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Synthesis, Biological Evaluation, and Structure–Activity Relationships of 2-[2-(Benzoylamino)benzoylamino]benzoic Acid Analogues as Inhibitors of Adenovirus Replication

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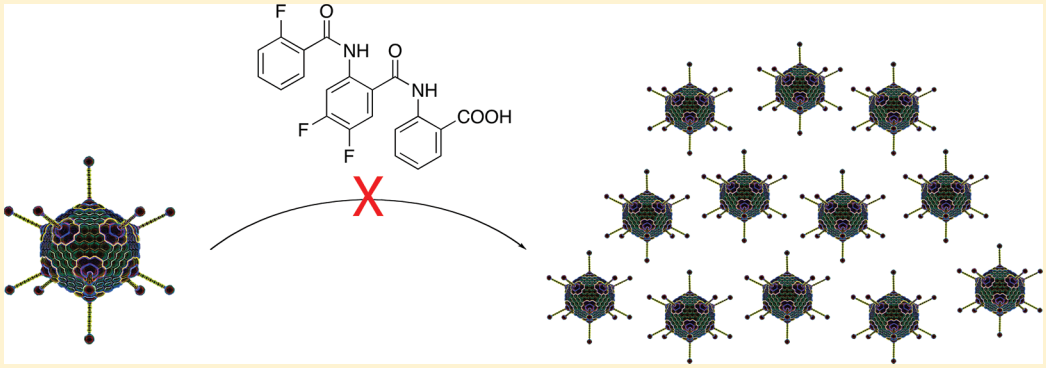
Supporting Information



ABSTRACT: 2-[2-Benzoylamino)benzoylamino]benzoic acid (**1**) was previously identified as a potent and nontoxic antiadenoviral compound (*Antimicrob. Agents Chemother.* **2010**, *54*, 3871). Here, the potency of **1** was improved over three generations of compounds. We found that the *ortho*, *ortho* substituent pattern and the presence of the carboxylic acid of **1** are favorable for this class of compounds and that the direction of the amide bonds (as in **1**) is obligatory. Some variability in the *N*-terminal moiety was tolerated, but benzamides appear to be preferred. The substituents on the middle and C-terminal rings were varied, resulting in two potent inhibitors, **35g** and **35j**, with EC_{50} = 0.6 μM and low cell toxicity.

1. INTRODUCTION

Adenovirus infections are very common and can occur throughout the year. Children in particular are commonly infected in the respiratory tract and alimentary tract, and adenovirus can be considered endemic in the pediatric population. The human adenoviruses have more than 50 different serotypes that fall into seven species, A–G, and all belong to the *Mastadenovirus* genus of the family *Adenoviridae*. Adenoviruses are associated with a wide variety of clinical symptoms in humans such as acute upper respiratory disease, gastroenteritis, hemorrhagic cystitis, and conjunctivitis.[2−4] An infection can result in severe disease, but adenovirus infections are most often self-limited in immunocompetent individuals. In immunocompromised individuals, the situation is quite different. For these people, including patients immunosuppressed for hematopoietic stem cell or solid organ transplantation, AIDS patients, and patients with genetic immunodeficiencies, an adenovirus infection might result in disseminated disease and multiple organ failure.[5,6] Case fatality rates above 50% have been reported for immunocompromised children with adenovirus infections, and in pediatric bone marrow transplant recipients in particular, the incidence of adenovirus infection is higher than that for adult recipients.[5,7] Several different adenovirus serotypes have been isolated from immunocompromised patients, most often from species A, B, or C.[5,8,9] Serotypes from species B are mainly associated with renal syndromes in these patients and species C serotypes with hepatitis. Reports of a species A serotype, Ad31, have increased in recent years, and this serotype often occurs in patients infected with multiple adenovirus serotypes.[10,11]

Today, there are no approved specific antiviral compounds for treatment of adenovirus infections. Various drugs, such as ribavirin, cidofovir, and ganciclovir, have been tested in clinical settings or in animal models with variable results. Of the currently used antiviral agents, cidofovir appears to be most promising against adenovirus infections,[12−16] but the outcome

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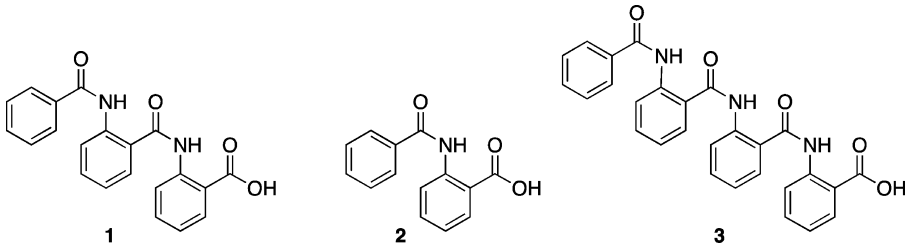
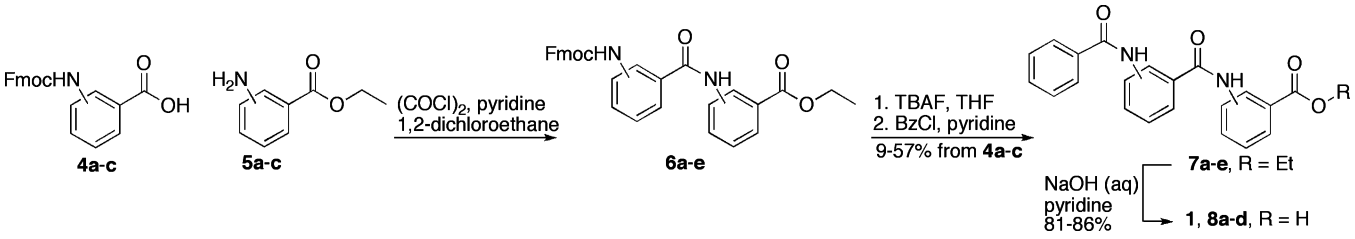
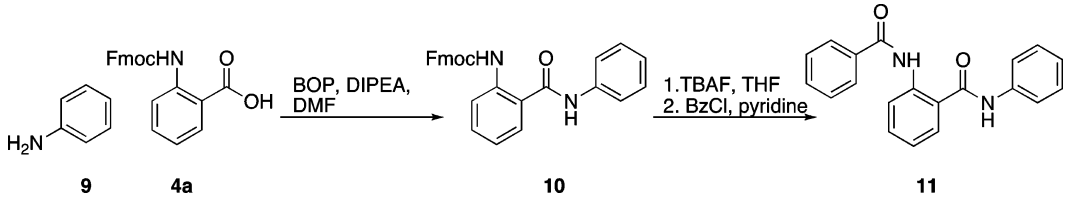


Figure 1. Hit structure (**1**) obtained from screening of a library of 9800 small molecules for inhibition of adenovirus replication, together with the inactive compounds **2** and **3**.[1]

Scheme 1. Synthesis of Esters 7a−e and Acids 1 and 8a−d



Scheme 2. Synthesis of 11



of cidofovir-treated hematopoietic stem cell recipients with adenovirus infections has been found to be poor.[17]

Antiviral compounds to be considered as drug candidates must be assessed in whole cell systems that allow uptake and distribution of the compound to all required cellular compartments. We have therefore assayed the antiadenoviral activity in both heteroploid and normal diploid cells representing epithelial tumors, hematopoietic malignant cells, and diploid human fibroblasts. We selected adenovirus type 5, which is ubiquitous and often used as a representative of this family, and the antiadenoviral activity was assessed in A549 cells throughout the study.

We have previously identified 2-[2-benzoylamino)-benzoylamino]benzoic acid (**1**, Figure 1) as a potent antiadenoviral compound with low cellular toxicity.[1] In the present study, we designed, synthesized, and evaluated three generations of inhibitors, 42 compounds in total. We established structure−activity relationships and identified inhibitors with improved potency.

2. RESULTS AND DISCUSSION

In the quality control of the commercial screening hit compound **1**, the material turned out to be composed of a mixture of **1**, **2**, and **3** (Figure 1). These were separated and re-evaluated as pure compounds. We discovered that only the trimeric structure (**1**) was active, whereas the dimeric (**2**) and the tetrameric (**3**) structures were inactive.[1] Thus, the synthesized and purchased substances that are presented here are all trimeric analogues of **1**.

2.1. Chemistry: First-Generation Compounds. To determine the importance of the *ortho*, *ortho* substituent pattern, **1** was scrambled to give compounds **8a−d** (Scheme 1, Table 1). First, the carboxylic acid of the right-hand ring was moved to the *meta* (**8a**) or *para* (**8b**) position while maintaining the *ortho* pattern on the central ring. Second, the substituents on the central ring were moved to the *meta* (**8c**) and *para* (**8d**) positions while maintaining the *ortho* pattern on the right-hand ring. A general synthetic route to compounds **1** and **8a−d** is presented in Scheme 1. Activation of acids **4a−c** with oxalyl chloride and coupling to anilines **5a−c** gave amides **6a−e**. Subsequent fluoride-mediated removal of the 9-fluorenylmethyloxycarbonyl (Fmoc) group,[18] followed by benzoylation in a one-pot, two-step procedure, afforded esters **7a−e** at 9−57% yield from **4a−c** after HPLC purification (Scheme 1). Portions of the ethyl esters **7a−e** were saponified and the acids **1** and **8a−d** were isolated in 81−86% yield after HPLC purification (Scheme 1).

To determine whether the carboxylic acid moiety of the lead compound **1** was critical for biological activity, the reference compound **11** without the carboxylic acid was synthesized (Scheme 2). The route was essentially as described in Scheme 1, with the difference that the coupling of **9** with **4a** was performed using the coupling reagent (benzotriazol-1-yloxy)-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) to give **10**.

In addition, a small set of commercial compounds, **12a−g**, with additional structural variation, was included in the evaluation (Table 2).

