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Removal of Metabolic Liabilities Enables Development of Derivatives of Procaspase-Activating Compound 1 (PAC-1) with Improved Pharmacokinetics

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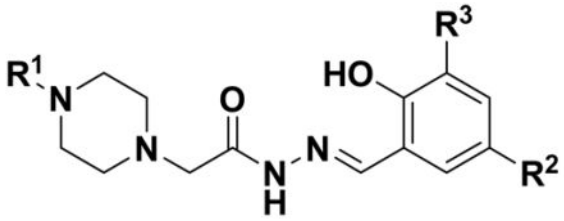
Abstract

Procaspase-Activating Compound 1 (**PAC-1**) is an *ortho*-hydroxy-*N*-acylhydrazone that induces apoptosis in cancer cells by chelation of labile inhibitory zinc from procaspase-3. **PAC-1** has been assessed in a wide variety of cell culture experiments and in vivo models of cancer, with promising results, and a Phase 1 clinical trial in cancer patients has been initiated (NCT02355535). For certain applications, however, the in vivo half-life of **PAC-1** could be limiting. Thus, with the goal of developing a compound with enhanced metabolic stability, a series of **PAC-1** analogues was designed containing modifications that systematically block sites of metabolic vulnerability. Evaluation of the library of compounds identified four potentially superior candidates with comparable anticancer activity in cell culture, enhanced metabolic stability in liver microsomes, and improved tolerability in mice. In head-to-head experiments with **PAC-1**, pharmacokinetic evaluation in mice demonstrated extended elimination half-lives and greater AUC values for each of the four compounds, suggesting them as promising candidates for further development.

Graphical abstract



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Supporting Information

Full biological protocols, copies of NMR spectra, LC traces for liver microsome experiments, formation curves for IC_{50} determination and zinc binding determination. This material is available free of charge via the Internet at http://pubs.acs.org.

Introduction

The development of personalized therapeutics has emerged as a promising strategy in anticancer drug discovery. Translocation, mutation, and abnormal expression of genes can produce unique proteins that exist only in tumors, and the selective modulation of these proteins can kill cancer cells with little effect on healthy cells, minimizing adverse side effects.[1] As evasion of apoptosis is a hallmark of cancer,[2, 3] many recent efforts to develop new anticancer drugs have focused on inhibition of antiapoptotic proteins, including MDM2,[4] Bcl-2,[5, 6] and XIAP.[7] Similarly, small molecules capable of enhancing the activity of proapoptotic proteins hold promise for the treatment of cancer. One target that has received considerable attention is procaspase-3,[8–11] a member of the caspase family of proteases critical to apoptosis. Both the intrinsic and extrinsic pathways of apoptosis converge to activate executioner caspases-3, -6, and -7, from their proenzyme forms.[12, 13] The low frequency of procaspase-3 mutations in cancer,[14] its downstream location relative to apoptotic proteins that are frequently mutated,[13] and the overexpression of procaspase-3 in a number of cancer types, including lymphoma,[15, 16] leukemia,[17, 18] melanoma,[19, 20] glioblastoma,[21, 22] pancreatic cancer,[23] liver cancer,[24] lung cancer,[25–27] breast cancer,[28–31] esophageal cancer,[32] and colon cancer,[8, 33–35] have made the small molecule-mediated activation of procaspase-3 an attractive strategy for personalized medicine.[8–11]

Procaspase-Activating Compound 1 (**PAC-1**, **1**, Figure 1) was identified via a high-throughput screen for compounds that could enhance procaspase-3 enzymatic activity in vitro.[8] **PAC-1** has been shown to be cytotoxic against a diverse array of cancer cells in culture, including cell lines derived from hematopoietic tumors (lymphoma,[8, 36–43] leukemia,[8, 38, 42–47] and multiple myeloma[47]), carcinomas of diverse origin (breast,[8, 38, 42–46, 48–50] renal,[8] adrenal,[8, 51] colon, [8, 42, 47, 49, 50, 52] lung,[8, 42–50, 52–55] cervical,[38, 47] gastric, [42, 43, 47, 49, 50, 52] ovarian,[47] liver,[42, 43, 47] prostate,[42, 43] and gallbladder[42, 43]), and other solid tumor histologies (melanoma,[8, 38, 42, 43] osteosarcoma,[47] neuroblastoma,[8, 47, 49, 50] and glioblastoma[42, 43]). **PAC-1** and derivatives also induce apoptosis in patient-derived samples from colon cancer[8] and chronic lymphocytic leukemia,[18] and have anticancer efficacy in multiple murine tumor models[8, 42, 43, 48, 54–56] and in pet dogs with cancer.[38]

The *ortho*-hydroxy-*N*-acylhydrazone was identified as the key pharmacophore of **PAC-1** through extensive studies of structure-activity relationships (SAR).[36, 37] Several **PAC-1** derivatives containing this motif have comparable activity in vitro and in cell culture, but modification of the core results in a loss of activity.[37] The *ortho*-hydroxy-*N*-acylhydrazone is known to chelate metals, including iron,[57] copper,[58] and zinc,[59] and many divalent metal cations are also known to inhibit procaspase[36] and caspase[60–63] enzymes. In particular, zinc from the labile zinc pool, which is bound loosely to certain proteins, co-localizes with procaspase-3 in cells[64] and inhibits the enzymatic activity of both procaspase-3[36] and caspase-3,[61] and a putative binding site on procaspase-3/caspase-3 for labile zinc ions has been identified.[65] The mechanism of action of **PAC-1** most likely involves the chelation of labile zinc from procaspase-3, relieving the zinc-mediated inhibition and allowing procaspase-3 to process itself to the active form.[36, 37, 41] Using genetically-encoded zinc sensors, **PAC-1** has been shown to mobilize the labile zinc pool in cancer cells.[41] Providing

further support to this direct procaspase-3 activation mechanism, cells treated with **PAC-1** or a derivative show cleaved procaspase-3 and poly-ADP ribose polymerase-1 prior to release of cytochrome *c* from the mitochondria or cleavage of initiator procaspases-8 and -9,[8, 42, 43, 66] and **PAC-1**-mediated apoptosis occurs regardless of the status of Bcl-2 family proteins.[67, 68] Because of this unique mechanism, **PAC-1** is increasingly being used as a tool to directly activate procaspase-3 in a variety of biological settings.[66, 67, 69, 70] In addition, **PAC-1** and derivatives have shown synergy with experimental therapeutics[18, 48] and with the anticancer drug paclitaxel.[55]

Despite the potential for promiscuity and/or instability with certain *ortho*-hydroxy-*N*-acylhydrazones,[71] **PAC-1** shows minimal inhibitory activity towards zinc-dependent enzymes, including matrix metalloproteinases-9 and -14,[72] and a derivative of **PAC-1** showed minimal inhibition toward carboxypeptidase A and histone deacetylases.[18] These results are consistent with the known modest affinity of **PAC-1** for zinc,[36] allowing for a high degree of selectivity for chelation of zinc ions from the labile pool over essential zinc ions in canonical zinc binding sites within metalloproteins. In addition, **PAC-1** is stable in aqueous solution; degradation of **PAC-1** is observed only when the compound is subjected to extremes in temperature and pH outside of relevant physiological ranges.[73]

Pharmacokinetic studies with **PAC-1** in mice and dogs revealed that serum concentrations of approximately 10 μM can be achieved with few adverse events,[39] with transient neuroexcitation observed only at elevated doses when **PAC-1** is administered via IV or IP injection.[38] A Phase 1 clinical trial of **PAC-1** given orally to cancer patients has been initiated (NCT02355535). A sulfonamide-containing derivative of **PAC-1**, called **S-PAC-1** (**2**, Figure 1), is well tolerated by mice at doses of 350 mg/kg or higher via IP injection, with peak plasma concentrations of 3.5 mM at this dose.[38] It is likely that the improved safety profile is due in large part to the decreased ability of **S-PAC-1** to cross the blood-brain barrier, as compared to **PAC-1**.[41] In addition to the ability of **S-PAC-1** to induce cell death to a variety of cancer cell lines in culture,[38] recent efforts have demonstrated the potential for **S-PAC-1** to sensitize cancer cells in culture to ionizing radiation.[74] Encouragingly, **S-PAC-1** was effective in reducing or stabilizing tumor growth in four out of six canine patients with spontaneously occurring lymphoma, and the compound was well tolerated in all six dogs.[38] These results demonstrate the potential for procaspase-3 activation as a safe and promising anticancer strategy.

While studies with **PAC-1** and **S-PAC-1** have been encouraging, a challenge in using these compounds in animals is the relatively short in vivo half-lives of both **PAC-1** (2.1 ± 0.3 h in dogs)[39] and **S-PAC-1** (1.09 ± 0.02 h in dogs)[38] following IV administration. A study in rats identified three main pathways of metabolism for **PAC-1**, including oxidative *N*-debenzylation, olefin oxidation, and arene oxidation (Figure 2).[75] While many of these metabolites may be active based on the predicted structure-activity relationships, the alcohols and secondary amines resulting from these metabolites provide sites for conjugation, including sulfation and glucuronidation; these conjugates are then cleared from circulation. The metabolic liabilities present in **PAC-1** likely contribute to its pharmacokinetic profile, necessitating relatively large doses to achieve therapeutic levels in

vivo. A **PAC-1** analogue lacking some of these liabilities may allow for lower dosing, which could potentially reduce off-target toxicity. In this work, we describe the design, synthesis, and evaluation of a family of **PAC-1** derivatives with the goal of enhancing metabolic stability, and we report on promising compounds with enhanced metabolic stability in vitro and in vivo.

Results

Library Design

The structure-activity relationships of **PAC-1** indicate that modifications to the aryl rings can be tolerated, as long as the core *ortho*-hydroxy-*N*-acylhydrazone remains intact.[8, 36–38] The synthetic strategy that has been adopted to access these active compounds involves the late-stage condensation of a hydrazide and an aldehyde to form the key *ortho*-hydroxy-*N*-acylhydrazone.[8, 37, 38, 40, 42–46, 49, 51, 53] This strategy was useful for the generation of a large combinatorial library of 837 diverse **PAC-1** analogues.[40] However, for this study, we sought a more focused library design, with an emphasis on the creation of derivatives with systematic removal of the metabolic liabilities. The library (Figure 3) consists of 45 **PAC-1** analogues (**1–45**), constructed from nine hydrazides (**46a-i**) and five aldehydes (**47a-e**). In order to avoid oxidative *N*-debenzylation, the benzyl moiety was modified to a benzoyl (as in **46c**, **46e**, **46g**, and **46i**), hypothesized to be more resistant to oxidation.[76] In order to avoid olefin oxidation, the allyl group was changed to a propyl group (as in **47b** and **47e**) or removed entirely (as in **47c**). Finally, in order to block arene oxidation, building blocks were introduced containing nitrile (**46d-e**), fluorine (**46f-g**, **47c-e**), and trifluoromethyl (**46h-i**) substituents. Multiple derivatives were synthesized containing only one modification to the **PAC-1** core, so that the effect of individual changes could be systematically evaluated.

Compound Synthesis

Synthesis of the library involved the construction of the nine hydrazides and five aldehydes, followed by the condensation of each hydrazide with each aldehyde to give the **PAC-1** derivatives. The hydrazides were synthesized according to Scheme 1a. The synthesis began with the alkylation of piperazine (**48**) with ethyl chloroacetate (**49**) to form monosubstituted piperazine **50**. Compound **50** was then reacted with substituted benzyl or benzoyl halides to give disubstituted piperazines **51a-i** in high yields. Reaction of the esters with hydrazine then gave hydrazides **46a-i**.

Synthesis of the aldehydes is shown in Scheme 1b. Both salicylaldehyde (**52**) and 5-fluorosalicylaldehyde (**47c**) were alkylated with allyl bromide to give allyloxybenzaldehydes **53a-b** in high yields. These compounds underwent Claisen rearrangements upon heating at 200°C, yielding aldehydes **47a** and **47d** in approximately 50% yield. Finally, hydrogenation with diphenyl sulfide as a catalyst poison allowed for chemoselective reduction of the olefins,[77] giving aldehydes **47b** and **47e** in high yield. As shown in Scheme 1c, each of the hydrazides (**46a-i**) was condensed with each of the aldehydes (**47a-e**) in the presence of a catalytic amount of HCl to give **PAC-1** derivatives **1–45**, the structures of which are given in Table 1. Chromatographic purification of the library members yielded the **PAC-1** analogues in high purity (97% average purity). All derivatives were at least 95% pure except

compounds **20**, **22**, and **24**; because this standard of purity was not met for these three compounds, they were excluded from further evaluation.

Evaluation of PAC-1 analogues

Upon completion of the synthesis of the 45 **PAC-1** derivatives, the compounds were evaluated in biological assays. First, the ability of the compounds to induce cell death in U-937 (human lymphoma) cells in culture was determined (Table 1 and Supporting Information). Each of the compounds induced dose-dependent cell death under these conditions, and most of the compounds were approximately as potent as **PAC-1** and **S-PAC-1**, confirming the previously determined SAR.[8, 37, 38, 40]

The metabolic stability of the compounds was then evaluated in rat liver microsomes. The compounds were incubated with liver microsomes for 3 hours at 10 μM, and the metabolites were observed by LC/MS, with (±)-propranolol hydrochloride as a positive control;[78] approximately 20% of the control remained. The results of this assay are shown in Table 1. Compounds that contained benzoyl substituents were significantly more stable than analogous compounds containing benzyl groups; for example, compound **3** was more stable than **PAC-1**, and compound **32** was more stable than compound **31**. The propyl-containing compounds were less stable than the allyl-containing compounds (e.g., **PAC-1** was more stable than compound **10**). In addition, **S-PAC-1** was relatively stable in the liver microsomes, despite the short in vivo half-life of the compound.[38] This suggests that clearance mechanisms other than oxidative metabolism are responsible for the elimination of **S-PAC-1** from treated animals.

