2. HRMS (ESI) calcd for $C_{19}H_{20}Cl_2N_3O_5$, 440.0780 $(M + H)^+$; found, 440.0774.

5-Chloro-*N*-((2*S*,3*R*)-1-((2-chloro-4-nitrophenyl)amino)-3-methyl-1-oxopentan-2-yl)-2-hydroxybenzamide (62). Compound **62** was prepared by a procedure similar to that used to prepare compound **56** starting from 2-chloro-4-nitroanilin, Fmoc-*L*-Ile-OH and 5-chloro-2-methoxybenzoic acid. The corresponding intermediate (2*S*,3*R*)-2-amino-*N*-(2-chloro-4-nitrophenyl)-3-methylpentanamide **55g** was obtained as a yellow solid (1.3 g, 78% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.65 (s, 1H), 8.57 (d, *J* = 9.3 Hz, 1H), 8.05 (d, *J* = 2.4 Hz, 1H), 7.94 (dd, *J* = 9.3, 2.7 Hz, 1H), 3.41 (d, *J* = 3.3 Hz, 1H), 2.09 – 1.96 (m, 1H), 1.36 – 1.20 (m, 1H), 1.19 – 1.00 (m, 1H), 0.93 (d, *J* = 6.9 Hz, 3H), 0.79 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.6, 142.3, 140.3, 124.4, 123.1, 122.5, 119.0, 60.3, 37.9, 23.7, 16.0, 11.8.

Compound **62** was obtained as an off-white solid (170 mg, 72% in two steps). HPLC purity 98.7% (t_R = 19.38 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.75 (s, 1H), 8.65 (d, *J* = 9.0 Hz, 1H), 8.42 (s, 1H), 8.32 (d, *J* = 2.1 Hz, 1H), 8.19 (dd, *J* = 9.0, 2.7 Hz, 1H), 7.44 – 7.35 (m, 2H), 6.96 (d, *J* = 8.7 Hz, 1H), 6.88 (d, *J* = 7.5 Hz, 1H), 4.66 (t, *J* = 7.5 Hz, 1H), 2.25 – 2.10 (m, 1H), 1.79 – 1.62 (m, 1H), 1.45 – 1.27 (m, 1H), 1.10 (d, *J* = 6.6 Hz, 3H), 1.03 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$+CD_3OD) δ 170.5, 168.7, 158.5, 143.6, 140.0, 134.2, 127.4, 125.0, 124.2,

123.7, 123.3, 121.4, 119.4, 116.1, 59.2, 36.0, 25.1, 15.8, 10.9. HRMS (ESI) calcd for $C_{19}H_{20}Cl_2N_3O_5$, 440.0780 $(M + H)^+$; found, 440.0773.

(*S*)-5-Chloro-*N*-(1-((2-chloro-4-nitrophenyl)amino)-4-(methylthio)-1-oxobutan-2-yl)-2-hydroxybenzamide (63). Compound **63** was prepared by a procedure similar to that used to prepare compound **56** starting from 2-chloro-4-nitroanilin, Fmoc-*L*-Met-OH and 5-chloro-2-methoxybenzoic acid. The corresponding intermediate (*S*)-2-amino-*N*-(2-chloro-4-nitrophenyl)-4-(methylthio)butanamide **55h** was obtained as ayellow solid (1.53 g, 87% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.73 (s, 1H), 8.73 (d, *J* = 9.0 Hz, 1H), 8.28 (d, *J* = 2.4 Hz, 1H), 8.14 (dd, *J* = 9.0, 2.4 Hz, 1H), 3.82 – 3.68 (m, 1H), 2.78 – 2.59 (m, 2H), 2.42 – 2.27 (m, 1H), 2.13 (s, 3H), 1.97 – 1.71 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7, 143.0, 140.5, 124.9, 123.7, 123.0, 119.6, 55.0, 33.5, 30.8, 15.4.

Compound **63** was obtained as a grey solid (130 mg, 73% in two steps). HPLC purity 99.1% (t_R = 19.08 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.76 (s, 1H), 9.02 (s, 1H), 8.65 (d, *J* = 9.3 Hz, 1H), 8.31 (d, *J* = 2.4 Hz, 1H), 8.18 (dd, *J* = 9.3, 2.4Hz, 1H), 7.50 – 7.36 (m, 3H), 6.97 (d, *J* = 9.0 Hz, 1H), 5.11 – 5.01 (m, 1H), 2.92 – 2.79 (m, 1H), 2.77 – 2.65 (m, 1H), 2.38 – 2.28 (m, 2H), 2.21 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.6, 169.5, 160.5, 143.7, 140.0, 135.2, 125.6, 125.1, 124.0, 123.6, 123.3, 120.8, 120.6, 114.4, 53.7, 30.5, 29.8, 15.5. HRMS (ESI) calcd for $C_{18}H_{18}Cl_2N_3O_5S$, 458.0344 $(M + H)^+$; found, 458.0340.