2.2. Biology: First-Generation Compounds. Compounds **1**, **7a−e**, **8a−8d**, **11** (Table 1), and **12a−g** (Table 2) were screened for inhibition of adenoviral replication essentially as described previously.[1] Briefly, A549 cells were infected with

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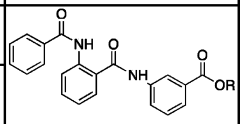
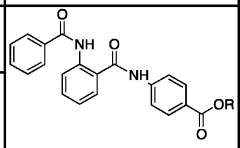
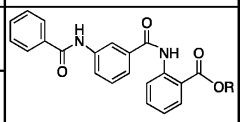
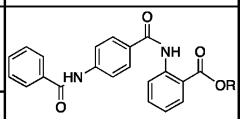
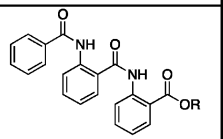
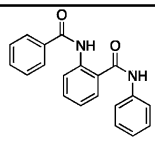
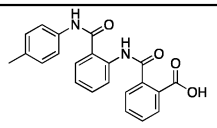
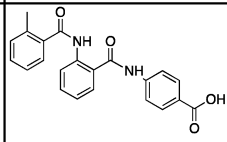
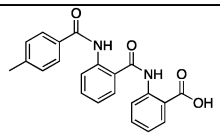
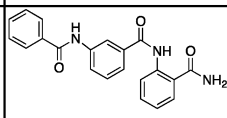
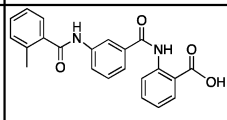
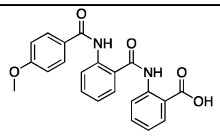
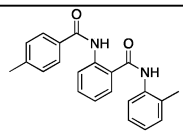
Table 1. Inhibition of Adenovirus 5 Replication with Synthesized Compounds

Compound	Structure	Percentage Inhibition at 15 μM[a]
7a		0[b]
8a		8 ± 3
7b		8 ± 2[b]
8b		8 ± 7
7c		25 ± 10[b]
8c		39 ± 5
7d		15 ± 12
8d		47 ± 5
7e		c
1		96 ± 1[d]
11		18 ± 13[b]

[a]Percentage inhibition of Ad5 replication in A549 cells at a concentration of 15 μM. R denotes ethyl for compounds **7a–e** and H for compounds **1** and **8a–d**. See the Experimental Section for details. [b]Visible precipitation of compound in the assay mixture. [c]No evaluation was done due to poor solubility in DMSO and extremely poor water solubility. [d]Percentage inhibition for the synthesized compound. The commercial compound gave 91 ± 4% inhibition at 15 μM.

Ad5 and incubated with the compound for 24 h. Thereafter, DNA was prepared and quantitative real-time PCR was used to assess the amount of viral DNA synthesized. Compound **1** contains a right-hand ring with *ortho*-connected substituents and a central ring, also with *ortho*-connected substituents. Moving the carboxylic acid moiety to the *meta* (**8a**) or *para* (**8b**) position resulted in a sharp drop in activity. Changing the pattern of substitution on the central ring to *meta* (**8c**) or *para* (**8d**) resulted in reduced activity, although this was less dramatic than with **8a** and **8b**. This result indicates that there appears to be more room for changes in the left-hand part of the molecule. The wiggle room seen in the left-hand part of the

Table 2. Inhibition of Adenovirus 5 Replication with Commercial Compounds

Compound	Structure	Percent Inhibition	
		15 μM[a]	5 μM[a]
12a		0	0
12b		20 ± 10	0
12c		85 ± 4	0
12d		19 ± 7	0
12e		7 ± 5	5 ± 3
12f		67 ± 0.3	0
12g		12 ± 6	0

[a]Percentage inhibition of Ad5 replication in A549 cells at 5 μM and 15 μM. See the Experimental Section for details.

molecule was also evident, because **1**, **12c**, and **12f** showed similar activities (Table 1 and 2). The *ortho*, *ortho* substituent pattern thus appears to be favorable for the [(benzoylamino)-benzoylamino]benzoic acid class of inhibitors of adenovirus replication.

The esters **7a–e** corresponding to the acids **1** and **8a–d** were included to investigate the importance of the carboxylic acid. A comparison of the ester/acid pairs **7c**/**8c** and **7d**/**8d** showed that the acids were more active than the corresponding esters (Table 1). Interestingly, the ester **7c** and the corresponding amide **12d** show similar activity (Table 1 and 2). The preference for acid **8c** does not appear to be a steric effect because the terminal amide in **12d** and the carboxylic acid in **8c** will occupy very similar volumes. When comparing **1** with **8a**, **8b**, **11**, and **12g**, it even appears worse to have a carboxylate in

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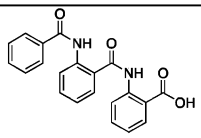
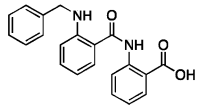
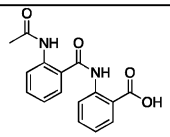
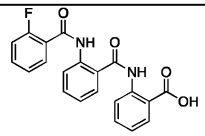
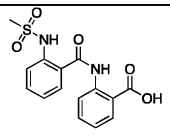
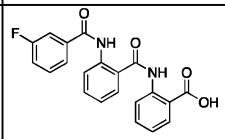
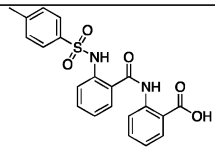
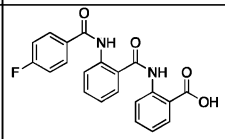
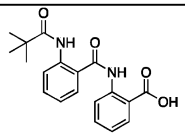
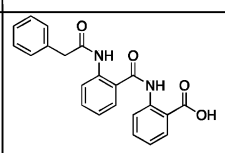
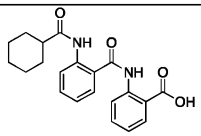
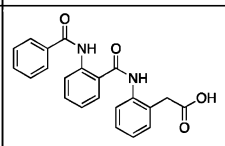
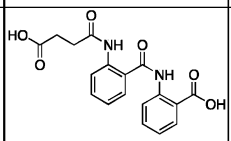
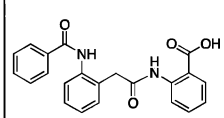
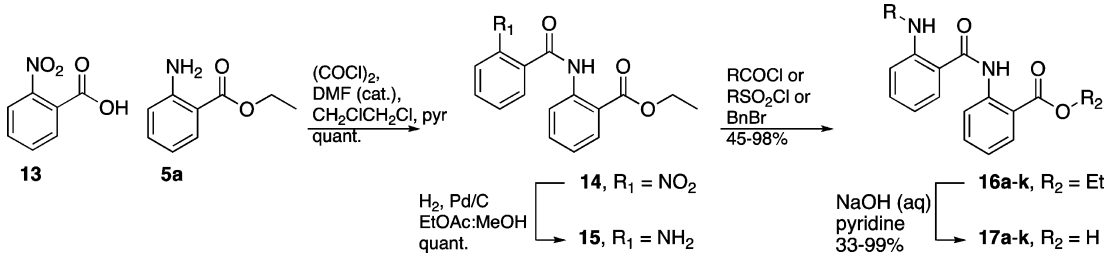
Table 3. Inhibition of Adenovirus 5 Replication with Second-Generation Compounds

Compound	Structure	Percentage inhibition		Compound	Structure	Percentage inhibition	
		15 μM [a]	5 μM [a]			15 μM [a]	5 μM [a]
1		96 ± 1	89 ± 5	**17g**		79 ± 5	44 ± 17
17a		24 ± 14	4 ± 11	**17h**		98 ± 1	96 ± 1
17b		23 ± 13	0	**17i**		n.d.[b]	41 ± 32
17c		8 ± 35	2 ± 24	**17j**		95 ± 1	76 ± 10
17d		52 ± 18	45 ± 7	**17k**		21 ± 12	32 ± 19
17e		55 ± 11	20 ± 1	**23**		0	0
17f		0	0	**28**		0	0

[a]Percentage inhibition of Ad5 replication in A549 cells at 5 and 15 μM. See the Experimental Section for details. [b]n.d. = not determined.

Scheme 3. Synthesis of Acids 17a–k



the *meta* (**8a**) or *para* (**8b**) position than none at all (**11**) or than a methyl group in the *ortho* position (**12g**) (Table 1 and 2). Reversing the direction of the amide bonds, as in going from the potent compound **12c** to **12a**, completely abolished antiviral activity (Table 2).

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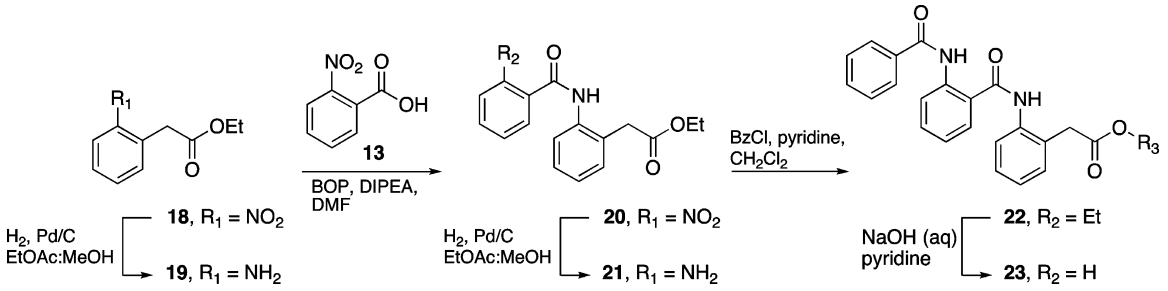
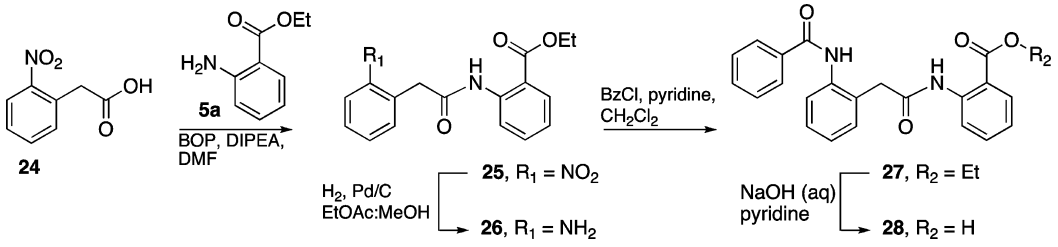
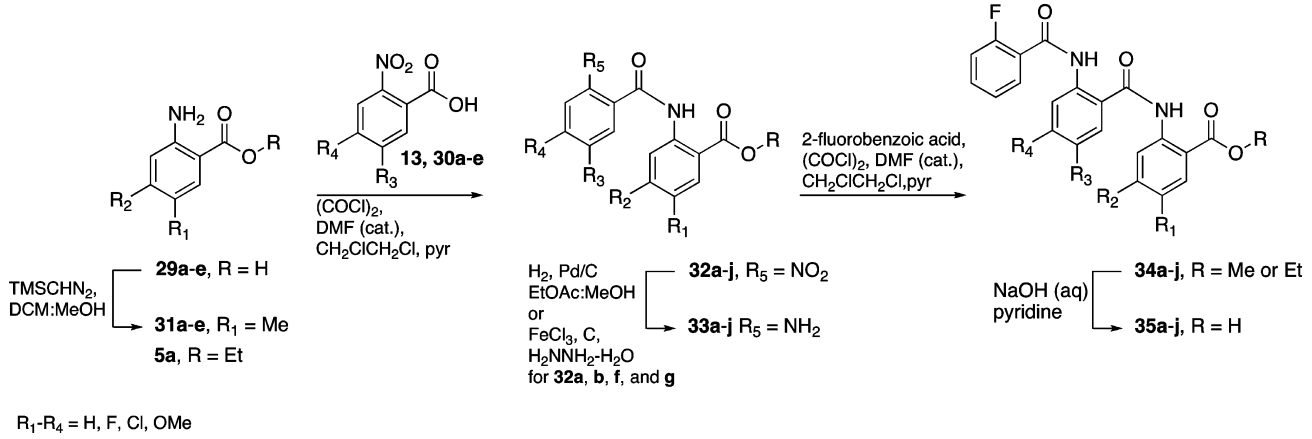
Scheme 4. Synthesis of Acid 23



Scheme 5. Synthesis of Acid 28



Scheme 6. Synthesis of Acids 35a–j



2.3. Chemistry: Second-Generation Compounds. On the basis of the first-generation inhibitors, we concluded that the *ortho*, *ortho* substituent pattern was preferable, that the free acid was beneficial, and that there appeared to be room for variation in the *N*-terminus. As a next step, compounds **17a–k** (Table 3) with variation at the *N*-terminus were designed and synthesized (Scheme 3). A more efficient and high-yielding route than the previous one (Scheme 1) was envisaged using the nitro group of **13** as protected aniline. Fortunately, coupling of **13** with **5a** followed by hydrogenation of the resulting product (**14**) gave **15** in quantative amounts. Compound **15** was subsequently reacted with some selected activated acids, sulfonyl chlorides, succinic anhydride, and benzyl bromide to give **16a–k** in 45–98% yield. The route was concluded with hydrolysis to give **17a–k** in 33–99% yield (Scheme 3, Table 3).