The results of selected liver microsome experiments are shown in Figure 4 (The full set of results is in the Supporting Information). **PAC-1** (Figure 4A) was 38% stable in the assay, and several metabolites, including an *N*-dealkylated product, a dihydroxylated product, and multiple monooxygenated products, were observed. **S-PAC-1** (Figure 4B) was found to be more stable than **PAC-1**, and fewer metabolites were observed. One of the modifications that improved stability to the greatest degree was the addition of the benzoyl in place of the benzyl substituent, as demonstrated with compound **3** (Figure 4C). This modification prevented the *N*-debenzylation completely and increased stability to 89% during the three-hour incubation. In addition, compounds with a benzoyl substituent had fewer monooxygenated species than **PAC-1**, as the amide likely acted to deactivate the aromatic ring towards oxidation. The addition of fluorine to the benzylidene ring, as in compound **19** (Figure 4D), was also successful in reducing the number of monooxygenated metabolites, and as expected, dihydroxylated metabolites were not formed from compounds lacking the allyl group. Finally, combining multiple modifications, as in compounds **7** (Figure 4E) and **32** (Figure 4F), led to highly stable compounds that gave significantly fewer metabolites in the liver microsome experiment.

Evaluation of toxicity in mice

Based on the comparable cytotoxicity and improved in vitro metabolic stability profile, several compounds were selected for further in vivo evaluation. In order to assess the in vivo tolerability of the compounds, they were administered to mice via intraperitoneal injection at

a dose of 200 mg/kg, the maximum tolerated dose of **PAC-1**.[48] Compounds were formulated at 5 mg/mL in a 200 mg/mL aqueous solution of 2-hydroxypropyl-β-cyclodextrin. The results of this experiment are shown in Table 1. Responses are graded as mild, moderate, or severe; compounds that were lethal to the mice at 200 mg/kg were also noted. Compounds **9** and **18** were lethal at a lower dose of 100 mg/kg, and compound **36** induced hemolysis in the animals, so the compounds containing the (trifluoromethyl)benzoyl substituent were not pursued further.

Secondary assays

Because of their high stability, comparable potency, and improved in vivo tolerability as compared to **PAC-1**, compounds **7**, **30**, **32**, and **41** were selected for further investigation. In order to confirm that the hit compounds act similarly to **PAC-1**, the compounds were evaluated for their ability to chelate zinc in vitro, activate executioner caspases in whole cells, and induce apoptosis in cancer cells. Zinc binding was determined using an EGTA titration experiment.[79] In this experiment, varying concentrations of $Zn(OTf)_2$ were added to each well of a 96-well plate with a HEPES-buffered solution containing EGTA and **PAC-1** derivative, and the fluorescence of the complex was analyzed, a slight variant of our previous protocol for assessment of zinc binding.[37] As shown in Table 2, **PAC-1** binds zinc with a K_d of 1.28 ± 0.03 nM, while **S-PAC-1** binds zinc with a K_d of 2.72 ± 0.13 nM. Each of the four new compounds displays affinity for zinc in the range of 1–2 nM. **PAC-1a**, an inactive derivative of **PAC-1** lacking the allyl and hydroxyl substituents,[8, 36, 37] does not bind zinc.

In addition, the ability of compounds to activate executioner caspases in whole cells was evaluated. Cells were treated with compound for 0 or 16 hours, then the cells were lysed, and cleavage of the fluorescent caspase-3/-7 substrate Ac-DEVD-AFC was analyzed via kinetic reads. The percent activity at 16 hours was normalized to the slope of each compound at 0 hours (0% activity) and the slope of the positive control compound staurosporine at 16 hours (100% activity). As shown in Table 2 and Figure 5, **PAC-1** induces the highest degree of caspase activation, while each of the other active compounds induces greater than 60% activation of the executioner caspases. As expected, treatment with DMSO alone or **PAC-1a** induces minimal caspase activity in the cells.

In order to determine the mode of cell death induced by the compounds, U-937 cells were treated with compounds at 50 μM for 12 hours, and viability was assessed by Annexin V-FITC/propidium iodide staining (Figure 6). Each of these compounds induced approximately 50% cell death under these conditions, and the presence of large populations in the lower right quadrants of the histograms (Annexin V-FITC positive, propidium iodide negative) confirms that the compounds induce apoptosis in these cancer cells.

As many **PAC-1** derivatives show activity against white blood cell cancer lines[8, 37, 38, 40, 42–47] and patient-derived leukemic lymphocytes[18] in culture, the compounds were evaluated for their ability to induce cell death in a panel of lymphoma and leukemia cell lines, including Jurkat (human leukemia), GL-1 (dog lymphoma), OSW (dog lymphoma), and EL4 (mouse lymphoma) cells, in order to complement the previously

determined IC_{50} values in U-937 (human lymphoma) cells. As shown in Table 3, the compounds displayed comparable potency against each given cell line. These results provide further support to the previously determined structure-activity relationships, as the modifications to improve metabolic stability had minimal effect on the activity of the new compounds, and further suggest the potential of **PAC-1** and derivatives for the treatment of white blood cell cancers.

Pharmacokinetics

Because compounds **7**, **30**, **32**, and **41** all chelate zinc, activate executioner caspases in whole cells, and induce apoptosis, all four of the hit compounds were studied further in vivo. The pharmacokinetics of the four compounds plus **PAC-1** and **S-PAC-1** were evaluated in mice at a dose of 25 mg/kg (IV injection), and the results are shown in Figure 7 and Table 4. **PAC-1** and **S-PAC-1** were cleared rapidly and were no longer detectable after 5 and 6 hours post-treatment, respectively. In contrast, detectable levels of each of the four new derivatives remained in circulation for at least 8 hours post-treatment.

The elimination half-life of **PAC-1** was 24.6 ± 0.9 minutes, and the half-life of **S-PAC-1** was 38.1 ± 3.3 minutes. Each of the four new derivatives displayed half-lives of at least 88 minutes, with compound **41** having the longest half-life at 122.3 ± 1.4 minutes. In addition, AUC values from intravenous administration for the four new derivatives were all significantly higher than that of **PAC-1**. Compounds **7**, **32**, and **41** were also found to display increased oral bioavailability as compared to **PAC-1** and **S-PAC-1**.

Discussion

The introduction of substituents designed to block oxidative metabolism is among the most attractive methods to achieve a favorable pharmacokinetic profile, as the drug can pass through the liver without being modified and remain in circulation for longer periods of time. The knowledge of the metabolites formed from **PAC-1** in vivo facilitated the design of a library of **PAC-1** derivatives whose members lacked many of the metabolic liabilities present on the parent compound. The flexible, modular nature of the **PAC-1** synthesis allowed for the rapid generation of 45 derivatives from nine hydrazides and five aldehydes.

Metal binders in medicine

Given the increased attention paid to so-called “PAINS” (pan-assay interference compounds), a further discussion of **PAC-1** and derivatives in relationship to PAINS compounds is warranted. The metal-binding ability of *ortho*-hydroxy-*N*-acylhydrazones can cause members of this class of compounds to appear as hits in screening assays due to interference with the assay screening system, rather than via specific interactions with biological targets.[71] In these cases, attempts to validate such hits will fail because the apparent activity of the screening hit is unrelated to the target. However, rather than interfering with the in vitro procaspase-3 enzymatic assay, the chelation of zinc from procaspase-3 in vitro by **PAC-1** is highly biologically relevant: **PAC-1** is directly modulating zinc, an endogenous inhibitor of procaspase-3, and the binding site on procaspase-3/caspase-3 for this inhibitory zinc has been identified.[65] Through this

"inhibiting the inhibitor" mechanism, **PAC-1** is akin to compounds that bind to other endogenous apoptotic inhibitors and induce apoptosis, such as those binding MDM2[4] and XIAP.[7]

PAC-1 also chelates labile zinc in cancer cells in culture, as determined by detailed experiments with genetically encoded fluorescent sensors specific for zinc.[41] This removal of zinc leads to the observed anticancer effect in cell culture. **PAC-1** shows no activity toward several other enzymes as assessed by in vitro assays,[80] **PAC-1** and derivatives do not affect the activity of proteins that rely on tightly-bound zinc,[18, 72] and **PAC-1** derivatives that do not bind zinc in vitro are inactive in cells.[37] As shown by multiple investigators, treatment of cells with **PAC-1** or a derivative results in the cleavage of procaspase-3 prior to the cleavage of initiator procaspases-8 and -9,[42, 43] and co-treatment of **PAC-1** with a covalent inhibitor of caspase-9 does not prevent cleavage of procaspase-3,[66] further supporting the hypothesis that the proapoptotic activity of **PAC-1** is due to chelation of labile inhibitory zinc from procaspase-3, leading to the activation of procaspase-3 and apoptotic cell death.

While many metal chelators will interfere with in vitro enzyme assays, it would be inappropriate to disregard all metal chelators from consideration as drug candidates due to this in vitro artifact. Indeed, metal chelators have a rich history in drug discovery and have made a positive impact on many diseases through a diverse range of mechanisms and targets.[81] The many examples of therapeutic metal-binding compounds include the marketed drugs vorinostat (Zn^{2+}),[82] penicillamine (Cu^{2+}),[81] and the entire class of bisphosphonates (Ca^{2+}),[83] as well as the experimental therapeutics elesclomol (Cu^{2+}),[81] ML-133/Apto-253 (Zn^{2+}),[84] and triapine (Fe^{3+}/Fe^{2+}),[85] all of which rely on metal chelation in vivo for their mechanism of action. It is safe to say that many of these compounds would interfere with certain in vitro assays that are contingent upon metal-bound proteins. While it is reasonable for metal chelators and metal-chelating motifs to be structural alerts when examining screening hits, if the desired biological activity is metal chelation, then obviously metal chelation is the precise trait to look for in a screening hit.

Translational potential of PAC-1 derivatives

Cell culture evaluation of the compounds reported herein confirm previously determined structure-activity relationships, in that substituents can be introduced to the aromatic rings without abolishing activity if the core *ortho*-hydroxy-*N*-acylhydrazone remains intact. Removal of the allyl group leads to a diminution in cell culture potency, consistent with previous reports,[8, 37] although reduction to the fully saturated propyl group was tolerated in the cell culture experiment. It is likely that the increased hydrophobicity of the alkyl chain contributes to increased cell permeability, as the allyl group does not affect the ability of **PAC-1** to bind zinc.[37]

The benzoyl-containing compounds display similar cell culture activity to **PAC-1**. This substitution changes the electronics at both the arene and the piperazine nitrogen; the role of the benzylpiperazine in **PAC-1** activity merits further evaluation. Evaluation of the metabolic stability of the library members in rat liver microsomes suggested that *N*-

debenzylation was the main route of metabolism in vitro; the **PAC-1** derivatives containing benzoyl substituents were more stable than those containing benzyl substituents. These substitutions also reduced the extent of arene oxidation, providing further support for advancement of these compounds.

Four compounds (**7**, **30**, **32**, and **41**) were identified with favorable cell culture potency, in vitro metabolic stability, and in vivo tolerability. Each of these compounds contains the benzoyl substitution, as well as at least one arene substituent (fluorine and/or nitrile) not present on **PAC-1**. The introduction of fluorine is common in medicinal chemistry, especially the use of aryl fluorides to block undesired metabolic arene oxidation, as in the cholesterol-lowering drug ezetimibe.[86] Aryl nitriles are also commonly employed to accomplish this goal, as nitriles typically pass through the body unmodified, and the electron-withdrawing nature of the group deactivates the arene towards oxidative metabolism at other sites.[87] Trifluoromethyl groups can deactivate arenes similarly in certain cases,[88] and in vitro results with the (trifluoromethyl)benzoyl-containing **PAC-1** derivatives were encouraging. However, these compounds were not evaluated further due to unacceptable levels of toxicity in vivo.

Further evaluation of the four lead compounds demonstrates that they chelate zinc, activate executioner caspases in whole cells, and induce apoptosis similarly to **PAC-1** and **S-PAC-1**. The four derivatives display three- to five-fold higher elimination half-lives and up to two-fold higher overall compound exposure compared to **PAC-1**. Results from the liver microsome experiment are mostly consistent with the observed in vivo pharmacokinetic profiles: fewer metabolites formed from the new derivatives than from **PAC-1** in vitro, and the compounds remain in serum for longer periods of time than **PAC-1**. In contrast, **S-PAC-1** is stable in the liver microsome assay but has a relatively short in vivo half-life. This suggests that the main mode of clearance for **S-PAC-1** may not be via oxidative metabolism; instead, the compound may be excreted without modification. A more thorough understanding of this phenomenon may allow for the design of **PAC-1** derivatives that improve upon the pharmacokinetics even further than those described in this report. The rapid clearance of **PAC-1** and **S-PAC-1** from circulation makes them challenging to evaluate in certain efficacy models in vivo; these studies typically require large doses of compound that increase the potential for toxicity. The four novel derivatives remain in circulation for longer than either **PAC-1** or **S-PAC-1**, and thus offer promise as novel therapeutic agents for the treatment of cancer.

Experimental

Chemical Information

Materials and Methods

General: All reactions requiring anhydrous conditions were conducted under a positive atmosphere of nitrogen or argon in oven-dried glassware. Standard syringe techniques were used for anhydrous addition of liquids. Unless otherwise noted, all starting materials, solvents, and reagents were acquired from commercial suppliers and used without further purification. Flash chromatography was performed using 230–400 mesh silica gel.

Compound syntheses are discussed in the order in which they appear in the text. Syntheses of **46a**,[8] **46b**,[37] **46h**,[40] **47a**,[37] **50**,[37] **PAC-1** (**1**),[8] **S-PAC-1** (**2**),[37] and **PAC-1a**[8] have been described previously.

All NMR experiments were recorded either in $CDCl_3$ (Sigma or Cambridge), CD_3OD (Sigma), or $(CD_3)_2CO$ (Sigma or Cambridge) on a Varian Unity 500 MHz spectrometer with residual undeuterated solvent as the internal reference for ^{1}H-NMR and ^{13}C-NMR, and C_6F_6 as the internal reference for ^{19}F-NMR. Chemical shift, δ (ppm); coupling constants, *J* (Hz); multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, sext = sextet, m = multiplet, br = broad); and integration are reported. High-resolution mass spectral data was recorded on a Micromass Q-Tof Ultima hybrid quadrupole/time-of-flight ESI mass spectrometer or a Micromass 70-VSE at the University of Illinois Mass Spectrometry Laboratory. Compound purity was assessed by analytical HPLC (monitoring at 254 nm) on a Waters Alliance e2695 HPLC with a Waters XBridge C18 column, 4.6 × 150 mm. Mobile phase A was 0.1% F_3CCO_2H in H_2O, B was MeCN (solvent B). A gradient was run with 0% B for 1 min, then 0–100% B for 10 min, then constant 100% B for 5 min, then 100-0% B for 1 min, then constant 0% B for 5 min. All compounds evaluated in biological assays were ≥95% pure.