(*S*)-*tert*-Butyl 4-(5-chloro-2-hydroxybenzamido)-5-((2-chloro-4-nitrophenyl)amino)-5-oxopentanoate (64). To a solution of 2-chloro-4-nitroanilin (300 mg, 1.7 mmol) and Fmoc-Glu(O^{t}Bu)-OH (962 mg, 2.3 mmol) in 30 mL of toluene was added PCl_3 (310 mg, 2.3 mmol). The resulting mixture was stirred at 80 °C for 1 h. The mixture was diluted with 150 mL of DCM, washed with water (50 mL), dried (Na_2SO_4) and concentrated to give the intermediate.

The intermediate was dissolved in 20 mL of CH_3CN, and piperidine (254 mg, 3.0 mmol) was added. The mixture was stirred at r.t. overnight, and then concentrated. The residue was purified by column chromatography (Hex/EtOAc = 5/1 to 3/1) to give (*S*)-*tert*-butyl 4-amino-5-((2-chloro-4-nitrophenyl)amino)-5-oxopentanoate **55i** (380 mg, 61% in two steps) as yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.67 (s, 1H), 8.69 (d, *J* = 9.3 Hz, 1H), 8.23 (d, *J* = 2.7 Hz, 1H), 8.10 (dd, *J* = 9.3, 2.7 Hz, 1H), 3.58 (dd, *J* = 7.8, 4.8 Hz, 1H), 2.46 – 2.36 (m, 2H), 2.32 – 2.18 (m, 1H), 1.98 – 1.66 (m, 3H), 1.42 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.6, 172.5, 142.9, 140.5, 124.8, 123.6, 122.9, 119.6, 81.0, 55.6, 32.1, 29.9, 28.1 (3C).

To a solution of amine **55i** (100 mg, 0.28 mmol), 5-chloro-2-hydroxybenzoic acid (48 mg, 0.28 mmol) and DMAP (7 mg, 0.056 mmol) in 15 mL of DCM was added EDCI (107 mg, 0.56 mmol) at 0 °C. The resulting mixture was stirred at r.t. for 24 h and then concentrated. The residue was purified by preparative TLC to give compound **64** (27 mg, 18%) as a yellow solid. HPLC purity 98.4% (t_R = 19.94 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.94 (s, 1H), 9.32 (s, 1H), 8.62 (d, *J* = 9.3 Hz, 1H), 8.49 (d, *J* = 5.7 Hz, 1H), 8.28 (d, *J* = 2.4 Hz, 1H), 8.14 (dd, *J* = 9.3, 2.4 Hz, 1H), 7.62 (d, *J* = 2.1 Hz, 1H), 7.36 (dd, *J* = 9.0, 2.1 Hz, 1H), 6.94 (d, *J* = 8.7 Hz, 1H), 4.81 – 4.69 (m, 1H), 2.85 – 2.71 (m, 1H), 2.58 – 2.44 (m, 1H), 2.39 – 2.19 (m, 2H), 1.49 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.2, 170.3, 169.7, 160.5, 143.5, 140.3, 135.0, 126.0, 125.0, 124.0, 123.5, 123.3, 120.8, 120.3, 114.3, 82.9, 55.0, 32.0, 28.3 (3C), 25.6. HRMS (ESI) calcd for $C_{22}H_{24}Cl_2N_3O_7$, 512.0991 $(M + H)^+$; found, 512.0988.

(*S*)-*N*-(1-((3,5-Bis(trifluoromethyl)phenyl)amino)-3-methyl-1-oxobutan-2-yl)-5-chloro-2-hydroxybenzamide (65). Compound **65** was prepared by a procedure similar to that used to prepare compound **56** starting from 3,5-bis(trifluoromethyl)aniline, Fmoc-*L*-Val-OH and 5-chloro-2-methoxybenzoic acid. The corresponding intermediate (*S*)-2-amino-*N*-(3,5-

bis(trifluoromethyl)phenyl)-3-methylbutanamide **55j** was obtained as pale yellow oil (1.4 g, 94% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.01 (s, 1H), 8.12 (s, 2H), 7.56 (s, 1H), 3.40 (d, *J* = 3.5 Hz, 1H), 2.54 – 2.37 (m, 1H), 1.55 (s, 2H), 1.05 (d, *J* = 6.9 Hz, 3H), 0.87 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.4, 139.4, 132.5 (q, *J* = 33.2 Hz, 2C), 123.3 (q, *J* = 271.1 Hz, 2C), 119.1 (q, *J* = 3.2 Hz, 2C), 117.2 (m), 60.5, 30.8, 19.8, 16.0.