To investigate the presumptive binding pocket further, compounds **23** and **28** were synthesized (Schemes 4 and 5). These compounds and **17k** each contain an additional methylene group compared to the parent compound **1**. The added methylene group gives a slightly longer inhibitor overall and separates the amide–carboxylic acid in **23** and amide–amide in **28**, groups that commonly participate in directed hydrogen bonding to receptors. Apart from using phenylacetic acid, derivatives **18** and **24**, respectively, the route to **23** and **28** followed suit with the one presented in Scheme 3.

2.4. Biology: Second-Generation Inhibitors. Most of the second-generation inhibitors showed lower activity than **1** (Table 3). However, this screening indicated that compound **17h** is more active than **1** and that compound **17j** is of similar potency. Compounds **17d**, **17e**, and **17g** had diminished but not abolished activity, while the other compounds tested showed much lower potency. Elongation of the inhibitor with a methylene unit was unfavorable, regardless of the position of insertion, as seen for **17k**, **23**, and **28**.

2.5. Chemistry: Third-Generation Inhibitors. A further round of optimization to give a third generation of inhibitors was performed around **17h**, the most potent compound from the second generation. Here, the central and C-terminal rings were decorated with electron-donating substituents (OMe) or small and larger electron-withdrawing substituents (F, Cl). The synthetic route (Scheme 6) followed the one outlined for the second generation (Scheme 3) and gave compounds **35a–j** (Table 4). Where amino benzoate esters were not commercially available, a methyl esterification of the corresponding

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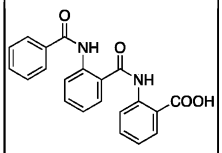
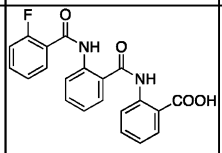
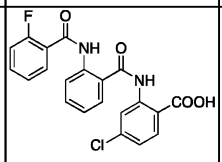
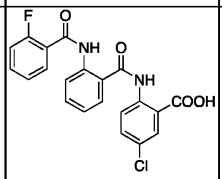
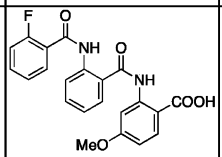
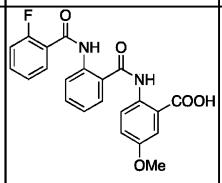
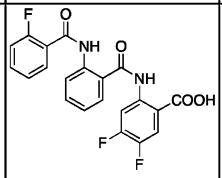
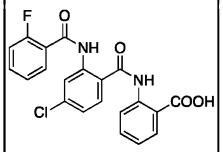
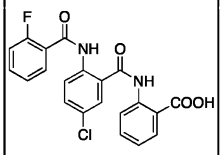
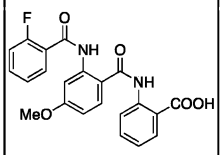
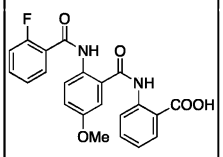
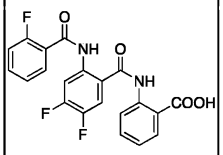
Table 4. Inhibition of Adenovirus 5 Replication with Third-Generation Compounds

Compound	Structure	Percentage inhibition	
		15 μM [a]	5 μM [a]
1		96 ± 1	89 ± 5
17h		98±1	96±1
35a		61±0	0
35b		0	0
35c		0	0
35d		78±3	28±20

Compound	Structure	Percentage inhibition	
		15 μM [a]	5 μM [a]
35e		0	0
35f		78±2	57±5
35g		91±2	89±2
35h		89±6	92±3
35i		97±1	91±6
35j		93±2	94±3

[a]Percentage inhibition of Ad5 replication in A549 cells at 5 and 15 μM. See the Experimental Section for details.

commercial amino benzoic acid was conveniently carried out using trimethylsilyldiazomethane.[19] Substantial amounts of byproduct were observed after hydrogenolysis of the 4,5-difluoro-2-nitrobenzamide **32e**, which was possibly a result of nucleophilic aromatic substitution of the unreacted activated difluoronitrobenzamide.

For the four chlorinated compounds **32a**, **32b**, **32f**, and **32g**, reduction with hydrogen gas over palladium on charcoal resulted in the corresponding undesirable *des*-chloro compound. A small screen to find conditions that avoided reducing the chloro substitutent was carried out: $NaBH_4$/Pd/C,[20] $SnCl_2/2H_2O$,[21] and $FeCl_3$/C/hydrazine.[22−24] $FeCl_3$/C/hydrazine conditions gave enough of the desired anilines **33f** and **33g** to move forward, although the method was less than ideal (20−46% yield). For nitro compounds **32a** and **32b**, with a methyl ester instead of an ethyl ester, no desired compound could be isolated. Rather, a crystalline, poorly soluble product in which the methyl ester was cleaved was isolated as the major product, presumably due to hydrazinolysis. Starting with the corresponding ethyl ester instead did give enough of the desired anilines **33a** and **33b**, although in very poor yields (1−10%), to obtain **35a** and **35b**.

2.6. Biology: Third-Generation Compounds. With the exception of **35d**, substitution on the C-terminal ring reduced activity. On the other hand, the central ring tolerated all the substituents tested, as shown for **35f−j**, and most of the compounds inhibited viral replication to the same extent as **1** and **17h**. The biological activity could not be correlated with the calculated physcial properties pK_a or log *P* (see Supporting Information). Interestingly, the variation in the calculated pK_a values differed substantially between models (two different empirical models and quantum mechanical (QM) calculation

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on different protonation states and conformations). The variation in calculated pK_a between models was larger than the variation seen between different compounds. The lack of correlation probably reflects the complexity of a whole-cell assay system, where any change in substituents and the corresponding change in physical properites of the compounds will affect for example solubility, membrane permeability, nonspecific protein binding, and target affinity.

2.7. EC_{50} and Toxicity of Selected Compounds. Host cell toxicity is an important factor in the development of antiviral agents. For compounds **1**, **17h**, and **35g–j**, toxicity was measured using the XTT test[25−27] and EC_{50} values for inhibition of viral replication were determined (Table 5). The most potent compounds **35g** and **35j** have EC_{50} values of 0.57 and 0.58 μM, respectively, and are thus more potent than both **1** and **17h**. The inhibitors **35g** and **35j** both show low toxicity at concentrations 2 orders of magnitude higher than their EC_{50} values. Importantly, the inhibitors are more potent than cidofovir, which is currently used in the clinic to treat adenovirus infections.

3. CONCLUDING REMARKS

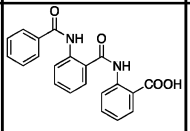
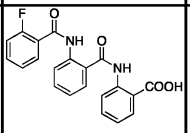
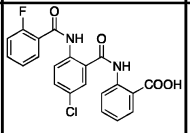
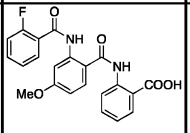
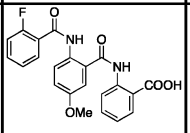
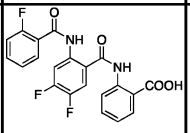
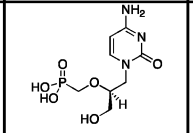
The potency of 2-[2-benzoylamino)benzoylamino]benzoic acid (**1**) was improved by screening three generations of designed and synthesized compounds and also seven commercial, structurally similar compounds. From the first generation, we conclude that the *ortho*, *ortho* substituent pattern and the presence of a carboxylic acid in **1** is favorable for this class of compounds and that the direction of the amide bonds as in **1** is obligate. Although there appears to be room for some variability in the N-terminal moiety of the compound class, a second set of designed compounds showed that the substituted benzamides are preferable, and compound **17h** showed improved activity over **1**. In a third generation, the substituents on the central and C-terminal aromatic rings were varied, resulting in potent inhibitors of intracellular viral replication with low cell toxicity. The most potent compounds **35g** and **35j** have EC_{50} values of 0.57 and 0.58 μM, respectively, and have low toxicity at concentrations 2 orders of magnitude higher than their EC_{50} values. In conclusion, we have shown that this class of compounds hold promise to be further developed into novel antiadenoviral drugs.

4. EXPERIMENTAL SECTION

4.1. Quantitative Real-Time PCR. The method used to establish the inhibitory effects of the compounds has been described elsewhere.[1] Briefly, A549 cells were infected with Ad5 and incubated with the compound for 24 h. Thereafter, DNA was prepared from the infected cells and quantitative real-time PCR was used to detect newly synthesized viral DNA. The principle of quantitative real-time PCR has been described previously,[28] as has the design of primers and probes for quantitative PCR analysis of various Ad types representing different adenovirus species.[29,30] Quantitative real-time PCR was carried out using a primer pair and a FAM-labeled probe for amplification and detection of the hexon gene. The amount of hexon gene DNA detected was normalized to the cellular RNaseP gene. Real-time PCR was performed in an ABI PRISM 7700 sequence detector (Applied Biosystems) and analyzed with Sequence Detector v1.7a software.