General Procedure A: Synthesis of dialkylated piperazines: To a round-bottom flask were added benzyl halide (1.0 equiv.), K_2CO_3 (3.0 equiv.), and acetone (0.2 M). The mixture was stirred, and **50** (1.5 equiv.) was added. The reaction mixture was stirred at reflux overnight. The reaction mixture was cooled to room temperature. The solid was filtered and washed with acetone. The filtrate was concentrated, and the product was purified by silica gel column chromatography.

General Procedure B: Synthesis of amides: To an oven-dried round-bottom flask were added **50** (1.0 equiv.), anhydrous tetrahydrofuran (0.2 M), and freshly distilled Et_3N (2.0 equiv.). The solution was stirred at 0°C under N_2, and the benzoyl chloride (1.0 equiv.) was added. The reaction mixture was stirred overnight at room temperature under N_2. The reaction mixture was diluted with EtOAc and washed with sat. $NaHCO_3$ (2x), H_2O, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The product was purified by silica gel column chromatography.

General Procedure C: Synthesis of hydrazides: To a round-bottom flask were added ethyl ester (1.0 equiv.) and EtOH or 2:1 EtOH:MeOH (0.5 M). The solution was stirred, and anhydrous hydrazine (4.0 equiv.) was added dropwise. The reaction mixture was stirred at reflux overnight. The reaction mixture was cooled to room temperature and concentrated. The resulting residue was partitioned between CH_2Cl_2/1:1 brine:0.1 M KOH. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2x). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. Purification by silica gel column chromatography or recrystallization yielded pure hydrazide.

Ethyl 4-Benzoyl-1-piperazineacetate (51c): Synthesized according to General Procedure B: **50** (2.45 g, 14.2 mmol, 1.0 equiv.), anhydrous tetrahydrofuran (70 mL, 0.2 M), freshly distilled Et_3N (4.0 mL, 28.4 mmol, 2.0 equiv.), benzoyl chloride (**54c**, 2.0 g, 1.7 mL, 1.0

equiv.). Purification by silica-gel column chromatography (50–100% EtOAc/hexanes) afforded **51c** (2.87 g, 73.1%) as a pale yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.41-7.38 (m, 5H), 4.19 (q, 2H, *J* = 7.0 Hz), 3.85 (br s, 2H), 3.48 (br s, 2H), 3.25 (s, 2H), 2.68 (br s), 2.54 (br s, 2H), 1.27 (t, 3H, *J* = 7.0 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$)δ 170.5, 170.2, 135.9, 129.9, 128.7, 127.3, 61.0, 59.4, 53.3 (br), 52.8 (br), 47.8 (br), 42.1 (br), 14.4. HRMS (ESI): 277.1552 $(M+H)^+$; calcd. for $C_{15}H_{21}N_2O_3$: 277.1552.

4-Benzoyl-1-piperazineacetohydrazide (46c): Synthesized according to General Procedure C: **51c** (2.87 g, 10.4 mmol, 1.0 equiv.), anhydrous hydrazine (1.31 mL, 41.6 mmol, 4.0 equiv.), EtOH (20 mL, 0.5 M). **46c** (1.41 g, 51.5%) was obtained as a white solid after extraction without further purification. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.39-7.34 (m, 5H), 3.84 (br s, 2H), 3.77 (br s, 2H), 3.43 (br s, 2H), 3.08 (s, 2H), 2.56 (br s, 2H), 2.44 (br s, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.5, 169.9, 135.5, 130.0, 128.7, 127.1, 60.6, 53.9 (br), 53.4 (br), 47.7 (br), 42.2 (br). HRMS (ESI): 263.1513 $(M+H)^+$; calcd. for $C_{13}H_{19}N_4O_2$: 263.1508.

Ethyl 4-(4-Cyanophenylmethyl)-1-piperazineacetate (51d): Synthesized according to General Procedure A: 4-(bromomethyl)benzonitrile (**54d**, 2.0 g, 10.2 mmol, 1.0 equiv.), **50** (2.64 g, 15.3 mmol, 1.5 equiv.), K_2CO_3 (4.22 g, 30.6 mmol, 3.0 equiv.), acetone (50 mL, 0.2 M). Purification by silica gel column chromatography (50–100% EtOAc/hexanes) afforded **51d** (2.71 g, 92.3%) as a yellow solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.60 (d, 2H, *J* = 8.0 Hz), 7.44 (d, 2H, *J* = 8.0 Hz), 4.18 (q, 2H, *J* = 7.0 Hz), 3.55 (s, 2H), 3.20 (s, 2H), 2.61 (br s, 4H), 2.51 (br s, 4H), 1.26 (t, 3H, *J* = 7.0 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.4, 144.4, 132.3, 129.7, 119.2, 111.0, 62.5, 60.8, 59.6, 53.1, 53.1, 14.4. HRMS (ESI): 288.1718 $(M+H)^+$; calcd. for $C_{16}H_{22}N_3O_2$: 288.1712.

4-(4-Cyanophenylmethyl)-1-piperazineacetohydrazide (46d): Synthesized according to General Procedure C: **51d** (2.71 g, 9.43 mmol, 1.0 equiv.), anhydrous hydrazine (1.18 mL, 37.7 mmol, 4.0 equiv.), EtOH (19 mL, 0.5 M). **46d** (1.73 g, 67.1%) was obtained as an off-white solid after extraction without further purification. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.10 (br s, 1H), 7.60 (d, 2H, *J* = 8.0 Hz), 7.43 (d, 2H, *J* = 8.5 Hz), 3.84 (br d, 2H, *J* = 5.0 Hz), 3.55 (s, 2H), 3.08 (s, 2H), 2.55 (br s, 4H), 2.46 (br s, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.6, 144.1, 132.4, 129.6, 119.1, 111.2, 62.5, 60.8, 53.8, 53.3. HRMS (ESI): 274.1673 $(M+H)^+$; calcd. for $C_{14}H_{20}N_5O$: 274.1668.

Ethyl 4-(4-Cyanobenzoyl)-1-piperazineacetate (51e): Synthesized according to General Procedure B: **50** (5.20 g, 30.2 mmol, 1.0 equiv.), anhydrous tetrahydrofuran (150 mL, 0.2 M), freshly distilled Et_3N (8.4 mL, 60.4 mmol, 2.0 equiv.), 4-cyanobenzoyl chloride (**54e**, 5.0 g, 30.2 mmol, 1.0 equiv.). Purification by silica gel column chromatography (0–10% MeOH/EtOAc) afforded **51e** (5.95 g, 65.4%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.68 (d, 2H, *J* = 8.0 Hz), 7.47 (d, 2H, *J* = 8.0 Hz), 4.14 (q, 2H, *J* = 7.0 Hz), 3.80 (br s, 2H), 3.37 (br s, 2H), 3.23 (s, 2H), 2.67 (br s, 2H), 2.53 (br s, 2H), 1.23 (t, 3H, *J* = 7.0 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.0, 168.3, 140.1, 132.5, 127.9, 118.2, 113.6, 60.9, 59.0, 52.9, 52.3, 47.5, 42.1, 14.3. HRMS (ESI): 302.1501 $(M+H)^+$; calcd. for $C_{16}H_{20}N_3O_3$: 302.1505.

4-(4-Cyanobenzoyl)-1-piperazineacetate (46e): Synthesized according to General Procedure C with modification as noted: **51e** (5.59 g, 18.6 mmol, 1.0 equiv.), anhydrous hydrazine (2.4 mL, 74.4 mmol, 4.0 equiv.), EtOH (35 mL, 0.5 M). After extraction with CH_2Cl_2, the aqueous layer was extracted with EtOAc (3x). **46e** (2.98 g, 55.8%) was obtained as an off-white solid after extraction without further purification. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.02 (br s, 1H), 7.70 (d, 2H, *J* = 8.5 Hz), 7.48 (d, 2H, *J* = 8.5 Hz), 3.86 (br d, 2H, *J* = 3.5 Hz), 3.78 (br s, 2H), 3.37 (br s, 2H), 3.11 (s, 2H), 2.60 (br s, 2H), 2.46 (br s, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.8, 168.4, 139.8, 132.6, 127.9, 118.1, 113.8, 60.6, 53.8 (br), 53.3 (br), 47.6 (br), 42.2 (br). HRMS (ESI): 288.1464 $(M+H)^+$; calcd. for $C_{14}H_{18}N_5O_2$: 288.1461.

Ethyl 4-(4-Fluorophenylmethyl)-1-piperazineacetate (51f): Synthesized according to General Procedure A: 4-fluorobenzyl chloride (**54f**, 2.5 g, 2.1 mL, 17.3 mmol, 1.0 equiv.), **50** (4.48 g, 26.0 mmol, 1.5 equiv.), K_2CO_3 (7.19 g, 52.0 mmol, 3.0 equiv.), acetone (90 mL, 0.2 M). Purification by silica gel column chromatography (gradient, 50–100% EtOAc/hexanes) afforded **51f** (3.66 g, 75.4%) as a yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.24-7.20 (m, 2H), 6.95-6.91 (m, 2H), 4.13 (q, 2H, *J* = 7.0 Hz), 3.42 (s, 2H), 3.15 (s, 2H), 2.55 (br s, 4H), 2.46 (br s, 4H), 1.21 (t, 3H, *J* = 7.0 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.3, 162.0 (d, $J_{C\text{-}F}$ = 243.5 Hz), 133.9, 130.6 (d, $J_{C\text{-}F}$ = 8.0 Hz), 115.0 (d, $J_{C\text{-}F}$ = 21.0 Hz), 62.2, 60.6, 59.6, 53.1, 52.8, 14.3. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −119.1. HRMS (ESI): 281.1659 $(M+H)^+$; calcd. for $C_{15}H_{22}FN_2O_2$: 281.1665.

4-(4-Fluorophenylmethyl)-1-piperazineacetohydrazide (46f): Synthesized according to General Procedure C: **51f** (3.0 g, 10.7 mmol, 1.0 equiv.), anhydrous hydrazine (1.4 mL, 42.8 mmol, 4.0 equiv.), EtOH (20 mL, 0.5 M). **46f** (2.59 g, 91.1%) was obtained as a white solid after extraction without further purification. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.15 (br s, 1H), 7.22-7.19 (m, 2H), 6.95-6.91 (m, 2H), 3.84 (br s, 2H), 3.40 (s, 2H), 3.01 (s, 2H), 2.47 (br s, 4H), 2.39 (br s, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.5, 162.0 (d, $J_{C\text{-}F}$ = 243.6 Hz), 133.7 (d, $J_{C\text{-}F}$ = 2.8 Hz), 130.6 (d, $J_{C\text{-}F}$ = 8.3 Hz), 115.1 (d, $J_{C\text{-}F}$ = 21.1 Hz), 62.0, 60.6, 53.7, 53.0. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −118.9. HRMS (ESI): 267.1630 $(M+H)^+$; calcd. for $C_{13}H_{20}FN_4O$: 267.1621.

Ethyl 4-(4-Fluorobenzoyl)-1-piperazineacetate (51g): Synthesized according to General Procedure B: **50** (2.58 g, 15.0 mmol, 1.0 equiv.), anhydrous tetrahydrofuran (30 mL, 0.5 M), freshly distilled Et_3N (4.2 mL, 30.0 mmol, 2.0 equiv.), 4-fluorobenzoyl chloride (**54g**, 1.8 mL, 15.0 mmol, 1.0 equiv.). Purification by silica gel column chromatography (50–100% EtOAc/hexanes) afforded **51g** (3.74 g, 84.7%) as a pale yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.38-7.34 (m, 2H), 7.06-7.01 (m, 2H), 4.13 (q, 2H, *J* = 7.0 Hz), 3.77 (br s, 2H), 3.43 (br s, 2H), 3.21 (s, 2H), 2.61 (br s, 2H), 2.52 (br s, 2H), 1.22 (t, 3H, *J* = 7.0 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.0, 169.4, 163.5 (d, $J_{C\text{-}F}$ = 248.1 Hz), 131.8, 129.5 (d, $J_{C\text{-}F}$ = 8.3 Hz), 115.6 (d, $J_{C\text{-}F}$ = 22.0 Hz), 60.8, 59.1, 52.8 (br), 47.8 (br), 42.2 (br), 14.3. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −113.4. HRMS (ESI): 295.1457 $(M+H)^+$; calcd. for $C_{15}H_{20}FN_2O_3$: 295.1458.

4-(4-Fluorobenzoyl)-1-piperazineacetohydrazide (46g): Synthesized according to General Procedure C: **51g** (3.73 g, 12.7 mmol, 1.0 equiv.), anhydrous hydrazine (1.6 mL, 50.8 mmol, 4.0 equiv.), EtOH (25 mL, 0.5 M). **46g** (2.28 g, 64.1%) was obtained as a white solid after

extraction without further purification. ^{1}H-NMR (500 MHz, $CDCl_3$) δ ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.09 (br s, 1H), 7.37-7.33 (m, 2H), 7.06-7.02 (m, 2H), 3.85 (br s, 2H), 3.70 (br s, 2H), 3.42 (br s, 2H), 3.06 (s, 2H), 2.48 (br s, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.8, 169.5, 163.5 (d, J_{C-F} = 249.1 Hz), 131.5 (d, J_{C-F} = 2.8 Hz), 129.4 (d, J_{C-F} = 9.1 Hz), 115.7 (d, J_{C-F} = 22.0 Hz), 60.6, 53.5 (br), 47.7 (br), 42.3 (br). ^{19}F-NMR (470 MHz, $CDCl_3$) δ −113.1. HRMS (ESI): 281.1409 $(M+H)^+$; calcd. for $C_{13}H_{18}FN_4O_2$: 281.1414.