Compound **65** was obtained as a yellow solid (148 mg, 62% in two steps). HPLC purity 99.5% (t_R = 19.90 min). ^{1}H NMR (300 MHz, CD_3OD+$CDCl_3$) δ 10.50 (s, 1H), 8.80 (d, *J* = 8.1 Hz, 1H), 8.15 (s, 2H), 7.89 (d, *J* = 2.1 Hz, 1H), 7.54 (s, 1H), 7.27 (dd, *J* = 8.7, 2.1 Hz, 1H), 6.86 (d, *J* = 9.0 Hz, 1H), 2.33 – 2.17 (m, 1H), 1.13 – 1.01 (m, 6H). ^{13}C NMR (75 MHz, CD_3OD+$CDCl_3$) δ 172.0, 167.6, 157.4, 140.5, 133.9, 132.7 (q, *J* = 33.2 Hz, 2C), 129.6, 125.0, 123.8 (q, *J* = 271.6 Hz, 2C), 120.2 (q, *J* = 3.5 Hz, 2C), 119.1, 118.6, 117.6 (m), 60.4, 31.9, 19.6, 18.6. HRMS (ESI) calcd for $C_{20}H_{18}ClF_6N_2O_3$, 483.0910 $(M + H)^+$; found, 483.0907.

(*S*)-5-Chloro-*N*-(1-((2-chloro-4-(trifluoromethyl)phenyl)amino)-3-methyl-1-oxobutan-2-yl)-2-hydroxybenzamide (66). Compound **66** was prepared by a procedure similar to that used to prepare compound **56** starting from 2-chloro-4-(trifluoromethyl)aniline, Fmoc-*L*-Val-OH and 5-chloro-2-methoxybenzoic acid. The corresponding intermediate (*S*)-2-amino-*N*-(2-chloro-4-(trifluoromethyl)phenyl)-3-methylbutanamide **55k** was obtained as a pale yellow solid (1.3 g, 86% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.47 (s, 1H), 8.67 (d, *J* = 8.7 Hz, 1H), 7.63 (s, 1H), 7.52 (d, *J* = 8.7 Hz, 1H), 3.45 (d, *J* = 3.3 Hz, 1H), 2.52 – 2.39 (m, 1H), 1.55 (s, 2H), 1.06 (d, *J* = 6.9 Hz, 3H), 0.89 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.4, 137.9, 126.3 (q, *J* = 3.9 Hz), 126.2 (q, *J* = 33.3 Hz), 125.0 (q, *J* = 3.8 Hz), 123.6 (q, *J* = 270.2 Hz), 123.1, 120.5, 61.0, 30.9, 19.8, 16.1.

Compound **66** was obtained as a pale yellow solid (142 mg, 71%). HPLC purity 99.7% (t_R = 19.56 min). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.82 (s, 1H), 8.53 (d, *J* = 8.7 Hz, 1H), 8.21 (s, 1H), 7.68 (s, 1H), 7.56 (d, *J* = 9.0 Hz, 1H), 7.44 (d, *J* = 2.1 Hz, 1H), 7.37 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.00 – 6.91 (m, 2H), 4.66 – 4.57 (m, 1H), 2.45 – 2.31 (m, 1H), 1.17 – 1.09 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.4, 169.3, 160.3, 137.0, 134.9, 127.5 (q, *J* = 33.6 Hz), 126.6 (q, *J* = 3.8 Hz), 125.4, 125.2 (q, *J* = 3.7 Hz), 123.9, 123.3 (q, *J* = 270.3 Hz), 123.3, 121.6, 120.4, 114.7, 59.9, 31.2, 19.4, 18.6. HRMS (ESI) calcd for $C_{19}H_{18}Cl_2F_3N_2O_3$, 449.0647 $(M + H)^+$; found, 449.0641.