XTT Toxicity Test. The toxic effect of the compounds on cells was determined with the XTT-based In Vitro Toxicology Assay Kit (Sigma-Aldrich). This method is based on the principle of conversion of 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2*H*-tetrazolium-5-carboxanilide to a water-soluble formazan derivative by mitochondrial dehydrogenase. Approximately 15000 A549 cells were seeded in each well of 96-well plates (Nunc, Roskilde, Denmark) on the day before addition of compounds. The next day, the growth medium was removed and compound was added to the cells in 100 μL of DMEM (Sigma-Aldrich) containing 0.75 g/L $NaHCO_3$, 20 mM HEPES, 1×

Table 5. Effective Concentrations and Toxicities of Compounds with Inhibitory Effects

Compound	Structure	EC_{50} [a] [μM]	Viability 30 μM [b]	Viability 60 μM [b]
1		3.7[c]	82	83
17h		2.3	85	83
35g		0.57	91	88
35h		2.3	92	94
35i		1.4	84	84
35j		0.58	96	96
Cidofovir		20[c]	100	98

[a]EC_{50} values for inhibition of Ad5 replication in host cells. See Experimental Section for details. [b]Percentage viable cells after 24 h at 30 and 60 μM. See Experimental Section for details. [c]Data acquired in our group using the same assay as for the other compounds reported here (Andersson et al.[1]). The baseline toxicity from the DMSO in the inhibitor solutions have been subtracted (2% toxicity with 0.15% DMSO corresponding to 30 μM inhibitor solutions and 12% toxicity with 0.30% DMSO corresponding to 60 μM inhibitor solutions).

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PEST, and 2% fetal bovine serum to give final concentrations of compound of 30 μM (with 0.15% DMSO) and 60 μM (with 0.30% DMSO). The cells were incubated in the presence of test compound or DMSO alone at 37 °C in 5% CO_2 for 24 h in total. Four hours prior to toxicity measurement, 50 μL of XTT was added to the cells. The intensity of the formazan dye was measured spectrophotometrically at a wavelength of 450 nm.[25−27]

4.2. General Chemical Procedures. LCMS was carried out with a Waters LC system equipped with an Xterra C_{18} column (50 mm × 19 mm, 5 μm, 125 Å), eluted with a linear gradient of CH_3CN in water, both of which contained formic acid (0.2%). A flow rate of 1.5 mL/min was used and detection was performed at 214 and 254 nm. Mass spectra were obtained on a Water micromass ZQ 2000 using positive and negative electrospray ionization. Semipreparative reversed-phase HPLC was performed on a Beckman System Gold HPLC with a Supelco Discovery BIO Wide Pore C-18 column, using gradients of MeCN (0.1% TFA) and water (0.1% TFA) with a flow rate of 11 mL min^{-1} and detection at 214 nm. ^1H NMR and ^{13}C NMR spectra were recorded with a Bruker DRX-400 or DRX-500 spectrometer. NMR experiments were conducted at 298 K in pyridine-d_5 (residual solvent peak = 7.20 ppm (δ_H) and 123.44 (δ_C)) and DMSO-d_6 (residual solvent peak = 2.50 ppm (δ_H)). Compounds **12a−g** where purchased from ChemBridge Corp., analyzed with LCMS, and used without further characterization. All target compounds were ≥95% pure according to HPLC UV-traces, except the low-active compounds **17i**, **35b**, **35e**, and **35h** that were 91−93% pure.

4.3. Synthetic Procedures. *Procedure A: Synthesis of an Acyl Chloride and Coupling to an Aniline (Exemplified by **6a**).* A catalytic amount of DMF was added to a stirred solution of Fmoc-aminobenzoic acid (100 mg, 278 μmol) and oxalyl chloride (0.49 mL, 5.57 mmol) in 1,2-dichloroethane (3 mL) at rt and under nitrogen atmosphere. After 40 min, the reaction mixture was concentrated, redissolved in 1,2-dichloroethane, and concentrated again. The crude product was immediately dissolved in 1,2-dichloroethane (4 mL) before pyridine (0.22 mL, 2.78 mmol) and ethyl aminobenzoate (184 mg, 1.11 mmol) were added. The reaction was stirred overnight at room temperature and under nitrogen atmosphere before being diluted with DCM and washed with HCl (aq, 1M), $NaHCO_3$ (aq, satd), and brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated. The crude amide was purified by short-pass column chromatography (SiO_2, toluene/acetone 98:2 → 95:5) and taken directly to the next reaction.

*Procedure B: Fmoc Deprotection and in Situ Coupling to an Acyl Chloride (Exemplified by **11**).* TBAF (1 M, 0.35 mL, 0.345 mmol) was added to a stirred solution of Fmoc-protected aniline (100 mg, 0.23 mmol) in THF (3 mL) at room temperature. The reaction was stirred overnight before pyridine (70 μL, 0.917 mmol) and BzCl (80 μL, 0.689 mmol) were added. After 3 h, the reaction was concentrated and purified by column chromatography (SiO_2, toluene/acetone 100:0 → 95:5) to give benzoylated product in 87% yield. The benzoylated product was purified further by RP-HPLC.

*Procedure C: Saponification (Exemplified by **8d**).* NaOH (aq, 2 M, 0.5 mL) was added to a stirred solution of the ethyl ester (8.5 mg, 21.9 μmol) in pyridine (2 mL) at room temperature. After 2 h, the pyridine was removed under reduced pressure, and the residue diluted with EtOAc and washed with HCl (aq, 1 M). The organic phase was dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by semipreparative RP-HPLC to give the carboxylic acid product in 85% yield.

*Procedure D: BOP-Activation of a Carboxylic Acid and Coupling to an Aniline (Exemplified by **10**).* Aniline (114 μL, 1.25 mmol) was added to Fmoc-aminobenzoic acid (300 mg, 835 μmol), BOP (652 mg, 1.25 mmol), and DIPEA (0.44 mL, 2.50 mmol) in DMF (8 mL) at room temperature. After 2 h, more aniline (228 μL, 2.50 mmol) was added and the reaction was stirred overnight. The reaction mixture was diluted with HCl (aq, 1 M, 70 mL) and loaded onto a conditioned C_{18} solid-phase extraction column (10 g). The column was washed with HCl (aq, 1 M, 70 mL) and H_2O (140 mL) before being eluted with methanol and acetone. The pooled organic fractions were concentrated and purified by column chromatography (SiO_2, toluene/acetone 100:0 → 95:5 → 90:10) to give the benzamide in 28% yield.

*Procedure E: Hydrogenation (Exemplified by **15**).* The nitro compound (2.24 g, 7.14 mmol) and Pd/C (220 mg) was dissolved/suspended in EtOAc (50 mL) and methanol (20 mL) and stirred vigorously in an atmosphere of H_2 (1 atm). After 5 h, the Pd/C was filtered off over a pad of Celite and florisil to leave pure aniline product in quantitative yield (1.98 g) over two steps.

*Procedure F: Coupling of an Acyl Chloride or Sulfonyl Chloride with an Aniline (Examplified by **16d**).* Pyridine (43 μL, 0.54 mmol) was added to a stirred solution of the aniline (50 mg, 0.18 mmol) in DCM (0.9 mL) at room temperature under nitrogen atmosphere. After 5 min, methanesulfonyl chloride (29.9 μL, 0.198 mmol) was added and the reaction was stirred overnight before being diluted with DCM and washed three times with HCl (aq, 1M), $NaHCO_3$ (aq, satd), and brine. The organic phase was dried over Na_2SO_4, concentrated, and purified by column chromatography (toluene/acetone 100:0 −> 98:2) to give the derivatized aniline in 60% yield (38 mg).

*Procedure G: Benzylation of an Aniline (Exemplified by **16i**).* DIPEA (61 μL, 0.54 mmol) was added to a stirred solution of the aniline (50 mg, 0.18 mmol) in DCM (0.9 mL) at room temperature under nitrogen atmosphere. After 5 min, benzyl bromide 11 (23 μL, 0.198 mmol) was added and the reaction mixture was heated in a microwave oven at 80 °C for 20 min and then 100 °C for 45 min before being diluted with DCM and washed three times with HCl (aq, 1M), $NaHCO_3$ (aq, satd), and brine. The organic phase was dried over Na_2SO_4, concentrated, and purified by column chromatography (toluene/acetone 100:0 −> 98:2) to give the benzylated aniline in 45% yield (30 mg).

*Procedure H: $TMSCH_2N_2$-Mediated Esterification (Examplified by **31e**).* $TMSCH_2N_2$ (1.9 mL, 3.82 mmol, 2.0 M in hexanes) was added to a stirred solution of the aminobenzoic acid (600 mg, 3.47 mmol) in 20 mL of DCM/MeOH (9:1) under nitrogen atmosphere at room temperature. After 30 min, the reaction mixture was quenched with glacial HOAc before being diluted with DCM and washed with $NaHCO_3$ (aq, satd). The organic phase was dried over Na_2SO_4, filtered, and concentrated to give the methyl aminobenzoate in 99% yield (644 mg).

*Procedure I: Acid-Catalyzed Esterification (Exemplified by **31a**).* 1-Amino-4-chloro-benzoic acid (500 mg, 2.9 mmol) and H_2SO_4 (conc, 0.5 mL) in ethanol (30 mL) was refluxed for 3 days before being concentrated to half-volume and diluted with $NaHCO_3$ (aq, satd) and extracted three times with DCM (even though the reaction had not yet finished). The pooled DCM fractions were washed with brine, dried over $MgSO_4$, concentrated, and purified by column chromatography (toluene/acetone 100:0 −> 98:2) to give the ethyl ester in 64% yield (370 mg).

*Procedure J: Nitro Reduction Using $FeCl_3$/C/Hydrazine (Exemplified by **33f**).* Hydrazine hydrate (0.80 mL, 16.5 mmol) was added to a stirred solution of nitro compound **32f** (275 mg, 0.789 mmol), charcoal (50 mg, 4.16 mmol), and $FeCl_3$ (38 mg, 0.23 mmol) in methanol (10 mL) at 65 °C under nitrogen. The temperature was raised to reflux and kept there for 3 h before all the solids were filtered off over Celite. The crude material was purified by column chromatography (toluene/acetone 100:0 −> 98:2) to give the aniline **33f** in 46% yield (115 mg).

4.4. Tabulated Compounds. *Ethyl 3-[(2-Benzoylamino)-benzoylamino]benzoate (**7a**).* **7a** was synthesized by procedures A and B.

^1H NMR (500 MHz; pyridine-d_5): δ 12.47 (s, 1H), 11.60 (s, 1H), 9.19 (br d, J = 8.4 Hz, 1H), 8.71 (pyr-d5 overlap, 1H), 8.30−8.28 (m, 3H), 8.16 (dd, J = 7.9, 1.3 Hz, 1H), 7.99 (dt, J = 7.7, 1.1 Hz, 1H), 7.53 (ddd, J = 8.5, 7.2, 1.4 Hz, 1H), 7.49 (t, J = 7.9 Hz, 1H), 7.44 (tt, J = 7.2, 1.2 Hz, 1H), 7.41−7.38 (m, 2H), 7.08 (td, J = 7.4, 1.0 Hz, 1H), 4.29 (q, J = 7.1 Hz, 2H), 1.20 (t, J = 7.1 Hz, 3H).

^{13}C NMR (126 MHz; pyridine-d_5): δ 169.17, 166.12, 165.41, 140.72, 139.53, 135.54, 133.02, 132.11, 131.71, 129.37, 129.19, 129.08, 127.72, 126.49, 125.86, 122.92, 121.72, 121.58, 61.16, 14. 23.