Ethyl 4-[4-(Trifluoromethyl)benzoyl]-1-piperazineacetate (51i): Synthesized according to General Procedure B: **50** (2.58 g, 15.0 mmol, 1.0 equiv.), anhydrous tetrahydrofuran (30 mL, 0.5 M), freshly distilled Et_3N (4.2 mL, 30.0 mmol, 2.0 equiv.), 4-(trifluoroemthyl)benzoyl chloride (**54i**, 2.2 mL, 15.0 mmol, 1.0 equiv.). Purification by silica-gel column chromatography (50–100% EtOAc/hexanes) afforded **51i** (4.01 g, 77.5%) as a yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.65 (d, 2H, J = 8.0 Hz), 7.49 (d, 2H, J = 8.0 Hz), 4.16 (q, 2H, J = 7.0 Hz), 3.82 (br s, 2H), 3.41 (br s, 2H), 3.24 (s, 2H), 2.68 (br s, 2H), 2.54 (br s, 2H), 1.24 (t, 3H, J = 7.0 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$)δ 170.1, 168.9, 139.4 131.8 (q, J_{C-F} = 32.6 Hz), 127.6, 125.7 (q, J_{C-F} = 3.8 Hz), 123.8 (q, J_{C-F} = 271.0 Hz), 60.9, 59.1, 53.0 (br), 52.5 (br), 47.6 (br), 42.2 (br), 14.3. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −66.0. HRMS (ESI): 345.1430 $(M+H)^+$; calcd. for $C_{16}H_{20}F_3N_2O_3$: 345.1426.

4-[4-(Trifluoromethyl)benzoyl]-1-piperazineacetohydrazide (46i): Synthesized according to General Procedure C: **51i** (4.00 g, 11.6 mmol, 1.0 equiv.), anhydrous hydrazine (1.5 mL, 46.4 mmol, 4.0 equiv.), EtOH (25 mL, 0.5 M). **46i** (2.35 g, 61.4%) was obtained as a white solid after extraction without further purification. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.09 (br s, 1H), 7.62 (d, 2H, J = 8.0 Hz), 7.45 (d, 2H, J = 8.0 Hz), 3.88 (br s, 2H) 3.75 (br s, 2H), 3.35 (br s, 2H), 3.07 (s, 2H), 2.56 (br s, 2H), 2.42 (br s, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$)δ 169.8, 168.9, 139.1 131.8 (q, J_{C-F} = 32.1 Hz), 127.5, 125.7 (q, J_{C-F} = 3.6 Hz), 123.7 (q, J_{C-F} = 271.1 Hz), 60.5, 53.7 (br), 53.2 (br), 47.6 (br), 42.2 (br). ^{19}F-NMR (470 MHz, $CDCl_3$) δ −66.0. HRMS (ESI): 331.1374 $(M+H)^+$; calcd. for $C_{14}H_{18}F_3N_4O_2$: 331.1382.

2-Hydroxy-3-propylbenzaldehyde (47b): To a round-bottom flask were added aldehyde **47a** (1.62 g, 10.0 mmol, 1.0 equiv.), 5% Pd/C (324 mg, 20 wt% of **47a**), diphenyl sulfide (17 μL, 0.10 mmol, 0.010 equiv.), and EtOAc (40 mL, 0.25 M). The reaction mixture was stirred overnight at room temperature under an atmosphere of H_2 (balloon pressure). The reaction mixture was filtered through Celite and washed thoroughly with EtOAc. The filtrate was concentrated to afford aldehyde **47b** (1.50 g, 91.7%) as a yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.27 (s, 1H), 9.88 (s, 1H), 7.41-7.38 (m, 2H), 6.95 (t, 1H, J = 7.5 Hz), 2.64 (t, 2H, J = 7.5 Hz), 1.65 (sext, 2H, J = 7.5 Hz), 0.96 (t, 3H, J = 7.5 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 197.0, 160.0, 137.4, 131.7, 131.4, 120.4, 119.6, 31.3, 22.7, 14.1. HRMS (EI): 164.08383 (M^+); calcd. for $C_{10}H_{12}O_2$: 164.08373.

5-Fluoro-2-(2-propenyloxy)benzaldehyde (53b): To a round bottom flask were added 5-fluorosalicylaldehyde (**47c**, 4.0 g, 28.5 mmol, 1.0 equiv.), potassium carbonate (4.92 g, 35.6 mmol, 1.25 equiv.), and DMF (20 mL). Allyl bromide (3.7 mL, 42.8 mmol, 1.5 equiv.) was added slowly to the mixture. The reaction mixture was stirred overnight at room temperature. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with water (2 × 25 mL),

0.1M KOH (2 × 25 mL), water (2 × 25 mL), and brine (2 × 25 mL), dried over $MgSO_4$, filtered, and concentrated to yield **53b** (4.80 g, 93.3%) as a pale yellow liquid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 10.47 (d, 1H, *J* = 3.0 Hz), 7.50 (dd, 1H, *J* = 3.0, 8.0 Hz), 7.23 (ddd, 1H, *J* = 3.0, 7.5, 11.0 Hz), 6.95 (dd, 1H, *J* = 4.0, 9.0 Hz), 6.06 (tdd, 1H, *J* = 5.0, 10.5, 17.5 Hz), 5.44 (qd, 1H, *J* = 1.5, 17.0 Hz), 5.34 (ddd, 1H, *J* = 1.5, 2.5, 10.5 Hz), 4.64 (td, 2H, *J* = 1.5, 5.0 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 188.8, 157.4 (d, $J_{C\text{-}F}$ = 1.9 Hz), 157.2 (d, $J_{C\text{-}F}$ = 240.5 Hz), 132.4, 126.1 (d, $J_{C\text{-}F}$ = 5.9 Hz), 122.6 (d, $J_{C\text{-}F}$ = 23.8 Hz), 118.5, 114.8 (d, $J_{C\text{-}F}$ = 7.1 Hz), 114.2 (d, $J_{C\text{-}F}$ = 23.1 Hz), 70.1. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −125.5. HRMS (EI): 180.05789 (M^+); calcd. for $C_{10}H_9FO_2$: 180.05866.

5-Fluoro-2-hydroxy-3-(2-propenyl)benzaldehyde (47d): **53b** (4.64 g, 25.8 mmol) was heated neat overnight at 200°C. The crude product was purified by silica gel column chromatography (gradient, 0–10% CH_2Cl_2/hexanes) to yield **47d** (2.24 g, 48.3%) as a bright yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.10 (s, 1H), 9.83 (s, 1H), 7.17 (dd, 1H, *J* = 3.0, 9.0 Hz), 7.11 (dd, 1H, *J* = 3.0, 7.5 Hz) 5.96 (tdd, 1H, *J* = 6.5, 10.0, 17.0 Hz), 5.16-5.14 (m, 1H), 5.12 (qd, 1H, *J* = 1.5, 11.0 Hz), 3.42 (d, 2H, *J* = 6.5 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 195.9 (d, $J_{C\text{-}F}$ = 2.5 Hz), 156.0 (d, $J_{C\text{-}F}$ = 1.0 Hz), 155.7 (d, $J_{C\text{-}F}$ = 238.8 Hz), 135.1, 131.6 (d, $J_{C\text{-}F}$ = 6.4 Hz), 124.8 (d, $J_{C\text{-}F}$ = 23.6 Hz), 119.8 (d, $J_{C\text{-}F}$ = 6.4 Hz), 117.3, 116.0 (d, $J_{C\text{-}F}$ = 22.3 Hz), 33.2. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −126.9. HRMS (EI): 180.05761 (M^+); calcd. for $C_{10}H_9FO_2$: 180.05866.

5-Fluoro-2-hydroxy-3-propylbenzaldehyde (47e): To a round-bottom flask were added aldehyde **47d** (1.10 g, 6.11 mmol, 1.0 equiv.), 5% Pd/C (220 mg, 20 wt% of **47d**), diphenyl sulfide (10 μL, 0.061 mmol, 0.010 equiv.), and EtOAc (25 mL, 0.25 M). The reaction mixture was stirred overnight at room temperature under an atmosphere of H_2 (balloon pressure). The reaction mixture was filtered through Celite and washed thoroughly with EtOAc. The filtrate was concentrated to afford aldehyde **47e** (991 mg, 89.3%) as a yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.06 (br s, 1H), 9.80 (s, 1H), 7.12 (dd, 1H, *J* = 3.0, 9.0 Hz), 7.06 (dd, 1H, *J* = 3.0, 7.5 Hz), 2.62 (t, 2H, *J* = 7.5 Hz), 1.63 (sext, 2H, *J* = 7.5 Hz), 0.96 (t, 3H, *J* = 7.5 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 195.9 (d, $J_{C\text{-}F}$ = 2.5 Hz), 156.3 (d, $J_{C\text{-}F}$ = 1.0 Hz), 155.5 (d, $J_{C\text{-}F}$ = 238.1 Hz), 133.9 (d, $J_{C\text{-}F}$ = 6.3 Hz), 124.7 (d, $J_{C\text{-}F}$ = 23.1 Hz), 119.6 (d, $J_{C\text{-}F}$ = 6.5 Hz), 115.4 (d, $J_{C\text{-}F}$ = 22.4 Hz), 31.2, 22.4, 14.0. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −127.3. HRMS (EI): 182.07392 (M^+); calcd. for $C_{10}H_{11}FO_2$: 182.07431.

General Procedure D: Synthesis of PAC-1 analogues: To a 16 × 150 mm test tube were added hydrazide (1.0 equiv.), aldehyde (1.0 equiv.), EtOH or 2:1 MeOH:MeCN (0.15 M), and 1.2 M HCl (7 mol%). The reaction mixture was shaken overnight at reflux on a Büchi Syncore parallel synthesizer. The reaction mixture was cooled to room temperature, concentrated, and purified by silica gel column chromatography or recrystallization to yield pure **PAC-1** analogue.

N′-[2-hydroxy-3-(2-propenyl)phenylmethylene]-4-benzoyl-1-piperazineacetohydrazide (3): Synthesized according to General Procedure D, but in a round-bottom flask: **46c** (262 mg, 1.0 mmol, 1.0 equiv.), **47a** (162 mg, 1.0 mmol, 1.0 equiv.), 1.2 M HCl (58 μL, 0.070 mmol, 0.070 equiv.), EtOH (7 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–10% MeOH/EtOAc) yielded **3** (284 mg, 69.8%) as a white solid. ^{1}H-NMR (500

MHz, $CDCl_3$) δ 11.19 (s, 1H), 9.94 (br s, 1H), 8.45 (s, 1H), 7.46-7.41 (m, 5H), 7.20 (d, 1H, *J* = 6.5 Hz), 7.08 (dd, 1H, *J* = 1.5, 7.5 Hz), 6.85 (t, 1H, *J* = 7.0 Hz), 6.03 (tdd, 1H, *J* = 6.5, 10.0, 16.5 Hz), 5.10-5.05 (m, 2H), 3.88 (br s, 2H), 3.58 (s, 2H), 3.52 (br s, 2H), 3.45 (d, 2H, *J* = 6.5 Hz), 3.25 (s, 2H), 2.68 (br s, 2H), 2.61 (br s, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.6, 165.4, 156.4, 151.6, 136.5, 135.4, 132.5, 130.2, 129.3, 128.8, 128.3, 127.1, 119.2, 116.9, 115.8, 61.0, 53.7, 53.1, 47.6, 42.1, 33.9. HRMS (ESI): 407.2077 $(M+H)^+$; calcd. for $C_{23}H_{27}N_4O_3$: 407.2083. HPLC purity: 95%.

N′-[2-Hydroxy-3-(2-propenyl)phenylmethylene]-4-(4-cyanophenylmethyl)-1-piperazineacetohydrazide (4): Synthesized according to General Procedure D: **46d** (273 mg, 1.0 mmol, 1.0 equiv.), **47a** (162 mg, 1.0 mmol, 1.0 equiv.), 1.2 M HCl (58 μL, 0.070 mmol, 0.070 equiv.), EtOH (7 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–15% MeOH/EtOAc) yielded **4** (367 mg, 87.7%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.25 (br s, 1H), 9.99 (br s, 1H), 8.40 (s, 1H), 7.61 (d, 2H, *J* = 8.0 Hz), 7.44 (d, 2H, *J* = 8.0 Hz), 7.18 (dd, 1H, *J* = 1.5, 7.5 Hz), 7.07 (dd, 1H, *J* = 1.5, 7.5 Hz), 6.84 (t, 1H, *J* = 7.5 Hz), 6.02 (tdd, 1H, *J* = 6.5, 10.0, 16.5 Hz), 5.11-5.04 (m, 2H), 3.58 (s, 2H), 3.44 (d, 2H, *J* = 7.0 Hz), 3.19 (s, 2H), 2.63 (br s, 4H), 2.53 (br s, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 165.9, 156.5, 151.5, 144.0, 136.6, 132.5, 132.4, 129.6, 129.3, 128.4, 119.2, 119.1, 117.0, 115.8, 111.2, 62.4, 61.1, 53.8, 53.2, 34.0. HRMS (ESI): 418.2242 $(M+H)^+$; calcd. for $C_{24}H_{28}N_5O_2$: 418.2243. HPLC purity: 96%.

N′-[2-Hydroxy-3-(2-propenyl)phenylmethylene]-4-(4-cyanobenzoyl)-1-piperazineacetohydrazide (5): Synthesized according to General Procedure D: **46e** (287 mg, 1.0 mmol, 1.0 equiv.), **47a** (162 mg, 1.0 mmol, 1.0 equiv.), 1.2 M HCl (58 μL, 0.070 mmol, 0.070 equiv.), EtOH (7 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–10% MeOH/EtOAc) yielded **5** (378 mg, 87.6%) as a light yellow solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.23 (br s, 1H), 9.98 (br s, 1H), 8.32 (s, 1H), 7.69 (d, 2H, *J* = 8.5 Hz), 7.48 (d, 2H, *J* = 8.0 Hz), 7.17 (d, 1H, *J* = 7.0 Hz), 6.99 (dd, 1H, *J* = 1.5, 8.0 Hz), 6.81 (t, 1H, *J* = 7.5 Hz), 5.98 (tdd, 1H, *J* = 6.5, 10.0, 17.0), 5.08-5.02 (m, 2H), 3.85 (br s, 2H), 3.42-3.39 (m, 4H), 3.23 (s, 2H), 2.68 (br s, 2H), 2.86 (br s, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 168.4, 165.3, 156.4, 151.7, 139.7, 136.5, 132.6, 132.6, 129.4, 128.2, 127.9, 119.3, 118.1, 116.8, 115.9, 113.8, 60.9, 53.7 (br), 53.3 (br), 47.5 (br), 42.1 (br), 33.9. HRMS (ESI): 432.2034 $(M+H)^+$; calcd. for $C_{24}H_{26}N_5O_3$: 432.2036. HPLC purity: 97%.