(*S*)-5-Chloro-*N*-(1-((3-fluoro-5-(trifluoromethyl)phenyl)amino)-3-methyl-1-oxobutan-2-yl)-2-hydroxybenzamide (67). Compound **67** was prepared by a procedure similar to that used to prepare compound **56** starting from 3-amino-5-fluorobenzotrifluoride, Fmoc-*L*-Val-OH and 5-chloro-2-methoxybenzoic acid. The corresponding intermediate (*S*)-2-amino-*N*-(3-fluoro-5-(trifluoromethyl)phenyl)-3-methylbutanamide **55l** was obtained as pale yellow oil (1.2 g, 89% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.87 (s, 1H), 7.81 (d, *J* = 10.5 Hz, 1H), 7.51 (s, 1H), 7.03 (d, *J* = 8.1 Hz, 1H), 3.38 (d, *J* = 3.3 Hz, 1H), 2.52 – 2.37 (m, 1H), 1.52 (s, 2H), 1.04 (d, *J* = 6.9 Hz, 3H), 0.86 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.2, 163.0 (d, *J* = 245.4 Hz), 140.2 (d, *J* = 11.3 Hz), 132.8 (qd, *J* = 33.3, 9.4 Hz), 123.3 (qd, *J* = 271.0, 3.3 Hz), 111.8 (m), 109.9 (d, *J* = 25.7 Hz), 107.9 (dq, *J* = 24.8, 3.8 Hz), 60.5, 30.8, 19.8, 16.0.

Compound **67** was obtained as an off-white solid (150 mg, 78%). HPLC purity 99.8% (t_R = 19.42 min). ^{1}H NMR (300 MHz, CD_3OD+$CDCl_3$) δ 10.30 (s, 1H), 8.71 (d, *J* = 8.1 Hz, 1H), 7.86 (d, *J* = 2.4 Hz, 1H), 7.72 – 7.60 (m, 2H), 7.25 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.01 (d, *J* = 8.4 Hz, 1H), 6.84 (d, *J* = 9.0 Hz, 1H), 4.57 – 4.48 (m, 1H), 2.31 – 2.15 (m, 1H), 1.09 – 0.99 (m, 6H). ^{13}C NMR (75 MHz, CD_3OD+$CDCl_3$) δ 171.7, 167.6, 163.1 (d, *J* = 245.0 Hz), 157.4, 141.0 (d, *J* =

11.0 Hz), 133.8, 133.0 (qd, *J* = 33.2, 9.3 Hz), 129.3, 124.9, 123.7 (qd, *J* = 270.5, 3.4 Hz), 119.0, 118.3, 112.7 (m), 110.7 (d, *J* = 26.0 Hz), 108.3 (dq, *J* = 24.8, 3.8 Hz), 60.3, 31.8, 19.6, 18.6. HRMS (ESI) calcd for $C_{19}H_{18}ClF_4N_2O_3$, 433.0942 $(M + H)^+$; found, 433.0934.

(*S*)-5-Chloro-*N*-(1-((3,4-difluorophenyl)amino)-3-methyl-1-oxobutan-2-yl)-2-hydroxybenzamide (68). Compound **68** was prepared by a procedure similar to that used to prepare compound **56** starting from 3,4-difluoroaniline, Fmoc-*L*-Val-OH and 5-chloro-2-methoxybenzoic acid. The corresponding intermediate (*S*)-2-amino-*N*-(3,4-difluorophenyl)-3-methylbutanamide **55m** was obtained as yellow oil (1.25 g, 69% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.55 (s, 1H), 7.74 – 7.64 (m, 1H), 7.18 – 6.99 (m, 2H), 3.34 (d, *J* = 3.9 Hz, 1H), 2.50 – 2.33 (m, 1H), 1.48 (s, 2H), 1.03 (d, *J* = 6.9 Hz, 3H), 0.86 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.8, 150.3 (dd, *J* = 245.1, 13.1 Hz), 146.9 (dd, *J* = 243.2, 12.8 Hz), 134.6 (dd, *J* = 8.8, 3.1 Hz), 117.2 (dd, *J* = 18.1, 1.2 Hz), 115.1 (dd, *J* = 5.8, 3.6 Hz), 109.2 (d, *J* = 21.5 Hz), 60.5, 30.9, 19.8, 16.1.

Compound **68** was obtained as a white solid (200 mg, 76%). HPLC purity 99.6% (t_R = 18.03 min). ^{1}H NMR (300 MHz, CD_3OD+$CDCl_3$) δ 9.99 (s, 1H), 8.66 (d, *J* = 7.8 Hz, 1H), 7.82 (d, *J* = 2.4 Hz, 1H), 7.66 – 7.54 (m, 1H), 7.26 – 6.95 (m, 3H), 6.81 (d, *J* = 8.7 Hz, 1H), 4.54 – 4.44 (m, 1H), 2.30 – 2.12 (m, 1H), 1.03 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (75 MHz, CD_3OD+$CDCl_3$) δ 170.7, 167.1, 156.9, 149.7 (dd, *J* = 244.7, 13.1 Hz), 146.8 (dd, *J* = 243.5, 12.8 Hz), 134.3 (dd, *J* = 8.6, 3.2 Hz), 133.2, 128.4, 124.2, 118.5, 117.3, 116.8 (d, *J* = 18.1 Hz), 115.7 (dd, *J* = 5.9, 3.6 Hz), 109.5 (d, *J* = 21.7 Hz), 59.6, 31.1, 18.9, 18.1. HRMS (ESI) calcd for $C_{18}H_{18}ClF_2N_2O_3$, 383.0974 $(M + H)^+$; found, 383.0966.