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ESIMS m/z calcd for $[C_{23}H_{19}N_2O_4]^-$, 387.14; found, 387.51.

*Ethyl 4-[(2-Benzoylamino)benzoylamino]benzoate (**7b**).* **7b** was synthesized by procedures A and B.

^{1}H NMR (400 MHz; pyridine-d_5): δ 12.37 (s, 1H), 11.67 (s, 1H), 9.16 (d, J = 8.4 Hz, 1H), 8.32−8.29 (m, 2H), 8.22 (d, J = 8.5 Hz, 2H), 8.14 (d, J = 8.6 Hz, 2H), 8.10 (d, J = 7.8 Hz, 1H), 7.54−7.41 (m, 4H), 7.04 (t, J = 7.6 Hz, 1H), 4.33 (q, J = 7.1 Hz, 2H), 1.25 (t, J = 7.1 Hz, 3H).

^{13}C NMR (100 MHz; pyridine-d_5): δ 169.19, 166.00, 165.40, 143.64, 140.60, 135.51, 133.08, 132.17, 130.84, 129.34, 129.11, 127.72, 126.63, 122.86, 121.75, 121.57, 121.17, 60.91, 14. 32.

ESIMS m/z calcd for $[C_{23}H_{19}N_2O_4]^-$, 387.14; found, 387.56.

*Ethyl 2-[(3-Benzoylamino)benzoylamino]benzoate (**7c**).* **7c** was synthesized by procedures A and B.

^{1}H NMR (500 MHz; pyridine-d_5): δ 12.28 (s, 1H), 11.26 (s, 1H), 9.21 (d, J = 8.3 Hz, 1H), 9.01 (s, 1H), 8.42 (br d, J = 7.4 Hz, 1H), 8.23 (d, J = 7.1 Hz, 2H), 8.07 (br d, J = 7.7 1H), 8.01 (br d, J = 7.2 Hz, 1H) 7.56−7.37 (m, 5H), 7.11 (br t, J = 7.3 Hz, 1H), 4.30 (q, J = 7.0 Hz, 2H), 1.24 (t, J = 7.0 Hz, 3H).

^{13}C NMR (126 MHz; pyridine-d_5): δ 168.57, 167.09, 165.44, 142.20, 140.99, 136.32, 136.06, 134.71, 131.75, 131.32, 129.61, 128.64, 128.27, 124.57, 122.87, 122.42, 120.75, 120.64, 116.07, 61.79, 14. 08.

ESIMS m/z calcd for $[C_{23}H_{19}N_2O_4]^-$, 387.14; found, 387.54.

*Ethyl 2-[(4-Benzoylamino)benzoylamino]benzoate (**7d**).* **7d** was synthesized by procedures A and B.

^{1}H NMR (500 MHz; pyridine-d_5): δ 12.29 (s, 1H), 11.32 (s, 1H), 9.28 (d, J = 8.4 Hz, 1H), 8.39−8.32 (m, 4H), 8.23−8.20 (m, 2H), 8.10 (dd, J = 8.0, 1.4 Hz, 1H), 7.58 (ddd, J = 8.6, 7.3, 1.3 Hz, 1H), 7.47−7.42 (m, 1H), 7.39 (t, J = 7.4 Hz, 2H), 7.11 (ddd, J = 8.1, 7.3, 0.8 Hz, 1H), 4.34 (q, J = 7.1 Hz, 2H), 1.26 (t, J = 7.1 Hz, 3H).

^{13}C NMR (126 MHz; pyridine-d_5): δ 168.69, 167.14, 165.03, 143.86, 142.49, 135.94, 134.79, 131.85, 131.34, 130.30, 128.76, 128.63, 128.32, 122.66, 120.63, 115.83, 61.78, 14. 09.

ESIMS m/z calcd for $[C_{23}H_{19}N_2O_4]^-$, 387.14; found, 387. 53.

*Ethyl 2-[(2-Benzoylamino)benzoylamino]benzoate (**7e**).* **7e** was synthesized by procedures A and B.

^{1}H NMR (360 MHz; pyridine-d_5): δ 12.66 (s, 1H), 12.34 (s, 1H), 9.28 (d, J = 8.4 Hz, 1H), 9.00 (d, J = 8.4 Hz, 1H), 8.35 (dd, J = 6.9, 1.3 Hz, 2H), 8.13 (app t, J = 8.4 Hz, 2H), 7.58 (app t, J = 7.8 Hz, 2H), 7.52−7.46 (m, 3H), 7.19 (app q, J = 7.6 Hz, 2H), 4.35 (q, J = 7.1 Hz, 2H), 1.27 (t, J = 7.1 Hz, 3H).

^{13}C NMR (91 MHz; pyridine-d_5): δ 168.6, 168.2, 165.4, 141.4, 141.2, 135.6, 134.7, 133.5, 132.2, 131.4, 129.1, 127.92, 127.77, 123.6, 121.8, 121.2, 121.0, 116.9, 62.0, 14.1.

ESIMS m/z calcd for $[C_{23}H_{21}N_2O_4]^+$, 389.14; found, 389.24.

*3-[(2-Benzoylamino)benzoylamino]benzoic Acid (**8a**).* **8a** was synthesized by procedures A, B, and C.

^{1}H NMR (400 MHz; pyridine-d_5): δ 12.87 (s, 1H), 11.98 (s, 1H), 9.57 (d, J = 8.4 Hz, 1H), 9.35 (t, J = 1.8 Hz, 1H), 8.68−8.61 (m, 4H), 8.51 (dd, J = 7.9, 1.4 Hz, 1H), 7.93−7.86 (m, 2H), 7.82−7.71 (m, 3H), 7.43 (td, J = 7.6, 1.0 Hz, 1H).

^{13}C NMR (100 MHz; pyridine-d_5): δ 169.17, 168.76, 165.40, 140.70, 139.48, 135.53, 133.46, 132.95, 132.09, 129.28, 129.24, 129.08, 127.72, 126.33, 126.09, 122.87, 121.77, 121.50.

ESIMS m/z calcd for $[C_{23}H_{19}N_2O_4]^-$, 359.10; found, 359.52.

*4-[(2-Benzoylamino)benzoylamino]benzoic Acid (**8b**).* **8b** was synthesized by procedures A, B, and C.

^{1}H NMR (500 MHz; pyridine-d_5): δ 12.41 (s, 1H), 11.65 (s, 1H), 9.18 (d, J = 8.5 Hz, 1H), 8.49 (d, J = 8.6 Hz, 2H), 8.33−8.30 (m, 2H), 8.18 (d, J = 8.6 Hz, 2H), 8.11 (dd, J = 7.9, 1.0 Hz, 1H), 7.53−7.42 (m, 4H), 7.04 (td, J = 7.5, 1.0 Hz, 1H).

^{13}C NMR (126 MHz; pyridine-d_5): δ 169.17, 168.59, 165.41, 143.23, 140.65, 135.55, 133.04, 132.15, 131.20, 129.34, 129.13, 128.41, 127.74, 122.84, 121.80, 121.55, 121.25.

ESIMS m/z calcd for $[C_{23}H_{19}N_2O_4]^-$, 359.10; found, 359.52.

*2-[(3-Benzoylamino)benzoylamino]benzoic Acid (**8c**).* **8c** was synthesized by procedures A, B, and C.

^{1}H NMR (500 MHz; pyridine-d_5): δ 13.33 (s, 1H), 11.22 (s, 1H), 9.36 (d, J = 8.4 Hz, 1H), 9.12 (s, 1H), 8.50 (d, J = 7.8 Hz, 1H), 8.33 (d, J = 8.0 Hz, 1H), 8.22 (d, J = 7.7 Hz, 2H), 8.13 (d, J = 7.6 Hz, 1H), 7.61−7.55 (pyridine-d_5 overlap, 1H), 7.48−7.38 (m, 4H), 7.17 (t, J = 7.6 Hz, 1H).

^{13}C NMR (126 MHz; pyridine-d_5): δ 173.33, 168.02, 166.43, 143.61, 141.87, 137.64, 137.14, 134.95, 133.12, 132.71, 130.52, 129.62, 129.24, 125.22, 123.71, 123.70, 121.59, 121.42, 119. 24.

ESIMS m/z calcd for $[C_{23}H_{19}N_2O_4]^-$, 359.10; found, 359.55.

*2-[(4-Benzoylamino)benzoylamino]benzoic Acid (**8d**).* **8d** was synthesized by procedures A, B, and C.

^{1}H NMR (500 MHz; pyridine-d_5): δ 13.31 (s, 1H), 11.23 (s, 1H), 9.42 (d, J = 8.2 Hz, 1H), 8.53 (d, J = 7.4 Hz, 1H), 8.44 (d, J = 8.5 Hz, 2H), 8.27 (d, J = 8.5 Hz, 2H), 8.18 (d, J = 7.1 Hz, 2H), 7.63−7.55 (pyridine-d_5 overlap, 1H), 7.45−7.36 (m, 3H), 7.20−7.14 (pyridine-d_5 overlap, 1H).

^{13}C NMR (126 MHz; pyridine-d_5): δ 172.54, 167.08, 165.18, 143.59, 142.91, 136.03, 133.97, 132.21, 131.81, 130.89, 128.84, 128.62, 128.29, 122.53, 120.60, 120.41, 118. 28.

ESIMS m/z calcd for $[C_{23}H_{19}N_2O_4]^-$, 359.10; found, 359.53.

*2-[2-(Benzoylamino)benzoylamino]benzoic Acid (**1**).* **17a** was synthesized by procedures A, E, F, and C.

^{1}H NMR (500 MHz; pyridine-d_5): δ 13.47 (s, 1H), 12.80 (s, 1H), 9.27 (d, J = 8.4 Hz, 1H), 9.14 (d, J = 8.3 Hz, 1H), 8.53 (dd, J = 7.9, 1.5 Hz, 1H), 8.36 (dd, J = 7.6, 1.8 Hz, 2H), 8.24 (dd, J = 7.9, 1.1 Hz, 1H), 7.59−7.55 (m, pyridine-d_5 overlap, 1H), 7.52−7.45 (m, 4H), 7.23 (dt, J = 7.6, 0.9 Hz, 1H), 7.10 (dt, J = 7.6, 0.9 Hz, 1H).

^{13}C NMR (126 MHz; pyridine-d_5): δ 172.47, 168.31, 165.43, 141.72, 141.40, 135.70, 133.88, 133.16, 132.23, 132.13, 129.13, 128.12, 127.78, 121.70, 121.39, 120.81, 119. 10.

ESIMS m/z calcd for $[C_{21}H_{17}N_2O_4]^+$, 361.11; found, 361.20.

*2-[2-(Benzoylamino)benzoylamino]benzene (**11**).* **11** was synthesized by procedures D and B.