N′-[2-Hydroxy-3-(2-propenyl)phenylmethylene]-4-(4-fluorophenylmethyl)-1-piperazineacetohydrazide (6): Synthesized according to General Procedure D: **46f** (133 mg, 0.50 mmol, 1.0 equiv.), **47a** (81 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–10% MeOH/EtOAc) followed by precipitation from Et_2O yielded **6** (182 mg, 89.0%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.26 (br s, 1H), 10.02 (br s, 1H), 8.41 (s, 1H), 7.29-7.26 (m, 2H), 7.19 (dd, 1H, *J* = 1.5, 7.5 Hz), 7.08 (dd, 1H, *J* = 1.5, 8.0 Hz), 7.02-6.99 (m, 2H), 6.85 (t, 1H, *J* = 7.5 Hz), 6.03 (tdd, 1H, *J* = 6.5, 10.0, 16.5 Hz), 5.11-5.04 (m, 2H), 3.50 (s, 2H), 3.45 (d, 2H, *J* = 6.5 Hz), 3.19 (s, 2H), 2.62 (br s, 4H), 2.51 (br s, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 166.0, 162.2 (d, $J_{C\text{-}F}$ = 243.9 Hz), 156.6, 151.5, 136.7, 133.7 (d, $J_{C\text{-}F}$ = 3.1 Hz), 132.5, 130.7 (d, $J_{C\text{-}F}$ = 7.8 Hz), 129.3, 128.4, 119.2, 117.0, 115.8, 115.3 (d, $J_{C\text{-}F}$ = 21.0 Hz), 62.2, 61.2, 53.9, 53.1, 34.0. ^{19}F-NMR (470

MHz, $CDCl_3$) δ −118.8. HRMS (ESI): 411.2203 $(M+H)^+$; calcd. for $C_{23}H_{28}FN_4O_2$: 411.2196. HPLC purity: 98%.

N′-[2-Hydroxy-3-(2-propenyl)phenylmethylene]-4-(4-fluorobenzoyl)-1-piperazineacetohydrazide (7): Synthesized according to General Procedure D: **46g** (140 mg, 0.50 mmol, 1.0 equiv.), **47a** (81 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–10% MeOH/EtOAc) yielded **7** (171 mg, 80.5%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.19 (br s, 1H), 9.91 (br s, 1H), 8.43 (s, 1H), 7.43-7.40 (m, 2H), 7.19 (dd, 1H, *J* = 1.0, 7.5 Hz), 7.12-7.09 (m, 2H), 7.06 (dd, 1H, *J* = 1.5, 8.0 Hz), 6.85 (t, 1H, *J* = 7.5 Hz), 6.02 (tdd, 1H, *J* = 6.5, 10.0, 16.5 Hz), 5.10-5.04 (m, 2H), 3.69 (br s, 4H), 3.44 (d, 2H, *J* = 6.5 Hz), 3.24 (s, 2H), 2.64 (br s, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.6, 165.4, 163.6 (d, $J_{C\text{-}F}$ = 249.1 Hz), 156.4, 151.6, 136.5, 132.5, 131.4 (d, $J_{C\text{-}F}$ = 3.4 Hz), 129.5 (d, $J_{C\text{-}F}$ = 8.4 Hz), 129.3, 128.2, 119.2, 116.8, 115.8, 115.8 (d, $J_{C\text{-}F}$ = 21.5 Hz), 60.9, 53.6 (br), 47.7 (br), 42.3 (br), 33.9. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −112.8. HRMS (ESI): 425.1989 $(M+H)^+$; calcd. for $C_{23}H_{26}FN_4O_3$: 425.1989. HPLC purity: 97%.

N′-[2-Hydroxy-3-(2-propenyl)phenylmethylene]-4-[4-(trifluoromethyl)phenylmethyl]-1-piperazineacetohydrazide (8): Synthesized according to General Procedure D: **46h** (158 mg, 0.50 mmol, 1.0 equiv.), **47a** (81 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–15% MeOH/EtOAc) yielded **8** (125 mg, 54.4%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.32 (br s, 1H), 10.11 (br s, 1H), 8.33 (s, 1H), 7.56 (d, 2H, *J* = 8.5 Hz), 7.43 (d, 2H, *J* = 8.0 Hz), 7.17 (dd, 1H, *J* = 1.5, 7.5 Hz), 7.04 (dd, 1H, *J* = 1.5, 8.0 Hz), 6.83 (t, 1H, *J* = 7.5 Hz), 6.02 (tdd, 1H, *J* = 6.5, 10.0, 16.5 Hz), 5.10-5.04 (m, 2H), 3.57 (s, 2H), 3.44 (d, 2H, *J* = 7.0 Hz), 3.19 (s, 2H), 2.63 (br s, 4H), 2.53 (br s, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 166.0, 156.4, 151.2, 142.4, 136.6, 132.4, 129.5 (q, $J_{C\text{-}F}$ = 32.0 Hz), 129.3, 129.3, 128.3, 125.3 (q, $J_{C\text{-}F}$ = 3.8 Hz), 123.9 (q, $J_{C\text{-}F}$ = 270.6 Hz), 119.2, 117.0, 115.8, 62.3, 61.0, 53.7, 53.1, 34.0. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −65.4. HRMS (ESI): 461.2160 $(M+H)^+$; calcd. for $C_{24}H_{28}F_3N_4O_2$: 461.2164. HPLC purity: 95%.

N′-[2-Hydroxy-3-(2-propenyl)phenylmethylene]-4-[4-(trifluoromethyl)benzoyl]-1-piperazineacetohydrazide (9): Synthesized according to General Procedure D: **46i** (165 mg, 0.50 mmol, 1.0 equiv.), **47a** (81 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–15% MeOH/EtOAc) yielded **9** (211 mg, 89.1%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.28 (br s, 1H), 10.13 (br s, 1H), 8.27 (s, 1H), 7.65 (d, 2H, *J* = 8.0 Hz), 7.48 (d, 2H, *J* = 8.0 Hz), 7.15 (d, 1H, *J* = 8.0 Hz), 6.94 (d, 2H, *J* = 7.0 Hz), 6.79 (t, 1H, *J* = 7.5 Hz), 5.97 (tdd, 1H, *J* = 6.5, 10.0, 17.0 Hz), 5.05-5.00 (m, 2H), 3.86 (br s, 2H), 3.43 (br s, 2H), 3.39 (d, 2H, *J* = 6.5 Hz), 3.21 (s, 2H), 2.66 (br s, 2H), 2.58 (br s, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.0, 165.4, 156.3, 151.5, 139.0, 136.4, 132.5, 131.9 (q, $J_{C\text{-}F}$ = 32.6 Hz), 129.3, 128.2, 127.5, 125.8 (q, $J_{C\text{-}F}$ = 3.5 Hz), 123.7 (q, $J_{C\text{-}F}$ = 271.1 Hz), 119.3, 116.8, 115.8, 60.8, 53.5 (br), 47.5 (br), 42.0 (br), 33.8. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −66.0. HRMS (ESI): 475.1964 $(M+H)^+$; calcd. for $C_{24}H_{26}F_3N_4O_3$: 475.1957. HPLC purity: 97%.

N′-(2-Hydroxy-3-propylphenylmethylene)-4-phenylmethyl-1-piperazineacetohydrazide (10): Synthesized according to General Procedure D, but in a round-bottom flask: **46a** (248 mg, 1.0 mmol, 1.0 equiv.), **47b** (164 mg, 1.0 mmol, 1.0 equiv.), 1.2 M HCl (58 μL, 0.070 mmol, 0.070 equiv.), EtOH (7 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–20% MeOH/EtOAc) yielded **10** (345 mg, 87.3%) as an off-white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.30 (s, 1H), 10.12 (br s, 1H), 8.31 (s, 1H), 7.35-7.30 (m, 4H), 7.30-7.25 (m, 1H), 7.17 (d, 1H, *J* = 7.5 Hz), 7.03 (d, 1H, *J* = 7.5 Hz), 6.82 (t, 1H, *J* = 7.5 Hz), 3.54 (s, 2H), 3.19 (s, 2H), 2.67 (t, 2H, *J* = 7.5 Hz), 2.62 (br s, 4H), 2.54 (br s, 4H), 1.67 (sext, 2H, *J* = 7.5 Hz), 0.97 (t, 3H, *J* = 7.5 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 165.9, 156.7, 151.2, 137.9, 132.5, 130.7, 129.2, 128.8, 128.4, 127.3, 118.9, 116.8, 62.9, 61.0, 53.7, 53.0, 32.0, 22.7, 14.2. HRMS (ESI): 395.2436 $(M+H)^+$; calcd. for $C_{23}H_{31}N_4O_2$: 395.2447. HPLC purity: 98%.

4-{4-[N′-(2-Hydroxy-3-propylphenylmethylene)hydrazinecarbonylmethyl]-1-piperazinylmethyl}benzenesulfonamide (11): Synthesized according to General Procedure D: **46b** (164 mg, 0.50 mmol, 1.0 equiv.), **47b** (82 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), 2:1 MeOH:MeCN (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–20% MeOH/EtOAc) yielded **11** (211 mg, 89.0%) as a white solid. ^{1}H-NMR (500 MHz, $(CD_3)_2CO$) δ 11.78 (s, 1H), 10.76 (br s, 1H), 8.48 (s, 1H), 7.84 (d, 2H, *J* = 8.5 Hz), 7.51 (d, 2H, *J* = 8.5 Hz), 7.17 (d, 1H, *J* = 7.0 Hz), 7.14 (dd, 1H, *J* = 1.5, 8.0 Hz), 6.82 (t, 1H, *J* = 7.5 Hz), 6.54 (br s, 2H), 3.59 (s, 2H), 3.17 (s, 2H), 2.64-2.59 (m, 6H), 2.52 (br s, 4H), 1.63 (sext, 2H, *J* = 7.5 Hz), 0.93 (t, 3H, *J* = 7.5 Hz). ^{13}C-NMR (125 MHz, $(CD_3)_2CO$) δ 166.3, 157.3, 150.9, 144.0, 143.8, 132.7, 130.8, 129.9, 129.6, 126.9, 119.6, 118.3, 62.6, 61.7, 54.3, 53.6, 32.5, 23.4, 14.2. HRMS (ESI): 474.2175 $(M+H)^+$; calcd. for $C_{23}H_{32}N_5O_4S$: 474.2175. HPLC purity: 95%.

N′-(2-Hydroxy-3-propylphenylmethylene)-4-benzoyl-1-piperazineacetohydrazide (12): Synthesized according to General Procedure D: **46c** (131 mg, 0.50 mmol, 1.0 equiv.), **47b** (82 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 50–100% EtOAc/hexanes, then 5% MeOH/EtOAc) yielded **12** (174 mg, 85.5%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.29 (s, 1H), 10.29 (br s, 1H), 8.23 (s, 1H), 7.41-7.34 (m, 5H), 7.13 (dd, 1H, *J* = 1.5, 7.5 Hz), 6.90 (dd, 1H, *J* = 1.5, 7.5 Hz), 6.76 (t, 1H, *J* = 7.5 Hz), 3.80 (br s, 2H), 3.47 (br s, 2H), 3.18 (s, 2H), 2.71-2.52 (m, 6H, Ar-CH_2-CH_2), 1.61 (sext, 2H, *J* = 7.5 Hz), 0.91 (t, 3H, *J* = 7.5 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.5, 165.5, 156.6, 151.5, 135.3, 132.5, 130.6, 130.1, 128.8, 128.7, 127.0, 118.9, 116.7, 60.8, 53.6 (br), 53.0 (br), 47.5 (br), 42.0 (br), 31.9, 22.7, 14.1. HRMS (ESI): 409.2238 $(M+H)^+$; calcd. for $C_{23}H_{29}N_4O_3$: 409.2240. HPLC purity: 98%.

N′-(2-Hydroxy-3-propylphenylmethylene)-4-(4-cyanophenylmethyl)-1-piperazineacetohydrazide (13): Synthesized according to General Procedure D: **46d** (273 mg, 1.0 mmol, 1.0 equiv.), **47b** (164 mg, 1.0 mmol, 1.0 equiv.), 1.2 M HCl (58 μL, 0.070 mmol, 0.070 equiv.), EtOH (7 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–15% MeOH/EtOAc) yielded **13** (373 mg, 88.8%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.19 (br s, 1H), 9.99 (br s, 1H), 8.37 (s, 1H), 7.60 (d, 2H, *J* = 8.0 Hz), 7.44 (d, 2H, *J* = 7.5 Hz), 7.16 (dd, 1H, *J* = 1.5, 7.5 Hz), 7.04 (dd, 1H, *J* =

1.5, 7.5 Hz), 6.82 (t, 1H, *J* = 7.5 Hz), 3.58 (s, 2H), 3.19 (s, 2H), 2.66-2.63 (m, 6H), 2.52 (br s, 4H), 1.64 (sext, 2H, *J* = 7.5 Hz), 0.95 (t, 3H, *J* = 7.5 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 165.8, 156.8, 151.6, 144.0, 132.7, 132.3, 130.8, 129.6, 128.9, 119.1, 119.0, 116.8, 111.2, 62.4, 61.1, 53.8, 53.2, 32.0, 22.8, 14.2. HRMS (ESI): 420.2396 $(M+H)^+$; calcd. for $C_{24}H_{30}N_5O_2$: 420.2400. HPLC purity: 97%.