(*S*)-*N*-(1-((3,5-Bis(trifluoromethyl)phenyl)amino)-1-oxo-3-phenylpropan-2-yl)-5-chloro-2-hydroxybenzamide (69). Compound **69** was prepared by a procedure similar to that

used to prepare compound **56** starting from 3,5-bis(trifluoromethyl)aniline, Fmoc-*L*-Phe-OH and 5-chloro-2-methoxybenzoic acid. The corresponding intermediate (*S*)-2-amino-*N*-(3,5-bis(trifluoromethyl)phenyl)-3-phenylpropanamide **55n** was obtained as a white solid (1.63 g, 99% in two steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.89 (s, 1H), 8.11 (s, 2H), 7.60 (s, 1H), 7.38 – 7.27 (m, 3H), 7.26 – 7.21 (m, 2H), 3.77 (dd, *J* = 9.3, 3.9 Hz, 1H), 3.37 (dd, *J* = 13.8, 4.0 Hz, 1H), 2.83 (dd, *J* = 13.8, 9.3 Hz, 1H), 1.56 (s, 2H).^{13}C NMR (75 MHz, $CDCl_3$) δ 173.2, 139.2, 137.2, 132.4 (q, *J* = 33.2 Hz, 2C), 129.4 (2C), 129.1 (2C), 127.3, 123.3 (q, *J* = 271.0 Hz, 2C), 119.2 (q, *J* = 2.9 Hz, 2C), 117.4 (m), 56.7, 40.5.

Compound **69** was obtained as a pale yellow solid (240 mg, 65%). HPLC purity 99.5% (t_R = 20.17 min). ^{1}H NMR (300 MHz, $CDCl_3$+CD_3OD) δ 8.04 (s, 2H), 7.86 – 7.83 (m, 1H), 7.58 (s, 1H), 7.33 – 7.20 (m, 6H), 6.87 (d, *J* = 9.0 Hz, 1H), 5.02 – 4.92 (m, 1H), 3.34 – 3.14 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$+CD_3OD) δ 171.2, 167.5, 157.6, 139.8, 136.4, 133.9, 132.4 (q, *J* = 33.2 Hz, 2C), 129.5 (2C), 128.9 (3C), 127.4, 124.6, 123.5 (q, *J* = 270.8 Hz, 2C), 120.1 (q, *J* = 3.2 Hz, 2C), 119.0, 117.6, 117.6, 56.2, 38.7. HRMS (ESI) calcd for $C_{24}H_{18}ClF_6N_2O_3$, 531.0910 $(M + H)^+$; found, 531.0909.

(*S*)-5-Chloro-*N*-(1-((2-chloro-4-nitrophenyl)amino)-3-methyl-1-oxobutan-2-yl)-2-(methylsulfonamido)benzamide (70). To a solution of (*S*)-2-amino-*N*-(2-chloro-4-nitrophenyl)-3-methylbutanamide **55c** (50 mg, 0.23 mmol), 5-chloro-2-(methylsulfonamido)benzoic acid **23** (63 mg, 0.25 mmol) and DIEA (59 mg, 0.46 mmol) in 10 mL of DCM was added HBTU (175 mg, 0.46 mmol) at 0 °C. The resulting mixture was stirred at r.t. overnight, and then concentrated. The residue was purified by preparative TLC (DCM/MeOH) to give compound **70** as pale yellow solid (81 mg, 70%). HPLC purity 98.0% (t_R = 18.57 min). ^{1}H NMR (300 MHz, $CDCl_3$+CD_3OD) δ 8.40 (d, *J* = 9.0 Hz, 1H), 8.28 (d, *J* = 2.4 Hz, 1H), 8.12 (dd, *J* = 9.3, 2.4 Hz,

1H), 7.78 (d, *J* = 2.1 Hz, 1H), 7.60 (d, *J* = 9.0 Hz, 1H), 7.48 – 7.42 (m, 1H), 4.52 (d, *J* = 8.1 Hz, 1H), 2.99 (s, 3H), 2.42 – 2.28 (m, 1H), 1.12 – 1.03 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$+CD_3OD) δ 171.1, 168.7, 144.2, 140.6, 137.6, 133.3, 129.6, 128.8, 125.4, 124.9, 123.4, 122.8 (2C), 122.1, 61.1, 39.9, 30.3, 19.7, 19.0. HRMS (ESI) calcd for $C_{19}H_{21}Cl_2N_4O_6S$, 503.0559 $(M + H)^+$; found, 503.0553.