^{1}H NMR (400 MHz; pyridine-d_5): δ 12.56 (s, 1H), 11.42 (s, 1H), 9.19 (d, J = 8.4 Hz, 1H), 8.32−8.27 (m, 2H), 8.10 (dd, J = 7.9, 1.3 Hz, 1H), 8.04−7.99 (m, 2H), 7.52−7.37 (m, 6H), 7.22−7.17 (pyridine-d_5 overlap, 1H), 7.03 (td, J = 7.6, 1.1 Hz, 1H).

^{13}C NMR (100 MHz; pyridine-d_5): δ 168.97, 165.34, 140.67, 139.29, 135.56, 132.81, 132.09, 129.22, 129.16, 129.09, 127.70, 125.04, 122.80, 122.33, 121.78, 121.39.

ESIMS m/z calcd for $[C_{23}H_{19}N_2O_4]^-$, 315.11; found, 315.52.

*2-[2-(Acetylamino)benzoylamino]benzoic Acid (**17a**).* **17a** was synthesized by procedures A, E, F, and C.

^{1}H NMR (500 MHz; pyridine-d_5): δ 13.33 (s, 1H), 11.56 (s, 1H), 9.15 (d, J = 8.3 Hz, 1H), 8.96 (d, J = 8.3 Hz, 1H), 8.54 (dd, J = 7.8, 1.4 Hz, 1H), 8.14 (dd, J = 7.9, 1.1 Hz, 1H), 7.64−7.61 (ddd, J = 7.0, 1.5 Hz, 1H), 7.45−7.42 (ddd, J = 1.2 Hz, 1H), 7.23 (t, J = 7.6 Hz, 1H), 7.05 (t, J = 7.2 Hz, 1H), 2.20 (s, 3H).

^{13}C NMR (126 MHz; pyridine-d_5): δ 172.47, 168.69, 167.89, 141.92, 140.74, 133.87, 132.75, 132.23, 128.01, 123.29, 122.10, 121.85, 120.59, 118.94, 25. 05.

ESIMS m/z calcd for $[C_{16}H_{15}N_2O_4]^+$, 299.10; found, 299.25.

*2-[2-(Methanesulfonylamino)benzoylamino]benzoic Acid (**17b**).* **17b** was synthesized by procedures A, E, F, and C.

^{1}H NMR (360 MHz; pyridine-d_5): δ 13.40 (s, 1H), 9.01 (d, J = 8.4 Hz, 1H), 8.50 (dd, J = 7.9, 1.4 Hz, 1H), 8.15 (d, J = 7.9 Hz, 1H), 8.03 (d, J = 8.3 Hz, 1H), 7.60 (td, J = 7.9, 1.3 Hz, 1H), 7.44 (t, J = 7.8 Hz, 1H), 7.23 (t, J = 7.7 Hz, 1H), 7.08 (t, J = 7.6 Hz, 1H), 3.31 (s, 3H).

^{13}C NMR (91 MHz; pyridine-d_5): δ 174.29, 169.26, 143.36, 142.11, 135.70, 135.07, 133.93, 130.29, 125.65, 124.17, 122.43, 120.68, 41. 70.

ESIMS m/z calcd for $[C_{15}H_{15}N_2O_5S]^+$, 335.35; found, 335.26.

*2-[2-(p-Toluenesulfonylamino)benzoylamino]benzoic Acid (**17c**).* **17c** was synthesized by procedures A, E, F, and C.

^{1}H NMR (500 MHz; pyridine-d_5): δ 13.13 (s, 1H), 9.01 (d, J = 8.4 Hz, 1H), 8.48 (dd, J = 7.9, 1.4 Hz, 1H), 8.02 (d, J = 8.2 Hz, 1H), 7.96 (dd, J = 7.8, 0.9 Hz, 1H), 7.92 (d, J = 8.2 Hz, 2H), 7.64−7.60 (ddd, J = 8.6, 7.1, 1.5 Hz, 1H), 7.41−7.38 (ddd, J = 8.3, 7.2, 1.1 Hz, 1H), 7.24−7.21 (ddd, J = 7.9, 7.1, 0.8 Hz, 1H), 7.03−6.97 (m, 3H), 1.95 (s, 3H).

^{13}C NMR (126 MHz; pyridine-d_5): δ 172.16, 167.23, 143.91, 141.39, 139.40, 136.83, 133.79, 132.78, 131.97 129.75, 127.90, 127.45, 124.52, 124.04, 123.32, 122.57, 120.39, 118.61, 20. 83.

ESIMS m/z calcd for $[C_{21}H_{19}N_2O_5S]^+$, 411.09; found, 411.14.

*2-[2-(Trimethylacetylamino)benzoylamino]benzoic Acid (**17d**).* **17d** was synthesized by procedures A, E, F, and C.

^{1}H NMR (500 MHz; pyridine-d_5): δ 13.34 (s, 1H), 11.94 (s, 1H), 9.14 (dd, *J* = 8.5, 1.1 Hz, 2H), 8.51 (dd, *J* = 7.9, 1.6 Hz, 1H), 8.16 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.62−7.58 (ddd, *J* = 8.7, 7.1, 1.6 Hz, 1H), 7.45−7.42 (ddd, *J* = 8.5, 7.2, 1.3 Hz, 1H), 7.23−7.20 (ddd, *J* = 8.3, 7.3, 1.0 Hz, 1H), 7.05−7.02 (ddd, *J* = 8.3, 7.3, 1.1 Hz, 1H), 1.39 (s, 9H).

^{13}C NMR (126 MHz; pyridine-d_5): δ 177.1, 172.3, 168.1, 141.7, 141.5, 133.8, 132.9, 132.1, 127.8, 123.2, 122.8, 121.4, 121.1, 120.5, 118.8, 27. 5.

ESIMS *m*/*z* calcd for $[C_{19}H_{21}N_2O_4]^+$, 341.14; found, 341.24.

*2-[[2-(Cyclohexanecarboxylamino)benzoyl]amino]benzoic Acid (**17e**).* **17e** was synthesized by procedures A, E, F, and C.

^{1}H NMR (400 MHz; pyridine-d_5): δ 13.28 (s, 1H), 11.71 (s, 1H), 9.15 (d, *J* = 8.3 Hz, 1H), 9.12 (dd, *J* = 8.5, 1.0 Hz, 1H), 8.52 (dd, *J* = 7.9, 1.6 Hz, 1H), 8.17 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.60 (ddd, *J* = 8.5, 7.2, 1.3 Hz, 1H), 7.45 (ddd, *J* = 8.5, 7.2, 1.3 Hz, 1H), 7.23 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H), 7.06 (ddd, *J* = 8.1, 7.0, 1.1 Hz, 1H), 2.45 (tt, *J* = 11.4, 3.6 Hz, 1H), 2.13−2.06 (m, 2H), 1.74−1.61 (m, 4H), 1.53−1.46 (m, 1H), 1.27−1.06 (m, 3H).

^{13}C NMR (126 MHz; pyridine-d_5): δ 175.7, 173.4, 169.1, 142.8, 142.3, 134.8, 133.9, 133.2, 129.0, 124.3, 124.0, 122.71, 122.52, 121.6, 120.0, 48.1, 30.9, 26.96, 26. 80.

ESIMS *m*/*z* calcd for $[C_{21}H_{23}N_2O_4]^+$, 367.16; found, 367.24.

*2-[2-(4-Carboxybutanoylamido)benzoyl]aminobenzoic Acid (**17f**).* **17f** was synthesized by procedures A, E, F, and C.

^{1}H NMR (500 MHz; pyridine-d_5): δ 13.31 (s, 1H), 11.83 (s, 1H), 9.16 (d, *J* = 8.3 Hz, 1H), 9.01 (d, *J* = 8.4 Hz, 1H), 8.53 (d, *J* = 7.8 Hz, 1H), 8.13 (d, *J* = 7.9 Hz, 1H), 7.62 (t, *J* = 7.5 Hz, 1H), 7.40 (t, *J* = 7.8 Hz, 1H), 7.23 (t, *J* = 7.8 Hz, 1H), 7.02 (t, *J* = 7.5 Hz, 1H), 3.05 (m, 4H).

^{13}C NMR (126 MHz; pyridine-d_5): δ 175.1, 172.4, 171.0, 167.9, 141.9, 140.9, 133.9, 132.8, 132.2, 128.0, 123.1, 121.8, 120.6, 118.9, 33.3, 30. 0.

ESIMS *m*/*z* calcd for $[C_{18}H_{17}N_2O_6]^+$, 357.10; found, 357.23.

*2-[2-(Benzylamino)benzoylamino]benzoic Acid (**17g**).* **17g** was synthesized by procedures A, E, G, and C.

^{1}H NMR (360 MHz; pyridine-d_5): δ 13.13 (s, 1H), 9.21 (d, *J* = 8.4 Hz, 1H), 8.51 (dd, *J* = 7.9, 1.4 Hz, 1H), 8.16 (d, *J* = 7.9 Hz, 1H), 7.61−7.58 (m, 1H), 7.49 (d, *J* = 7.4 Hz, 2H), 7.32 (t, *J* = 7.5 Hz, 2H), 7.25 (app q, *J* = 7.4 Hz, 2H), 7.16 (t, *J* = 7.4 Hz, 1H), 6.81 (d, *J* = 8.4 Hz, 1H), 6.65 (t, *J* = 7.5 Hz, 1H), 4.46 (s, 2H).

^{13}C NMR (91 MHz; pyridine-d_5): δ 172.4, 168.9, 150.9, 142.7, 139.8, 133.9, 133.5, 132.1, 128.9, 128.4, 127.55, 127.36, 122.5, 120.4, 118.1, 116.0, 115.6, 112.7, 47. 1.

ESIMS *m*/*z* calcd for $[C_{21}H_{19}N_2O_3]^+$, 347.13; found, 347.22.

*2-[2-(2-Fluorobenzoylamino)benzoylamino]benzoic Acid (**17h**).* **17h** was synthesized by procedures A, E, F, and C.

^{1}H NMR (360 MHz; pyridine-d_5): δ 13.43 (s, 1H), 12.47 (d, *J* = 6.1 Hz, 1H), 9.16 (d, *J* = 8.4 Hz, 2H), 8.52 (d, *J* = 7.8 Hz, 1H), 8.52 (d, *J* = 7.8 Hz, 1H), 8.25 (td, *J* = 7.7, 1.5 Hz, 1H), 8.19 (d, *J* = 7.9 Hz, 1H), 7.53 (d, *J* = 7.5 Hz, 1H), 7.49 (d, *J* = 7.6 Hz, 1H), 7.44−7.38 (m, 1H), 7.23−7.17 (m, 2H), 7.11 (t, *J* = 7.6 Hz, 1H).

^{13}C NMR (91 MHz; pyridine-d_5): δ 173.90, 169.15, 163.70, 162.01 (d, *J* = 251.0 Hz), 143.25, 141.75, 137.24, 136.97, 135.22, 135.16, 134.20, 133.60, 133.15, 129.43, 126.4 (d, *J* = 2.7 Hz), 125.34, 125.00, 124.72, 123.99, 122.02, 120.45, 118.1 (d, *J* = 24 Hz).