N′-(2-Hydroxy-3-propylphenylmethylene)-4-(4-cyanobenzoyl)-1-piperazineacetohydrazide (14): Synthesized according to General Procedure D: **46e** (287 mg, 1.0 mmol, 1.0 equiv.), **47b** (164 mg, 1.0 mmol, 1.0 equiv.), 1.2 M HCl (58 μL, 0.070 mmol, 0.070 equiv.), EtOH (7 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0-15% MeOH/EtOAc) yielded **14** (377 mg, 86.9%) as a light yellow solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.15 (br s, 1H), 9.92 (br s, 1H), 8.32 (s, 1H), 7.71 (d, 2H, *J* = 8.0 Hz), 7.49 (d, 2H, *J* = 7.5 Hz), 7.16 (d, 1H, *J* = 7.0 Hz), 6.98 (dd, 1H, *J* = 1.5, 7.5 Hz), 6.80 (t, 1H, *J* = 7.5 Hz), 3.86 (br s, 2H), 3.44 (br s, 2H), 3.24 (s, 2H), 2.70 (br s, 2H), 2.64-2.57 (m, 4H), 1.62 (sext, 2H, *J* = 7.5 Hz), 0.93 (t, 3H, *J* = 7.5 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 168.5, 165.2, 156.7, 152.0, 139.8, 132.9, 132.7, 130.8, 129.0, 127.9, 119.1, 118.1, 116.7, 113.9, 61.0, 53.5 (br), 47.5 (br), 42.1 (br), 32.0, 22.8, 14.2. HRMS (ESI): 434.2188 $(M+H)^+$; calcd. for $C_{24}H_{28}N_5O_3$: 434.2192. HPLC purity: 98%.

N′-(2-Hydroxy-3-propylphenylmethylene)-4-(4-fluorophenylmethyl)-1-piperazineacetohydrazide (15): Synthesized according to General Procedure D: **46f** (133 mg, 0.50 mmol, 1.0 equiv.), **47b** (82 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–20% MeOH/EtOAc) followed by precipitation from Et_2O yielded **15** (137 mg, 66.4%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.26 (br s, 1H), 10.09 (br s, 1H), 8.31 (s, 1H), 7.26 (dd, 2H, *J* = 6.0, 8.0 Hz), 7.16 (dd, 1H, *J* = 1.5, 6.5 Hz), 7.02-6.97 (m, 3H), 6.80 (t, 1H, *J* = 7.5 Hz), 3.48 (s, 2H), 3.18 (s, 2H), 2.65 (t, 2H, *J* = 7.5 Hz), 2.61 (br s, 4H), 2.50 (br s, 4H), 1.65 (sext, 2H, *J* = 7.5 Hz), 0.95 (t, 3H, *J* = 7.5 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 165.9, 162.1 (d, $J_{C\text{-}F}$ = 243.6 Hz), 156.7, 151.3, 133.7 (d, $J_{C\text{-}F}$ = 3.0 Hz), 132.5, 130.7, 130.6 (d, $J_{C\text{-}F}$ = 7.8 Hz), 128.9, 118.9, 116.8, 115.2 (d, $J_{C\text{-}F}$ = 21.0 Hz), 62.1, 61.0, 53.8, 53.0, 32.0, 22.8, 14.2. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −118.8. HRMS (ESI): 413.2361 $(M+H)^+$; calcd. for $C_{23}H_{30}FN_4O_2$: 413.2353. HPLC purity: 97%.

N′-(2-Hydroxy-3-propylphenylmethylene)-4-(4-fluorobenzoyl)-1-piperazineacetohydrazide (16): Synthesized according to General Procedure D: **46g** (140 mg, 0.50 mmol, 1.0 equiv.), **47b** (82 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–15% MeOH/EtOAc) yielded **16** (133 mg, 62.4%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.24 (br s, 1H), 10.17 (br s, 1H), 8.25 (s, 1H), 7.37 (dd, 2H, *J* = 5.5, 8.5 Hz), 7.13 (dd, 1H, *J* = 1.5, 7.5 Hz), 7.06 (t, 2H, *J* = 8.5 Hz), 6.91 (dd, 1H, *J* = 1.5, 7.5 Hz), 6.77 (t, 1H, *J* = 7.5 Hz), 3.83 (br s, 2H), 3.49 (br s, 2H), 3.20 (s, 2H), 2.62-2.58 (m, 6H), 1.60 (sext, 2H, *J* = 7.5 Hz), 0.91 (t, 3H, *J* = 7.5 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.6, 165.4, 163.6 (d, $J_{C\text{-}F}$ = 249.3 Hz), 156.6, 151.7, 132.6, 131.4 (d, $J_{C\text{-}F}$ = 3.4 Hz), 129.5 (d, $J_{C\text{-}F}$ = 8.5 Hz), 128.9, 119.0, 116.7, 115.8 (d, $J_{C\text{-}F}$ = 21.8 Hz), 60.9, 53.5 (br), 47.7 (br), 42.2 (br), 31.9, 22.7, 14.1. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −112.8. HRMS (ESI): 427.2141 $(M+H)^+$; calcd. for $C_{23}H_{28}FN_4O_3$: 427.2145. HPLC purity: 96%.

N′-(2-Hydroxy-3-propylphenylmethylene)-4-[4-(trifluoromethyl)phenylmethyl]-1-piperazineacetohydrazide (17): Synthesized according to General Procedure D: **46h** (158 mg, 0.50 mmol, 1.0 equiv.), **47b** (82 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–15% MeOH/EtOAc) yielded **17** (93.9 mg, 40.6%) as a yellow solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.23 (br s, 1H), 10.05 (br s, 1H), 8.33 (s, 1H), 7.57 (d, 2H, *J* = 8.0 Hz), 7.44 (d, 2H, *J* = 8.0 Hz), 7.16 (dd, 1H, *J* = 1.5, 7.5 Hz), 7.02 (dd, 2H, *J* = 1.5, 7.5 Hz), 6.81 (t, 1H, *J* = 7.5 Hz), 3.58 (s, 2H), 3.19 (s, 2H), 2.67-2.62 (m, 6H), 2.53 (br s, 4H), 1.65 (sext, 2H, *J* = 7.5 Hz), 0.95 (t, 3H, *J* = 7.5 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 165.9, 156.8, 151.5, 142.4, 132.6, 130.8, 129.6 (q, $J_{C\text{-}F}$ = 32.0 Hz), 129.3, 128.9, 125.4 (q, $J_{C\text{-}F}$ = 3.6 Hz), 124.4 (q, $J_{C\text{-}F}$ = 270.5 Hz), 119.0, 116.9, 62.4, 61.1, 53.8, 53.2, 32.0, 22.8, 14.2. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −65.5. HRMS (ESI): 463.2321 $(M+H)^+$; calcd. for $C_{24}H_{30}F_3N_4O_2$: 463.2321. HPLC purity: 98%.

N′-(2-Hydroxy-3-propylphenylmethylene)-4-[4-(trifluoromethyl)benzoyl]-1-piperazineacetohydrazide (18): Synthesized according to General Procedure D: **46i** (165 mg, 0.50 mmol, 1.0 equiv.), **47b** (82 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–15% MeOH/EtOAc) yielded **18** (216 mg, 90.8%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.24 (br s, 1H), 10.14 (br s, 1H), 8.23 (s, 1H), 7.64 (d, 2H, *J* = 8.0 Hz), 7.47 (d, 2H, *J* = 8.0 Hz), 7.13 (d, 1H, *J* = 8.0 Hz), 6.89 (d, 1H, *J* = 7.5 Hz), 6.76 (t, 1H, *J* = 7.5 Hz), 3.85 (br s, 2H), 3.43 (br s, 2H), 3.21 (s, 2H), 2.73-2.58 (m, 6H), 1.60 (sext, 2H, *J* = 7.5 Hz), 0.90 (t, 3H, *J* = 7.5 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.0, 165.4, 156.5, 151.6, 139.0, 132.7, 131.9 (q, $J_{C\text{-}F}$ = 32.5 Hz), 130.6, 128.8, 127.5, 125.8 (q, $J_{C\text{-}F}$ = 3.5 Hz), 123.7 (q, $J_{C\text{-}F}$ = 271.3 Hz), 119.0, 116.7, 60.8, 53.5 (br), 47.5 (br), 42.0 (br), 31.9, 22.7, 14.1. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −66.0. HRMS (ESI): 477.2108 $(M+H)^+$; calcd. for $C_{24}H_{28}F_3N_4O_3$: 477.2114. HPLC purity: 99%.

N′-(5-Fluoro-2-hydroxyphenylmethylene)-4-phenylmethyl-1-piperazineacetohydrazide (19): Synthesized according to General Procedure D: **46a** (124 mg, 0.50 mmol, 1.0 equiv.), **47c** (70 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–20% MeOH/EtOAc) yielded **19** (173 mg, 93.7%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 10.81 (br s, 1H), 10.13 (br s, 1H), 8.39 (s, 1H), 7.33-7.30 (m, 4H), 7.28-7.25 (m, 1H), 7.00 (dt, 1H, *J* = 3.0, 9.0 Hz), 6.94-6.89 (m, 2H), 3.55 (s, 2H), 3.19 (s, 2H), 2.63 (br s, 4H), 2.53 (br s, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 166.3, 155.9 (d, $J_{C\text{-}F}$ = 235.8 Hz), 154.8, 150.0, 137.9, 129.3, 128.5, 127.4, 118.9 (d, $J_{C\text{-}F}$ = 23.1 Hz), 118.4 (d, $J_{C\text{-}F}$ = 7.6 Hz), 117.6 (d, $J_{C\text{-}F}$ = 7.5 Hz), 116.1 (d, $J_{C\text{-}F}$ = 23.8 Hz), 63.0, 61.1, 53.9, 53.1. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −128.5. HRMS (ESI): 371.1877 $(M+H)^+$; calcd. for $C_{20}H_{24}FN_4O_2$: 371.1883. HPLC purity: 95%.

4-{4-[N′-(5-Fluoro-2-hydroxyphenylmethylene)hydrazinecarbonylmethyl]-1-piperazinylmethyl}benzenesulfonamide (20): Synthesized according to General Procedure D: **46b** (164 mg, 0.50 mmol, 1.0 equiv.), **47c** (70 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), 2:1 MeOH:MeCN (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–20% MeOH/EtOAc) yielded **20** (172 mg, 82.1%) as a

yellow solid. ^{1}H-NMR (500 MHz, $(CD_3)_2CO$) δ 11.33 (br s, 1H), 10.95 (br s, 1H), 8.49 (s, 1H), 7.85 (d, 2H, *J* = 8.0 Hz), 7.49 (d, 2H, *J* = 8.0 Hz), 7.12 (dd, 1H, *J* = 3.0, 9.0 Hz), 7.07 (dt, 1H, *J* = 3.0, 8.5 Hz), 6.91 (dd, 1H, *J* = 5.0, 9.0 Hz), 6.62 (br s, 2H), 3.55 (s, 2H), 3.19 (s, 2H), 2.59 (br s, 4H), 2.49 (br s, 4H). ^{13}C-NMR (125 MHz, $(CD_3)_2CO$) δ 166.8, 156.4 (d, $J_{C\text{-}F}$ = 233.5 Hz), 155.4, 155.1 (d, $J_{C\text{-}F}$ = 2.8 Hz), 143.9, 143.6, 129.9, 126.7, 119.2 (d, $J_{C\text{-}F}$ = 7.6 Hz), 118.7 (d, $J_{C\text{-}F}$ = 17.8 Hz), 118.6, 116.6 (d, $J_{C\text{-}F}$ = 23.9 Hz), 62.5, 61.5, 54.1, 53.4. ^{19}F-NMR (470 MHz, $(CD_3)_2CO$) δ −127.3. HRMS (ESI): 450.1609 $(M+H)^+$; calcd. for $C_{20}H_{25}FN_5O_4S$: 450.1611. HPLC purity: 92%.

N′-(5-Fluoro-2-hydroxyphenylmethylene)-4-benzoyl-1-piperazineacetohydrazide (21): Synthesized according to General Procedure D: **46c** (131 mg, 0.50 mmol, 1.0 equiv.), **47c** (70 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–20% MeOH/EtOAc) yielded **21** (157 mg, 81.6%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 10.89 (br s, 1H), 10.50 (br s, 1H), 8.21 (s, 1H), 7.40-7.33 (m, 5H), 6.93 (dt, 1H, *J* = 2.5, 8.5 Hz), 6.84 (dd, 1H, *J* = 4.5, 9.0 Hz), 6.74 (dd, 1H, *J* = 2.5, 8.5 Hz), 3.79 (br s, 2H), 3.48 (br s, 2H), 3.17 (s, 2H), 2.60 (br s, 2H), 2.53 (br s, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.5, 165.8, 155.7 (d, $J_{C\text{-}F}$ = 235.8 Hz), 154.5, 149.7, 135.3, 130.1, 128.7, 127.0, 118.8 (d, $J_{C\text{-}F}$ = 23.1 Hz), 118.2 (d, $J_{C\text{-}F}$ = 7.5 Hz), 117.6 (d, $J_{C\text{-}F}$ = 7.4 Hz), 116.0 (d, $J_{C\text{-}F}$ = 23.6 Hz), 60.7, 53.6 (br), 53.4 (br), 47.6 (br), 42.0 (br). ^{19}F-NMR (470 MHz, $CDCl_3$) δ −128.3. HRMS (ESI): 385.1674 $(M+H)^+$; calcd. for $C_{20}H_{22}FN_4O_3$: 385.1676. HPLC purity: 97%.

N′-(5-Fluoro-2-hydroxyphenylmethylene)-4-(4-cyanophenylmethyl)-1-piperazineacetohydrazide (22): Synthesized according to General Procedure D: **46d** (137 mg, 0.50 mmol, 1.0 equiv.), **47c** (70 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–20% MeOH/EtOAc) yielded **22** (169 mg, 85.5%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 10.84 (br s, 1H), 10.20 (br s, 1H), 8.31 (s, 1H), 7.56 (d, 2H, *J* = 8.5 Hz), 7.41 (d, 1H, *J* = 8.0 Hz), 6.95 (dt, 1H, *J* = 3.0, 9.0 Hz), 6.88-6.85 (m, 2H), 3.54 (s, 2H), 3.18 (s, 2H), 2.60 (br s, 4H), 2.50 (br s, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 166.2, 155.7 (d, $J_{C\text{-}F}$ = 235.9 Hz), 154.6, 149.6, 144.0, 132.2, 129.5, 118.9 (d, $J_{C\text{-}F}$ = 22.6 Hz), 118.6, 118.2 (d, $J_{C\text{-}F}$ = 7.6 Hz), 117.5 (d, $J_{C\text{-}F}$ = 7.5 Hz), 116.0 (d, $J_{C\text{-}F}$ = 23.8 Hz), 110.9, 62.2, 61.0, 53.6, 53.0. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −128.4. HRMS (ESI): 396.1838 $(M+H)^+$; calcd. for $C_{21}H_{23}FN_5O_2$: 396.1836. HPLC purity: 94%.