(*S*)-5-Chloro-N-(1-((2-chloro-4-nitrophenyl)amino)-1-oxo-3-phenylpropan-2-yl)-2-(methylsulfonamido)benzamide (71). Compound **71** was prepared by a procedure similar to that used to prepare compound **70** starting from (*S*)-2-amino-*N*-(2-chloro-4-nitrophenyl)-3-phenylpropanamide **55d** and 5-chloro-2-(methylsulfonamido)benzoic acid **23**. The title compound was obtained as a pale yellow solid (80 mg, 77%). HPLC purity 98.6% (t_R = 19.21 min). ^{1}H NMR (300 MHz, CD_3OD) δ 8.42 (d, *J* = 9.3 Hz, 1H), 8.25 (d, *J* = 2.4 Hz, 1H), 8.11 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.63 (d, *J* = 2.1 Hz, 1H), 7.55 (d, *J* = 1.8 Hz, 1H), 7.44 (dd, *J* = 8.7, 2.4 Hz, 1H), 7.32 – 7.19 (m, 5H), 5.04 (dd, *J* = 8.7, 6.9 Hz, 1H), 3.37 (dd, *J* = 13.8, 6.9 Hz, 1H), 3.17 (dd, *J* = 13.8, 8.7 Hz, 1H), 2.90 (s, 3H). ^{13}C NMR (75 MHz, CD_3OD) δ 171.1, 168.9, 144.3, 140.8, 137.7, 137.1, 133.4, 129.8, 129.7 (2C), 129.3 (2C), 129.0, 127.7, 125.5, 124.8, 123.6, 123.1, 122.6, 122.5, 56.8, 39.9, 37.2. HRMS (ESI) calcd for $C_{23}H_{21}Cl_2N_4O_6S$, 551.0559 $(M + H)^+$; found, 551.0557.

5-Chloro-*N*-(2-((2-chloro-4-nitrophenyl)amino)-2-oxoethyl)-2-(methylsulfonamido)benzamide (72). Compound **72** was prepared by a procedure similar to that used to prepare compound **70** starting from 2-amino-*N*-(2-chloro-4-nitrophenyl)acetamide **55a** and 5-chloro-2-(methylsulfonamido)benzoic acid **23**. The title compound was obtained as a pale yellow solid (54 mg, 60%). HPLC purity 98.5% (t_R = 17.14 min). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.74 (s, 1H), 10.03 (s, 1H), 9.42 (t, *J* = 5.1 Hz, 1H), 8.38 (d, *J* = 1.2 Hz, 1H), 8.29

– 8.19 (m, 2H), 7.97 (d, J = 1.8 Hz, 1H), 7.65 (dd, J = 9.0, 2.1 Hz, 1H), 7.57 (d, J = 9.0 Hz, 1H), 4.26 (d, J = 5.4 Hz, 2H), 3.14 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.3, 167.5, 143.4, 140.8, 137.5, 132.5, 128.3, 127.2, 125.0, 124.5, 123.6, 123.2, 122.0, 121.4, 43.5, 39.9. HRMS (ESI) calcd for $C_{16}H_{15}Cl_2N_4O_6S$, 461.0089 $(M + H)^+$; found, 461.0085.

Plaque Assay. Compounds were tested using low MOI infections (0.06 vp/cell) and at concentrations of 10 μM and in a dose−response assay ranging from 10 to 0.375 μM in a plaque assay. Briefly, 293β5 cells were seeded in 6-well plates at a density of 4×10^5 cells per well in duplicate for each condition. When cells reached 80-90% confluency, they were infected with HAdV5-GFP (0.06 vp/cell) and rocked for 2 h at 37 °C. After the incubation the inoculum was removed, and the cells were washed once with PBS. The cells were then carefully overlaid with 4 mL/well of equal parts of 1.6% (water/vol) Difco Agar Noble (Becton, Dickinson & Co., Sparks, MD) and 2× EMEM (Minimum Essential Medium Eagle, BioWhittaker) supplemented with 2×penicillin/streptomycin, 2× *L*-glutamine, and 10% FBS. The mixture also contained the drugs in concentrations ranging from 10 to 0.375 μM. Following incubation for 7 days at 37 °C, plates were scanned with a Typhoon FLA 9000 imager (GE Healthcare Life Sciences) and plaques were quantified with ImageJ.[61]