ESIMS *m*/*z* calcd for $[C_{21}H_{14}FN_2O_4]^-$, 377.09; found, 377.20.

*2-[2-(3-Fluorobenzoylamino)benzoylamino]benzoic Acid (**17i**).* **17i** was synthesized by procedures A, E, F, and C.

^{1}H NMR (500 MHz; pyridine-d_5): δ 13.90 (s, 1H), 12.82 (s, 1H), 9.10−9.07 (m, 2H), 8.56−8.52 (m, *J* = 1.0 Hz, 1H), 8.27 (d, *J* = 7.5 Hz, 1H), 8.05 (d, *J* = 7.8 Hz, 1H), 8.02 (dt, *J* = 9.6, 2.1 Hz, 1H), 7.51 (app dt, *J* = 11.0, 7.9 Hz, 2H), 7.44 (app td, *J* = 7.9, 5.8 Hz, 1H), 7.27 (td, *J* = 8.4, 2.4 Hz, 1H), 7.20−7.15 (m, 2H).

^{13}C NMR (126 MHz; pyridine-d_5): δ 168.04, 164.04, 164.02, 163.01 (d, *J* = 246.4 Hz), 140.66, 137.86, 137.81, 135.73, 135.54, 133.26, 132.97, 132.20, 131.03, 130.97, 128.17, 123.77, 123.36, 123.14, 123.12, 121.59, 120.50, 118.91 (d, *J* = 21.3 Hz, 3C), 114.81 (d, *J* = 23.0 Hz).

ESIMS *m*/*z* calcd for $[C_{21}H_{16}FN_2O_4]^+$, 379.35; found, 379.37.

*2-[2-(4-Fluorobenzoylamino)benzoylamino]benzoic Acid (**17j**).* **17j** was synthesized by procedures A, E, F, and C.

^{1}H NMR (360 MHz; pyridine-d_5): δ 13.61 (s, 1H), 12.78 (s, 1H), 9.23 (d, *J* = 8.3 Hz, 1H), 9.15 (d, *J* = 8.3 Hz, 1H), 8.56 (d, *J* = 7.7 Hz, 1H), 8.32 (dd, *J* = 8.0, 5.7 Hz, 2H), 8.25 (d, *J* = 7.8 Hz, 1H), 7.62−7.56 (m, 1H), 7.51 (t, *J* = 7.9 Hz, 1H), 7.27−7.20 (m, *J* = 7.5 Hz, 3H), 7.10 (t, *J* = 7.7 Hz, 1H).

^{13}C NMR (91 MHz; pyridine-d_5): δ 168.55, 165.35 (d, *J* = 251.0 Hz), 164.56, 141.96, 141.54, 134.03, 133.41, 132.54, 132.25, 130.65, 130.55, 128.42, 121.91, 121.66, 121.03, 116.40, 116. 16.

ESIMS *m*/*z* calcd for $[C_{21}H_{16}FN_2O_4]^+$, 379.35; found, 379.43.

*2-[2-(Phenylacetylamino)benzoylamino]benzoic Acid (**17k**).* **17k** was synthesized by procedures A, E, F, and C.

^{1}H NMR (500 MHz; pyridine-d_5): δ 13.25 (s, 1H), 11.72 (s, 1H), 9.11 (dd, *J* = 8.4, 0.5 Hz, 1H), 8.95 (d, *J* = 8.1 Hz, 1H), 8.53 (dd, *J* = 7.9, 1.5 Hz, 1H), 8.09 (dd, *J* = 7.9, 1.2 Hz, 1H), 7.67−7.64 (ddd, *J* = 7.0, 1.6 Hz, 1H), 7.40−7.35 (m, 3H), 7.29−7.23 (m, 3H), 7.04−7.01 (ddd, *J* = 7.0, 1.0 Hz, 2H), 3.93 (s, 2H).

^{13}C NMR (126 MHz; pyridine-d_5): δ 172.36, 169.81, 167.73, 141.87, 140.66, 133.88, 132.72, 132.20, 129.98, 129.02, 127.85, 127.31, 123.34, 123.30, 122.18, 121.78, 120.60, 118.88, 45. 73.

ESIMS *m*/*z* calcd for $[C_{22}H_{19}N_2O_4]^+$, 375.13; found, 375.18.

*2-[2-(Benzoylamino)benzoylamino]phenylacetic Acid (**23**).* **23** was synthesized by procedures E, D, E, F, and C.

^{1}H NMR (400 MHz, pyridine-d_5): δ 12.94 (s, 1H), 11.59 (s, 1H), 9.29 (d, *J* = 8.5 Hz, 1H), 8.36 (d, *J* = 7.8 Hz, 1H), 8.29 (d, *J* = 7.4 Hz, 2H), 7.99 (d, *J* = 7.8 Hz, 1H), 7.56−7.49 (m, 2H), 7.44−7.40 (m, 1H), 7.37−7.33 (m, 3H), 7.24 (t, *J* = 7.6 Hz, 1H), 7.08 (t, *J* = 7.6 Hz, 1H), 4.10 (s, 2H).

^{13}C NMR (400 MHz, pyridine-d_5): δ 176.4, 169.4, 166, 142, 137.8, 133.6, 132.6, 132.2, 131.7, 129.7, 129.3, 128.6, 128.3, 127.4, 127.2, 122.0, 121.4, 40.5.

^{13}C NMR (100 MHz, pyridine-d_5): δ 175.5, 168.8, 165.3, 141.5, 137.4, 135.6, 133.1, 132.0, 131.7, 131.4, 129.1, 128.8, 128.1, 127.8, 126.82, 126.65, 123.0, 121.4, 120.8, 40.1.

ESIMS *m*/*z* calcd for $[C_{22}H_{17}N_2O_4]^+$, 373.13; found, 373.20.

*2-[2-(Benzoylamino)phenylacetylamino]benzoic Acid (**28**).* **28** was synthesized by procedures D, E, F, and C.

^{1}H NMR (400 MHz, pyridine-d_5): δ 12.72 (s, 1H), 11.30 (s, 1H), 8.95 (d, *J* = 8.4 Hz, 1H), 8.48−8.45 (m, 2H), 8.41 (dd, *J* = 7.7, 1.5 Hz, 1H), 8.37 (d, *J* = 8.0 Hz, 1H), 7.51−7.44 (m, 5H), 7.37−7.33 (m, 1H), 7.13 (t, *J* = 7.5 Hz, 2H), 4.03 (s, 2H).

^{13}C NMR (100 MHz, pyridine-d_5): δ 172.1, 171.3, 165.9, 141.7, 138.5, 135.6, 135.3, 133.8, 132.03, 131.90, 131.3, 128.97, 128.83, 128.28, 128.17, 125.54, 125.43, 120.5, 43. 6.

ESIMS *m*/*z* calcd for $[C_{22}H_{19}N_2O_4]^+$, 375.13; found, 375.22.

*2-[2-(2-Fluorobenzoylamino)benzoylamino]-4-chlorobenzoic Acid (**35a**).* **35a**was synthesized by procedures I, A, J, A, and C.

^{1}H NMR (500 MHz; pyridine-d_5): δ 14.04 (s, 1H), 12.39 (d, *J* = 6.1 Hz, 1H), 9.25 (d, *J* = 1.7 Hz, 1H), 9.14 (d, *J* = 8.3 Hz, 1H), 8.49 (d, *J* = 8.4 Hz, 1H), 8.24−8.21 (m, 2H), 7.50 (t, *J* = 7.8 Hz, 1H), 7.40−7.36 (m, 1H), 7.23−7.09 (m, 4H).

^{13}C NMR (126 MHz; pyridine-d_5): δ 171.89, 167.76, 162.24 (d, *J* = 2.5 Hz), 160.27 (d, *J* = 250.9 Hz), 142.56, 140.24, 138.43, 133.59 (d, *J* = 8.8 Hz), 133.38, 132.73, 131.49 (d, *J* = 1.6 Hz), 128.07, 124.83 (d, *J* = 3.4 Hz), 123.73, 122.95, 122.57, 122.47, 122.40, 119.87, 118.91, 116.58 (d, *J* = 23.1 Hz).

ESIMS *m*/*z* calcd for $[C_{21}H_{13}ClFN_2O_4]^-$, 411.06; found, 411.16.

*2-[2-(2-Fluorobenzoylamino)benzoylamino]-5-chlorobenzoic Acid (**35b**).* **35b** was synthesized by procedures I, A, J, A, and C.

Compound **35b** was only available in very small quantities. Consequently, some signals are lost in the noise or obscured by solvent signals.

^{1}H NMR (500 MHz; pyridine-d_5): δ 12.69 (s, 1H), 9.19 (d, *J* = 8.5 Hz, 1H), 9.12 (d, *J* = 8.8 Hz, 1H), 8.79 (s, 2H), 8.28 (d, *J* = 7.7 Hz, 1H), 8.23 (t, *J* = 7.1 Hz, 1H), 7.51−7.46 (m, 2H), 7.42−7.37 (m, 1H), 7.04−7.01 (m, 1H).

^{13}C NMR (126 MHz; pyridine-d_5): δ 168.65, 141.60, 141.33, 134.65 (d, *J* = 8.5 Hz), 133.43, 132.96, 132.61, 132.11, 129.38, 125.96 (d, *J* = 3.4 Hz), 123.28, 122.58, 117.73 (d, *J* = 22.9 Hz).

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ESIMS m/z calcd for $[C_{21}H_{13}ClFN_2O_4]^-$, 411.06; found, 411.18.

*2-[2-(2-Fluorobenzoylamino)benzoylamino]-4-methoxybenzoic Acid (**35c**).* **35d** was synthesized by procedures H, A, E, A, and C.

^{1}H NMR (400 MHz, pyridine-d_5): δ 13.39 (s, 1H, CONH), 12.35 (s, 1H, CONH), 9.13 (d, J = 8.3 Hz, 1H), 8.85 (d, J = 2.5 Hz, 1H), 8.48 (d, J = 8.9 Hz, 1H), 8.23 (t, J = 8.0 Hz, 1H), 8.18 (d, J = 7.5 Hz, 1H), 7.50 (t, J = 8.1 Hz, 1H), 7.36−7.42 (q, (d, J = 7.6, 15.0 Hz, 1H), 7.16−7.20 (under pyridine-d_5, 2H), 7.11−7.14 (m, 1H), 6.83−6.86 (dd, J = 3.4, 8.7 Hz, 1H), 3.75 (s, 3H, −ArOCH_3).

^{13}C NMR (400 MHz, pyridine-d_5): δ 172.1, 167.9, 164.1, 162.2, 160.4 (d, J = 249.9 Hz), 143.6, 140.2, 133.8, 133.7 (d, J = 8.3 Hz), 132.7, 131.6, 127.9, 124.9, 124.8, 123.9, 122.9, 122.6, 116.6 (d, J = 24.2 Hz), 110.9, 109.0, 105.8, 55.3.