N′-(5-Fluoro-2-hydroxyphenylmethylene)-4-(4-cyanobenzoyl)-1-piperazineacetohydrazide (23): Synthesized according to General Procedure D: **46e** (144 mg, 0.50 mmol, 1.0 equiv.), **47c** (70 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–20% MeOH/EtOAc) yielded **23** (144 mg, 70.1%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 10.79 (br s, 1H), 10.16 (br s, 1H), 8.28 (s, 1H), 7.67 (d, 2H, *J* = 8.0 Hz), 7.47 (d, 2H, *J* = 8.5 Hz), 6.95 (dt, 1H, *J* = 3.0, 8.0 Hz), 6.85 (dd, 1H, *J* = 4.5, 9.0 Hz), 6.79 (dd, 1H, *J* = 3.0, 8.5 Hz), 3.82 (br s, 2H), 3.41 (br s, 2H), 3.22 (s, 2H), 2.67 (br s, 2H), 2.54 (br s, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 168.4, 165.5, 155.7 (d, $J_{C\text{-}F}$ = 236.1 Hz), 154.5, 149.9, 139.7, 132.6, 127.8, 119.0 (d, $J_{C\text{-}F}$ = 23.1 Hz), 118.2 (d, $J_{C\text{-}F}$ = 7.6 Hz), 118.1, 117.4 (d, $J_{C\text{-}F}$ = 7.5 Hz), 116.0 (d, $J_{C\text{-}F}$ = 23.6 Hz), 113.7, 60.8, 53.4 (br), 52.7 (br), 47.4 (br), 42.0 (br). ^{19}F-NMR (470 MHz,

$CDCl_3$) δ −128.1. HRMS (ESI): 410.1623 $(M+H)^+$; calcd. for $C_{21}H_{21}FN_5O_3$: 410.1628. HPLC purity: 96%.

N′-(5-Fluoro-2-hydroxyphenylmethylene)-4-(4-fluorophenylmethyl)-1-piperazineacetohydrazide (24): Synthesized according to General Procedure D: **46f** (133 mg, 0.50 mmol, 1.0 equiv.), **47c** (70 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–20% MeOH/EtOAc) yielded **24** (152 mg, 78.2%) as a pale yellow solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 10.83 (br s, 1H), 10.19 (br s, 1H), 8.33 (s, 1H), 7.25 (dd, 2H, *J* = 5.5, 8.5 Hz), 6.99-6.95 (m, 3H), 6.90-6.86 (m, 2H), 3.47 (s, 2H), 3.18 (s, 2H), 2.60 (br s, 4H), 2.49 (br s, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 166.3, 162.1 (d, $J_{C\text{-}F}$ = 243.9 Hz), 155.8 (d, $J_{C\text{-}F}$ = 236.0 Hz), 154.7 (d, $J_{C\text{-}F}$ = 1.4 Hz), 149.7 (d, $J_{C\text{-}F}$ = 2.6 Hz), 133.7 (d, $J_{C\text{-}F}$ = 3.0 Hz), 130.6 (d, $J_{C\text{-}F}$ = 7.9 Hz), 118.8 (d, $J_{C\text{-}F}$ = 23.3 Hz), 118.3 (d, $J_{C\text{-}F}$ = 7.6 Hz), 117.6 (d, $J_{C\text{-}F}$ = 7.5 Hz), 116.0 (d, $J_{C\text{-}F}$ = 23.8 Hz), 115.2 (d, $J_{C\text{-}F}$ = 21.1 Hz), 62.1, 61.0, 53.8, 52.9. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −118.8, −128.4. HRMS (ESI): 389.1787 $(M+H)^+$; calcd. for $C_{20}H_{23}F_2N_4O_2$: 389.1789. HPLC purity: 94%.

N′-(5-Fluoro-2-hydroxyphenylmethylene)-4-(4-fluorobenzoyl)-1-piperazineacetohydrazide (25): Synthesized according to General Procedure D: **46g** (140 mg, 0.50 mmol, 1.0 equiv.), **47c** (70 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–20% MeOH/EtOAc) yielded **25** (101 mg, 50.1%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 10.81 (br s, 1H), 10.25 (br s, 1H), 8.29 (s, 1H), 7.38 (dd, 2H, *J* = 5.5, 8.5 Hz), 7.07 (t, 2H, *J* = 8.5 Hz), 6.98-6.94 (m, 1H), 6.86 (dd, 1H, *J* = 4.0, 9.0 Hz), 6.79 (dd, 1H, *J* = 2.0, 8.0 Hz), 3.63 (br s, 4H), 3.21 (s, 2H), 2.59 (br s, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.7, 165.7, 163.6 (d, $J_{C\text{-}F}$ = 249.4 Hz), 155.8 (d, $J_{C\text{-}F}$ = 236.3 Hz), 154.6 (d, $J_{C\text{-}F}$ = 0.9 Hz), 150.0 (d, $J_{C\text{-}F}$ = 2.3 Hz), 131.3 (d, $J_{C\text{-}F}$ = 3.4 Hz), 129.5 (d, $J_{C\text{-}F}$ = 8.4 Hz), 119.0 (d, $J_{C\text{-}F}$ = 23.1 Hz), 118.3 (d, $J_{C\text{-}F}$ = 7.5 Hz), 117.5 (d, $J_{C\text{-}F}$ = 7.3 Hz), 116.0 (d, $J_{C\text{-}F}$ = 24.6 Hz), 115.8 (d, $J_{C\text{-}F}$ = 21.9 Hz), 60.9, 53.5, 47.7, 42.2. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −112.6, −128.2. HRMS (ESI): 403.1573 $(M+H)^+$; calcd. for $C_{20}H_{21}F_2N_4O_3$: 403.1582. HPLC purity: 96%.

N′-(5-Fluoro-2-hydroxyphenylmethylene)-4-[4-(trifluoromethyl)phenylmethyl]-1-piperazineacetohydrazide (26): Synthesized according to General Procedure D: **46h** (158 mg, 0.50 mmol, 1.0 equiv.), **47c** (70 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–20% MeOH/EtOAc) yielded **26** (194 mg, 88.6%) as a pale yellow solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 10.83 (br s, 1H), 10.17 (br s, 1H), 8.34 (s, 1H), 7.56 (d, 2H, *J* = 8.0 Hz), 7.43 (d, 2H, *J* = 8.0 Hz), 6.98 (dt, 1H, *J* = 3.0, 8.0 Hz), 6.91-6.86 (m, 2H), 3.57 (s, 2H), 3.19 (s, 2H), 2.62 (br s, 4H), 2.52 (br s, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 166.3, 155.8 (d, $J_{C\text{-}F}$ = 236.0 Hz), 154.7 (d, $J_{C\text{-}F}$ = 1.5 Hz), 149.8 (d, $J_{C\text{-}F}$ = 2.4 Hz), 142.4 (d, $J_{C\text{-}F}$ = 0.8 Hz), 129.5 (q, $J_{C\text{-}F}$ = 32.1 Hz), 129.3, 125.3 (q, $J_{C\text{-}F}$ = 3.8 Hz), 124.4 (q, $J_{C\text{-}F}$ = 270.6 Hz), 118.9 (d, $J_{C\text{-}F}$ = 23.0 Hz), 118.3 (d, $J_{C\text{-}F}$ = 7.6 Hz), 117.6 (d, $J_{C\text{-}F}$ = 7.5 Hz), 116.1 (d, $J_{C\text{-}F}$ = 23.6 Hz), 62.3, 61.0, 53.8, 53.1. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −65.4, −128.4. HRMS (ESI): 439.1765 $(M+H)^+$; calcd. for $C_{21}H_{23}F_4N_4O_2$: 439.1757. HPLC purity: 96%.

N′-(5-Fluoro-2-hydroxyphenylmethylene)-4-[4-(trifluoromethyl)benzoyl]-1-piperazineacetohydrazide (27): Synthesized according to General Procedure D: **46i** (165 mg, 0.50 mmol, 1.0 equiv.), **47c** (70 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–20% MeOH/EtOAc) yielded **27** (173 mg, 76.5%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 10.80 (br s, 1H), 10.20 (br s, 1H), 8.28 (s, 1H), 7.65 (d, 2H, *J* = 8.0 Hz), 7.48 (d, 2H, *J* = 7.5 Hz), 6.96 (dt, 1H, *J* = 2.5, 8.0 Hz), 6.87 (dd, 1H, *J* = 4.5, 8.5 Hz), 6.79 (dd, 1H, *J* = 2.5, 8.0 Hz), 3.84 (br s, 2H), 3.44 (br s, 2H), 3.22 (s, 2H), 2.70 (br s, 2H), 2.55 (br s, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.1, 165.6, 155.8 (d, $J_{C\text{-}F}$ = 236.3 Hz), 154.6 (d, $J_{C\text{-}F}$ = 1.3 Hz), 150.0 (d, $J_{C\text{-}F}$ = 1.9 Hz), 138.9, 132.0 (q, $J_{C\text{-}F}$ = 32.6 Hz), 127.5, 125.8 (q, $J_{C\text{-}F}$ = 3.6 Hz), 123.7 (q, $J_{C\text{-}F}$ = 271.3 Hz), 119.0 (d, $J_{C\text{-}F}$ = 23.1 Hz), 118.3 (d, $J_{C\text{-}F}$ = 7.6 Hz), 117.4 (d, $J_{C\text{-}F}$ = 7.5 Hz), 116.0 (d, $J_{C\text{-}F}$ = 23.8 Hz), 60.8, 53.5 (br), 47.5 (br), 42.0 (br). ^{19}F-NMR (470 MHz, $CDCl_3$) δ −66.0, −128.1. HRMS (ESI): 453.1552 $(M+H)^+$; calcd. for $C_{21}H_{21}F_4N_4O_3$: 453.1550. HPLC purity: 97%.

N′-[5-Fluoro-2-hydroxy-3-(2-propenyl)phenylmethylene]-4-phenylmethyl-1-piperazineacetohydrazide (28): Synthesized according to General Procedure D: **46a** (124 mg, 0.50 mmol, 1.0 equiv.), **47d** (90 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–20% MeOH/EtOAc) yielded **28** (186 mg, 90.8%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.16 (s, 1H), 10.20 (br s, 1H), 8.28 (s, 1H), 7.34-7.30 (m, 4H), 7.28-7.25 (m, 1H), 6.91 (dd, 1H, *J* = 3.0, 9.0 Hz), 6.74 (dd, 1H, *J* = 3.0, 8.0 Hz), 5.98 (tdd, 1H, *J* = 6.5, 10.0, 16.5 Hz), 5.13-5.08 (m, 2H), 3.54 (s, 2H), 3.42 (d, 2H, *J* = 7.0 Hz), 3.20 (s, 2H), 2.63 (br s, 4H), 2.53 (br s, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 166.2, 155.5 (d, $J_{C\text{-}F}$ = 235.8 Hz), 152.5 (d, $J_{C\text{-}F}$ = 1.4 Hz), 149.8 (d, $J_{C\text{-}F}$ = 2.5 Hz), 137.9, 135.7, 130.2 (d, $J_{C\text{-}F}$ = 6.8 Hz), 129.2, 128.4, 127.3, 119.0 (d, $J_{C\text{-}F}$ = 23.1 Hz), 116.8 (d, $J_{C\text{-}F}$ = 7.9 Hz), 116.6, 113.9 (d, $J_{C\text{-}F}$ = 23.5 Hz), 62.9, 61.0, 53.8, 53.0, 33.8. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −128.6. HRMS (ESI): 411.2191 $(M+H)^+$; calcd. for $C_{23}H_{28}FN_4O_2$: 411.2196. HPLC purity: 99%.

4-{4-[N′-(5-Fluoro-2-hydroxy-3-(2-propenyl)phenylmethylene)hydrazinecarbonylmethyl]-1-piperazinylmethyl}benzensulfonamide (29): Synthesized according to General Procedure D: **46b** (164 mg, 0.50 mmol, 1.0 equiv.), **47d** (90 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–20% MeOH/EtOAc) yielded **29** (214 mg, 87.2%) as a white solid. ^{1}H-NMR (500 MHz, $(CD_3)_2CO$) δ 11.73 (br s, 1H), 10.94 (br s, 1H), 8.46 (s, 1H), 7.85 (d, 2H, *J* = 8.5 Hz), 7.48 (d, 2H, *J* = 8.5 Hz), 6.96 (d, 2H, *J* = 9.0 Hz), 6.62 (br s, 2H), 5.99 (tdd, 1H, *J* = 6.5, 10.0, 16.5 Hz), 5.10 (qd, 1H, *J* = 1.5, 17.0 Hz), 5.04 (qd, 2H, *J* = 1.5, 10.0 Hz), 3.55 (s, 2H), 3.40 (d, 2H, *J* = 7.0 Hz), 3.19 (s, 2H), 2.59 (br s, 4H), 2.49 (br s, 4H). ^{13}C-NMR (125 MHz, $(CD_3)_2CO$) δ 166.8, 156.1 (d, $J_{C\text{-}F}$ = 233.6 Hz), 153.1 (d, $J_{C\text{-}F}$ = 1.3 Hz), 149.6, 143.9, 143.5, 136.6, 130.5 (d, $J_{C\text{-}F}$ = 7.0 Hz), 129.8, 126.7, 118.7 (d, $J_{C\text{-}F}$ = 23.1 Hz), 118.4 (d, $J_{C\text{-}F}$ = 8.0 Hz), 116.5, 114.6 (d, $J_{C\text{-}F}$ = 23.6 Hz), 62.5, 61.5, 54.1, 53.4, 34.2. ^{19}F-NMR (470 MHz, $(CD_3)_2CO$) δ −127.4. HRMS (ESI): 490.1930 $(M+H)^+$; calcd. for $C_{23}H_{29}FN_5O_4S$: 490.1924. HPLC purity: 98%.