Entry Assay. The anti-HAdV activity was measured in an entry assay using human A549 epithelial cells (3×10^5 cells/well in corning black wall, clear bottom 96-well plates) infected with HAdV5-GFP (2000 vp/cell) in the presence 50 μM of the candidates and in a dose-response assay. A standard infection curve was generated in parallel by infecting cells in the absence of compounds using serial 2-fold dilutions of virus. All reactions were done in triplicate. Cells, virus, and drugs were incubated for 48 h at 37 °C and 5% CO_2. Infection, as measured by

HAdV5-mediated GFP expression, was analyzed using a Typhoon 9410 imager (GE Healthcare Life Sciences) and quantified with ImageQuantTL (GE Healthcare Life Sciences).

Cytotoxicity Assay. The cytotoxicity of the compounds was analyzed by commercial kit AlamarBlue® (Invitrogen, Ref. DAL1025). A549 cells at a density of 5×10^3 cells per well in 96-well plates were seeded. Decreasing concentrations of each derivative (200 μM, 150 μM, 100 μM, 80 μM, 60 μM, 40 μM, 30 μM, 20 μM, 10 μM, 5 μM, 2.5 μM, 0 μM) were diluted in 100 μL of Dulbecco's Modified Eagle Medium (DMEM). Cells were then incubated at 37 °C for 48 h following the kit protocol. The cytotoxic concentration 50 (CC_{50}) value was obtained using the statistical package GraphPad Prism. This assay was performed in duplicate.

Virus Yield Reduction. A549 cells (1.5×10^5 cells/well in a 24-well plate) were incubated 24 h in 500 μL of complete DMEM and they were infected with wild-type HAdV5 (100 vp/cell) when more than 90% of confluency were observed. Infected cells were incubated 48 h at 37 °C in 500 μL of complete DMEM containing 25 μM of either compounds or the same volume of DMSO (positive control). After 48 h, cells were harvested and subjected to three rounds of freeze/thaw. Serial dilutions of clarified lysates were titrated on A549 cells (3×10^4 cells/well), and $TCID_{50}$ values were calculated using an end-point dilution method (Reed and Muench, 1938).

Time of Addition Curve Study. The anti-HAdV effect of derivatives at different points of time was measured in a time-curve assay using 293β5 cells (3×10^5 cells/well in corning black wall, clear bottom 96-well plates) infected with HAdV5-GFP (2.000 vp/cell) in the presence of different concentration of either derivatives (according to their CC_{50} value) or the same volume of DMSO (positive control). Parallel samples of HAdV-5 were incubated with or without the selected derivatives on ice for 1 h. Virus was then added to 293β5 cells and incubated at 37 °C.

The derivatives were added at the indicated time points before or during this incubation. After a total of 2 h at 37 °C, cells were incubated for an additional 48 h at 37 °C and 5% CO_2 before being analyzed for GFP expression using the Typhoon 9410 imager (GE Healthcare Life Sciences) as above.

Nuclear-Associated HAdV Genomes. The nuclear delivery of HAdV genomes was assessed by real-time PCR following nuclear isolation from infected cells. 1×10^6 A549 cells in 6-well plates were infected with wild-type HAdV5 at MOI 2,000 vp/cell in the presence of 50 μM of the derivates, or the same volume of DMSO for positive control. Forty-five minutes after infection, A549 cells were trypsinized and collected and then washed twice with PBS. Then, cytoplasmic and nuclear fractions were separated using a hypotonic buffer solution and NP-40 detergent. The cell pellet was resuspended in 500 μL of 1×hypotonic buffer (20 mM Tris-HCl pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$) and incubated for 15 min at 4 °C. Then, 25 μL of NP-40 was added and the samples were vortexed. The homogenates were centrifuged for 10 min at 835g at 4 °C. Following the removal of the cytoplasmic fraction (supernatant), HAdV DNA was isolated from the nuclear fraction (pellet) and from the cytoplasmic fraction using the QIAamp DNA Mini Kit (QIAGEN, Valencia, CA).