ESIMS m/z calcd for $[C_{22}H_{18}FN_2O_5]^+$, 409.38; found, 409.39.

*2-[2-(2-Fluorobenzoylamino)benzoylamino]-5-methoxybenzoic Acid (**35d**).* **35e** was synthesized by procedures H, A, E, A, and C.

^{1}H NMR (400 MHz, pyridine-d_5): δ 13.62 (s, 1H), 12.62 (d, J = 5.9 Hz, 1H), 9.17 (d, J = 8.4 Hz, 1H), 9.13 (d, J = 9.2 Hz, 1H), 8.26−8.20 (m, 2H), 8.18 (d, J = 3.0 Hz, 1H), 7.48 (t, J = 7.7 Hz, 1H), 7.43−7.37 (m, 1H), 7.22−7.16 (m, under pyridine-d_5, 3H), 7.09 (t, J = 7.6 Hz, 1H), 3.70 (s, 3H).

^{13}C NMR (100 MHz, pyridine-d_5): δ 172.38, 167.05, 162.19 (d, J = 2.7 Hz), 160.34 (d, J = 250.9 Hz), 155.25, 140.21, 135.42, 133.62, 133.53, 132.34, 131.55, 131.52, 127.85, 124.85 (d, J = 3.4 Hz), 123.74, 123.68, 122.89, 122.30, 121.93, 119.20, 116.58 (d, J = 23.2 Hz), 116.17, 55.19.

ESIMS m/z calcd for $[C_{22}H_{18}FN_2O_5]^+$, 409.38; found, 409.45.

*2-[2-(2-Fluorobenzoylamino)benzoylamino]-4,5-difluorobenzoic Acid (**35e**).* **35a** was synthesized by procedures H, A, E, A, and C.

^{1}H NMR (400 MHz, pyridine-d_5): δ 13.73 (s, 1H), 12.37 (d, J = 5.4 Hz, 1H), 9.14 (d, J = 8.4 Hz, 1H), 9.05 (app q, J = 7.3 Hz, 1H), 8.33 (t, J = 9.9 Hz, 1H), 8.25 (t, J = 7.2 Hz, 1H), 8.19 (d, J = 7.4 Hz, 1H), 7.51 (t, J = 7.3 Hz, 1H), 7.42 (q, J = 5.8 Hz, 1H), 7.25−7.23 (m, 2H), 7.14 (t, J = 7.5 Hz, 1H).

^{13}C NMR (100 MHz, pyridine-d_5): δ 171.01, 167.67, 162.36, 160.44 (d, J = 250.7 Hz, 14C), 152.44 (dd, J = 250.0, 13.0 Hz, 15C), 145.39 (dd, J = 245.0, 13.0 Hz, 15C), 140.32, 138.92, 138.82, 133.88, 133.80, 132.97, 131.70, 128.09, 125.03 (d, J = 3.0 Hz), 123.96, 122.66, 122.49, 120.41 (d, J = 19.1 Hz), 116.75 (d, J = 23.4 Hz), 109.32 (d, J = 24.0 Hz).

ESIMS m/z calcd for $[C_{21}H_{14}F_3N_2O_4]^+$, 415.33; found, 415.37.

*2-[4-Chloro-2-(2-fluorobenzoylamino)benzoylamino]benzoic Acid (**35f**).* **35f** was synthesized by procedures A, J, A, and C.

^{1}H NMR (500 MHz; pyridine-d_5): δ 13.42 (s, 1H), 12.51 (d, J = 6.4 Hz, 1H), 9.26 (d, J = 2.1 Hz, 1H), 9.09 (d, J = 8.3 Hz, 1H), 8.51 (dd, J = 7.8, 1.5 Hz, 1H), 8.22 (td, J = 7.7, 1.7 Hz, 1H), 8.07 (d, J = 8.5 Hz, 1H), 7.55−7.52 (m, 1H, under pyridine-d_5), 7.44−7−39 (m, 1H), 7.22−7.17 (m, 3H), 7.09 (dd, J = 8.5, 2.1 Hz, 1H).

^{13}C NMR (126 MHz; pyridine-d_5): δ 172.42, 166.73, 162.34 (d, J = 2.2 Hz), 160.36 (d, J = 251.0 Hz), 141.52, 141.32, 138.22, 133.95, 133.88, 133.68, 132.04, 131.63 (d, J = 1.6 Hz), 129.22, 124.93 (d, J = 3.4 Hz), 123.59, 123.12, 121.90, 120.80, 120.47, 118.96, 116.62 (d, J = 23.4 Hz).

ESIMS m/z calcd for $[C_{21}H_{13}ClFN_2O_4]^-$, 411.06; found, 411.15.

*2-[5-Chloro-2-(2-fluorobenzoylamino)benzoylamino]benzoic Acid (**35g**).* **35g** was synthesized by procedures A, J, A, and C.

^{1}H NMR (500 MHz; pyridine-d_5): δ 13.39 (s, 1H), 12.28 (d, J = 6.2 Hz, 1H), 9.07 (t, J = 8.7 Hz, 2H), 8.51 (d, J = 7.8 Hz, 1H), 8.22 (td, J = 7.6, 1.5 Hz, 1H), 8.19 (d, J = 2.3 Hz, 1H), 7.53 (m, 2H), 7.43−7.39 (m, 1H), 7.22−7.16 (m, 4H).

^{13}C NMR (126 MHz; pyridine-d_5): δ 172.21, 166.12, 162.13 (d, J = 2.2 Hz), 160.35 (d, J = 250.7 Hz), 141.32, 138.73, 133.82, 133.75, 133.58, 132.26, 132.03, 131.63 (d, J = 2.0 Hz), 128.41, 127.88, 124.88 (d, J = 3.2 Hz), 124.49, 123.99, 120.52, 119.35, 116.57 (d, J = 23.1 Hz).

ESIMS m/z calcd for $[C_{21}H_{13}ClFN_2O_4]^-$, 411.06; found, 411.15.

*2-[4-Methoxy-2-(2-fluorobenzoylamino)benzoylamino]benzoic Acid (**35h**).* **35h** was synthesized by procedures A, E, A, and C.

^{1}H NMR (400 MHz, pyridine-d_5): δ 13.25 (s, 1H), 12.82 (d, J = 4.6 Hz, 1H), 9.07 (d, J = 8.3 Hz, 1H), 8.87 (br s, 1H), 8.47 (d, J = 7.8 Hz, 1H), 8.20−8.15 (m, 2H), 7.51 (t, J = 7.8 Hz, 1H), 7.47−7.40 (m, 1H), 7.25−7.15 (m, under pyridine-d_5, 3H), 6.73 (dd, J = 8.8, 2.5 Hz, 1H), 3.72 (s, 3H).

^{13}C NMR (100 MHz, pyridine-d_5): δ 172.38, 167.60, 163.14, 162.59 (d, J = 2.0 Hz), 160.27 (d, J = 250.8 Hz), 142.49, 141.86, 133.80, 133.71, 132.06, 131.39 (d, J = 1.5 Hz), 129.59, 124.97 (d, J = 3.3 Hz), 122.96, 120.44, 118.64, 116.66 (d, J = 23.1 Hz), 114.32, 109.68, 107.16, 55. 32.

ESIMS m/z calcd for $[C_{22}H_{18}FN_2O_5]^+$, 409.38; found, 409.45.

*2-[5-Methoxy-2-(2-fluorobenzoylamino)benzoylamino]benzoic Acid (**35i**).* **35i** was synthesized by procedures A, E, A, and C.

^{1}H NMR (360 MHz, pyridine-d_5): δ 13.35 (s, 1H), 12.21 (d, J = 6.6 Hz, 1H), 9.15 (d, J = 8.3 Hz, 1H), 9.08 (d, J = 9.1 Hz, 1H), 8.49 (d, J = 7.7 Hz, 1H), 8.27 (t, J = 7.5 Hz, 1H), 7.78 (d, J = 2.3 Hz, 1H), 7.54 (t, J = 8.0 Hz, 1H), 7.44−7.38 (m, 1H), 7.23−7.17 (m, under pyridine-d_5, 4H), 3.77 (s, 3H).

^{13}C NMR (91 MHz, pyridine-d_5): δ 172.41, 167.30, 161.94, 160.76 (d, J = 250.0 Hz), 155.82, 141.79, 133.87, 133.61 (d, J = 8.7 Hz), 133.45, 132.16, 131.76, 124.98 (d, J = 3.1 Hz), 124.43, 124.33, 123.61, 123.30, 120.47, 118.87, 118.67, 116.66 (d, J = 24.1 Hz), 112.61, 55. 40.

ESIMS m/z calcd for $[C_{22}H_{18}FN_2O_5]^+$, 409.11; found, 409.39.

*2-[4,5-Difluoro-2-(2-fluorobenzoylamino)benzoylamino]benzoic Acid (**35j**).* **35j** was synthesized by procedures A, E, A, and C.

^{1}H NMR (360 MHz; pyridine-d_5): δ 14.10 (s, 1H), 12.58 (d, J = 6.3 Hz, 1H), 9.13−9.03 (m, 2 H), 8.58 (d, J = 7.7 Hz, 1H), 8.23 (td, J = 7.8, 1.3 Hz, 1H), 8.01 (t, J = 9.7 Hz, 1H), 7.49 (t, J = 8.1 Hz, 1H), 7.43 (q, J = 6.7 Hz, 1H), 7.25−7.13 (m, 3H).

^{13}C NMR (91 MHz; pyridine-d_5): δ 174.51, 165.64, 162.38, 160.39 (d, J = 251.0 Hz), 145.5 (dd, J = 246.0, 13.8 Hz), 141.35, 137.97, 137.86, 134.14, 134.05, 132.64, 132.34, 131.79, 125.08 (d, J = 2.7 Hz, 1C), 123.30, 122.41, 120.23, 119.21, 116.93 (d, J = 19.0 Hz), 116.78 (d, J = 24.0 Hz), 111.30 (d, J = 23.6 Hz).

ESIMS m/z calcd for $[C_{21}H_{12}F_3N_2O_4]^-$, 413.08; found, 413. 19.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Copies of NMR spectra and calculated physical chemical properties can be found in the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

Ad, adenovirus; BOP, (benzotriazol-1-yloxy)tris-(dimethylamino)phosphonium hexafluorophosphate; DCM, dichloromethane; DIPEA, diisopropylethylamine; DMEM, Dulbecco/Vogt Modified Eagle's Minimal Essential Medium; DMF, dimethyl formamide; DMSO, dimethyl sulfoxide; EC_{50}, effective concentration 50%; FAM, carboxyfluorescein; Fmoc, 9-fluorenylmethyloxycarbonyl; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HPLC, high performance liquid chromatography; PCR, polymerase chain reaction; PEST, penicillin−streptomycin; QM, quantum mechanics; XTT, 2,3-

bis[2-methoxy-4-nitro-5-sulfophenyl]-2*H*-tetrazolium-5-carboxanilide

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