DNA Quantification by Real-Time PCR. A549 cells (1.5×10^5 cells/well in a 24-well plate) were incubated 24 h in 500 μL of complete DMEM and they were infected with wild-type HAdV5 (100 vp/cell) when more than 90% of confluency were observed. Infected cells were incubated 24 h at 37 °C in 500 μL of complete DMEM containing 25μM of either compounds or the same volume of DMSO (positive control). All samples were done in duplicate. After 24 h of incubation at 37 °C, DNA was purified from the cell lysate with the QIAamp DNA Mini Kit (QIAGEN, Valencia, CA) following the manufacturer's instructions. TaqMan primers and

probes for a common region of the HAdV5 were designed with the GenScript Real-Time PCR (TaqMan) Primer Design software (GenScript). Oligonucleotides sequences were: AQ1: 5'-GCC ACG GTG GGG TTT CTA AAC TT-3'; AQ2: 5'-GCC CCA GTG GTC TTA CAT GCA CAT-3'; Probe: 6-FAM-5'-TGC ACC AGA CCC GGG CTC AGG TAC TCC GA-3'-TAMRA. Real-time PCR mixtures consisted of 9.5 μL of the purified DNA, AQ1 and AQ2 at a concentration of 200 nM each and Probe at a concentration of 50 nM in a total volume of 25 μL. The PCR cycling protocol was 95 °C for 3 min followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s. Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as internal control. Oligonucleotides sequences for GAPDH and conditions were those previously reported by Henke-Gendo et al. (2012).

For quantification, gene fragments from hexon, and GAPDH were cloned into the pGEM-T Easy vector (Promega) and known concentrations of template were used to generate a standard curve in parallel for each experiment. All assays were performed in thermal cycler LightCycler® 96 System (Roche).

Statistical Analyses. One-way ANOVA tests (Dunnet method) were carried out using the GraphPad Prism 6. We considered a statistical significance with a P value under 0.05. This statistical significance was pointed out with asterisk in graphs, and the numbers of them indicate the level of significance (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$).

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

^{1}H, ^{13}C NMR spectra of all new compounds (PDF)

Molecular formulation strings and some data (CSV)

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

HAdVs, human adenoviruses; SAR, structure-activity relationship; allo-HSCT, allogenic hematopoietic stem cell transplant; SOT, solid-organ transplant; BCV, brincidofovir; CMV, cytomegalovirus; HTS, high-throughput screening; HPMPA, 9-(3-hydroxy-2-phosphonyl-methoxy-propyl)-adenine; HIV, human immunodeficiency virus; EC_{50}, half maximal effective concentration; IC_{50}, half maximal inhibitory concentration; CC_{50}, cytotoxic concentration 50%; p.i., post-infection; TLC, thin layer chromatography; UV, ultraviolet; TMS, tetramethylsilane; HRMS, high-resolution mass spectrometry; HPLC, high-performance liquid chromatography; HBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; DIEA, *N*,*N*-diisopropylethylamine; DCM, dichloromethane; DMAP, 4-(dimethylamino)pyridine; DMSO, dimethyl sulfoxide; EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; EtOAc, ethyl acetate

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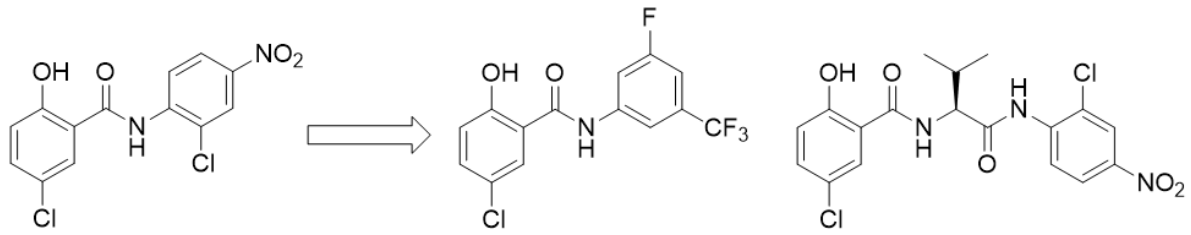
Table of Contents (TOC) Graphic



6 (Niclosamide)

Plaque Assay (HAdV-5):
IC_{50} = 0.6 ± 0.05 μM
A549 cell: CC_{50} = 22.9 ± 9.8 μM
Selectivity Index = 38.2

17 (JMX0312)

Plaque Assay (HAdV-5):
IC_{50} = 0.18 ± 0.01 μM
A549 cell: CC_{50} = 120.0 ± 33.6 μM
Selectivity Index = 666.7

58 (JMX0281)

Plaque Assay (HAdV-5):
IC_{50} = 0.45 ± 0.06 μM
A549 cell: CC_{50} = 200 ± 1.9 μM
Selectivity Index = 444.4