N′-[5-Fluoro-2-hydroxy-3-(2-propenyl)phenylmethylene]-4-benzoyl-1-piperazineacetohydrazide (30): Synthesized according to General Procedure D: **46c** (131 mg, 0.50 mmol, 1.0 equiv.), **47d** (90 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–15% MeOH/EtOAc) yielded **30** (186 mg, 87.8%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.17 (s, 1H), 10.45 (br s, 1H), 8.19 (s, 1H), 7.40-7.33 (m, 5H), 6.86 (dd, 1H, *J* = 3.0, 9.0 Hz), 6.60 (dd, 1H, *J* = 3.0, 8.5 Hz), 5.91 (tdd, 1H, *J* = 6.5, 9.5, 18.0 Hz), 5.09-5.03 (m, 2H), 3.80 (br s, 2H), 3.47 (br s, 2H), 3.35 (d, 2H, *J* = 6.5 Hz), 3.19 (s, 2H), 2.56 (br s, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.5, 165.6, 155.4 (d, $J_{C\text{-}F}$ = 235.5 Hz), 152.4 (d, $J_{C\text{-}F}$ = 1.4 Hz), 150.1 (d, $J_{C\text{-}F}$ = 1.8 Hz), 135.6, 135.3, 130.1, 130.1, 128.7, 127.0, 119.0 (d, $J_{C\text{-}F}$ = 23.0 Hz), 116.8 (d, $J_{C\text{-}F}$ = 7.9 Hz), 116.5, 113.9 (d, $J_{C\text{-}F}$ = 23.5 Hz), 60.7, 53.6 (br), 47.6 (br), 42.0 (br), 33.7. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −128.5. HRMS (ESI): 425.1991 $(M+H)^+$; calcd. for $C_{23}H_{26}FN_4O_3$: 425.1989. HPLC purity: 97%.

N′-[5-Fluoro-2-hydroxy-3-(2-propenyl)phenylmethylene]-4-(4-cyanophenylmethyl)-1-piperazineacetohydrazide (31): Synthesized according to General Procedure D: **46d** (137 mg, 0.50 mmol, 1.0 equiv.), **47d** (90 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–15% MeOH/EtOAc) yielded **31** (164 mg, 75.3%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.12 (br s, 1H), 10.16 (br s, 1H), 8.28 (s, 1H), 7.57 (d, 2H, *J* = 8.0 Hz), 7.42 (d, 2H, *J* = 8.0 Hz), 6.88 (dd, 1H, *J* = 3.0, 9.0 Hz), 6.72 (dd, 1H, *J* = 3.0, 8.0 Hz), 5.94 (tdd, 1H, *J* = 6.5, 10.0, 17.0 Hz), 5.09-5.05 (m, 2H), 3.55 (s, 2H), 3.38 (d, 2H, *J* = 7.0 Hz), 3.19 (s, 2H), 2.62 (br s, 4H), 2.51 (br s, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 166.0, 155.5 (d, $J_{C\text{-}F}$ = 235.5 Hz), 152.5 (d, $J_{C\text{-}F}$ = 1.4 Hz), 149.9, 144.0, 135.7, 132.2, 130.1 (d, $J_{C\text{-}F}$ = 6.8 Hz), 129.5, 119.0 (d, $J_{C\text{-}F}$ = 23.0 Hz), 119.0, 116.8 (d, $J_{C\text{-}F}$ = 7.8 Hz), 116.5, 113.9 (d, $J_{C\text{-}F}$ = 23.5 Hz), 111.0, 62.3, 60.9, 53.8, 53.1, 33.8. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −128.6. HRMS $(M+H)^+$; 436.2144 (M+1); calcd. for $C_{24}H_{27}FN_5O_2$: 436.2149. HPLC purity: 95%.

N′-[5-Fluoro-2-hydroxy-3-(2-propenyl)phenylmethylene]-4-(4-cyanobenzoyl)-1-piperazineacetohydrazide (32): Synthesized according to General Procedure D: **46e** (144 mg, 0.50 mmol, 1.0 equiv.), **47d** (90 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–15% MeOH/EtOAc) yielded **32** (196 mg, 87.2%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.10 (br s, 1H), 10.21 (br s, 1H), 8.20 (s, 1H), 7.65 (d, 2H, *J* = 8.0 Hz), 7.45 (d, 2H, *J* = 8.5 Hz), 6.84 (dd, 1H, *J* = 3.0, 9.0 Hz), 6.61 (dd, 1H, *J* = 3.0, 8.0 Hz), 5.88 (tdd, 1H, *J* = 7.0, 10.0, 16.5 Hz), 5.03-5.00 (m, 2H), 3.81 (br s, 2H), 3.40 (br s, 2H), 3.31 (d, 2H, *J* = 6.5 Hz), 3.21 (s, 2H), 2.66 (br s, 2H), 2.54 (br s, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 168.3, 165.4, 155.4 (d, $J_{C\text{-}F}$ = 235.9 Hz), 152.3, 150.1, 139.7, 135.4, 132.5, 130.0 (d, $J_{C\text{-}F}$ = 6.8 Hz), 127.7, 119.1 (d, $J_{C\text{-}F}$ = 23.1 Hz), 118.0, 116.6 (d, $J_{C\text{-}F}$ = 7.9 Hz), 116.5, 113.8 (d, $J_{C\text{-}F}$ = 23.5 Hz), 113.6, 60.7, 53.3 (br), 47.3 (br), 42.0 (br), 33.6. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −128.3. HRMS (ESI): 450.1931 $(M+H)^+$; calcd. for $C_{24}H_{25}FN_5O_3$: 450.1941. HPLC purity: 97%.

N′-[5-Fluoro-2-hydroxy-3-(2-propenyl)phenylmethylene]-4-(4-fluorophenylmethyl)-1-piperazineacetohydrazide (33): Synthesized according to General Procedure D: **46f** (133

mg, 0.50 mmol, 1.0 equiv.), **47d** (90 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–20% MeOH/EtOAc) yielded **33** (176 mg, 82.0%) as a yellow solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.15 (br s, 1H), 10.22 (br s, 1H), 8.26 (s, 1H), 7.25 (dd, 2H, *J* = 5.5, 8.5 Hz), 6.98 (t, 2H, *J* = 8.5 Hz), 6.89 (dd, 1H, *J* = 3.0, 9.0 Hz), 6.72 (dd, 1H, *J* = 3.0, 8.0 Hz), 5.95 (tdd, 1H, *J* = 6.5, 10.0, 17.0 Hz), 5.10-5.06 (m, 2H), 3.47 (s, 2H), 3.39 (d, 2H, *J* = 6.5 Hz), 3.18 (s, 2H), 2.61 (br s, 4H), 2.49 (br s, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 166.2, 162.1 (d, $J_{C\text{-}F}$ = 243.8 Hz), 155.5 (d, $J_{C\text{-}F}$ = 235.5 Hz), 152.5 (d, $J_{C\text{-}F}$ = 0.9 Hz), 149.8, 135.7, 133.6 (d, $J_{C\text{-}F}$ = 3.0 Hz), 130.6 (d, $J_{C\text{-}F}$ = 7.8 Hz), 130.1 (d, $J_{C\text{-}F}$ = 6.8 Hz), 119.0 (d, $J_{C\text{-}F}$ = 23.0 Hz), 116.8 (d, $J_{C\text{-}F}$ = 7.8 Hz), 116.5, 115.1 (d, $J_{C\text{-}F}$ = 21.0 Hz), 113.9 (d, $J_{C\text{-}F}$ = 23.5 Hz), 62.1, 61.0, 53.7, 52.9, 33.8. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −118.8, −128.6. HRMS (ESI): 429.2095 $(M+H)^+$; calcd. for $C_{23}H_{27}F_2N_4O_2$: 429.2102. HPLC purity: 98%.

N′-[5-Fluoro-2-hydroxy-3-(2-propenyl)phenylmethylene]-4-(4-fluorobenzoyl)-1-piperazineacetohydrazide (34): Synthesized according to General Procedure D: **46g** (140 mg, 0.50 mmol, 1.0 equiv.), **47d** (90 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–15% MeOH/EtOAc) yielded **34** (163 mg, 73.8%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.10 (br s, 1H), 10.23 (br s, 1H), 8.25 (s, 1H), 7.38 (dd, 2H, *J* = 5.5, 8.5 Hz), 7.07 (t, 2H, *J* = 8.5 Hz), 6.88 (dd, 1H, *J* = 3.0, 9.0 Hz), 6.65 (dd, 1H, *J* = 3.0, 8.5 Hz), 5.92 (tdd, 1H, *J* = 6.5, 9.5, 17.0 Hz), 5.10-5.04 (m, 2H), 3.82 (br s, 2H), 3.50 (br s, 2H), 3.36 (d, 2H, *J* = 6.5 Hz), 3.21 (s, 2H), 2.59 (br s, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.7, 165.6, 163.6 (d, $J_{C\text{-}F}$ = 249.8 Hz), 155.5 (d, $J_{C\text{-}F}$ = 235.6 Hz), 152.5 (d, $J_{C\text{-}F}$ = 1.4 Hz), 150.3 (d, $J_{C\text{-}F}$ = 2.5 Hz), 135.6, 131.3 (d, $J_{C\text{-}F}$ = 3.5 Hz), 130.2 (d, $J_{C\text{-}F}$ = 6.9 Hz), 129.5 (d, $J_{C\text{-}F}$ = 8.4 Hz), 119.2 (d, $J_{C\text{-}F}$ = 23.1 Hz), 116.7 (d, $J_{C\text{-}F}$ = 7.8 Hz), 116.6, 115.9 (d, $J_{C\text{-}F}$ = 21.8 Hz), 113.9 (d, $J_{C\text{-}F}$ = 23.5 Hz), 60.9, 53.6 (br), 47.7 (br), 42.2 (br), 33.8. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −112.6, −128.4. HRMS (ESI): 443.1886 $(M+H)^+$; calcd. for $C_{23}H_{25}F_2N_4O_3$: 443.1895. HPLC purity: 98%.

N′-[5-Fluoro-2-hydroxy-3-(2-propenyl)phenylmethylene]-4-[4(trifluoromethyl)phenylmethyl]-1-piperazineacetohydrazide (35): Synthesized according to General Procedure D: **46h** (158 mg, 0.50 mmol, 1.0 equiv.), **47d** (90 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–20% MeOH/EtOAc) yielded **35** (176 mg, 73.7%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.14 (br s, 1H), 10.19 (br s, 1H), 8.28 (s, 1H), 7.56 (d, 2H, *J* = 8.0 Hz), 7.43 (d, 2H, *J* = 8.0 Hz), 6.90 (dd, 1H, *J* = 3.0, 9.0 Hz), 6.72 (dd, 2H, *J* = 3.0, 8.0 Hz), 5.96 (tdd, 1H, *J* = 6.5, 10.0, 17.0 Hz), 5.11-5.08 (m, 2H), 3.57 (s, 2H), 3.40 (d, 2H, *J* = 6.5 Hz), 3.20 (s, 2H), 2.63 (br s, 4H), 2.53 (br s, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 166.1, 155.6 (d, $J_{C\text{-}F}$ = 235.5 Hz), 152.5 (d, $J_{C\text{-}F}$ = 1.3 Hz), 149.9 (d, $J_{C\text{-}F}$ = 2.4 Hz), 142.4, 135.7, 130.2 (d, $J_{C\text{-}F}$ = 6.8 Hz), 129.5 (q, $J_{C\text{-}F}$ = 32.0 Hz), 129.3, 125.3 (q, $J_{C\text{-}F}$ = 3.8 Hz), 124.4 (q, $J_{C\text{-}F}$ = 270.5 Hz), 119.1 (d, $J_{C\text{-}F}$ = 23.1 Hz), 116.9 (d, $J_{C\text{-}F}$ = 7.8 Hz), 116.6, 114.0 (d, $J_{C\text{-}F}$ = 23.5 Hz), 62.3, 61.0, 53.8, 53.1, 33.8. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −65.4, −128.5. HRMS (ESI): 479.2066 $(M+H)^+$; calcd. for $C_{24}H_{27}F_4N_4O_2$: 479.2070. HPLC purity: 96%.

N′-[5-Fluoro-2-hydroxy-3-(2-propenyl)phenylmethylene]-4-[4-(trifluoromethyl)benzoyl]-1-piperazineacetohydrazide (36): Synthesized according to General Procedure D: **46i** (165 mg, 0.50 mmol, 1.0 equiv.), **47d** (90 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–15% MeOH/EtOAc) yielded **36** (139 mg, 56.3%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.07 (br s, 1H), 10.14 (br s, 1H), 8.26 (s, 1H), 7.66 (d, 2H, J = 8.5 Hz), 7.50 (d, 2H, J = 8.0 Hz), 6.89 (dd, 1H, J = 3.0, 9.0 Hz), 6.66 (dd, 1H, J = 3.0, 8.5 Hz), 5.93 (tdd, 1H, J = 7.0, 10.0, 16.5 Hz), 5.10-5.04 (m, 2H), 3.86 (br s, 2H), 3.45 (br s, 2H), 3.37 (d, 2H, J = 6.5 Hz), 3.23 (s, 2H), 2.69 (br s, 2H), 2.57 (br s, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.1, 165.5, 155.6 (d, $J_{C\text{-}F}$ = 235.9 Hz), 152.5, 150.4 (d, $J_{C\text{-}F}$ = 2.1 Hz), 138.9, 135.6, 132.0 (q, $J_{C\text{-}F}$ = 32.6 Hz), 130.3 (d, $J_{C\text{-}F}$ = 6.8 Hz), 127.5, 125.9 (q, $J_{C\text{-}F}$ = 3.8 Hz), 123.7 (q, $J_{C\text{-}F}$ = 271.1 Hz), 119.3 (d, $J_{C\text{-}F}$ = 23.0 Hz), 116.7 (d, $J_{C\text{-}F}$ = 4.6 Hz), 116.7, 114.0 (d, $J_{C\text{-}F}$ = 23.5 Hz), 60.9, 53.6 (br), 47.5 (br), 42.2 (br), 33.8. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −66.0, −128.4. HRMS (ESI): 493.1868 $(M+H)^+$; calcd. for $C_{24}H_{25}F_4N_4O_3$: 493.1863. HPLC purity: 97%.

N′-(5-Fluoro-2-hydroxy-3-propylphenylmethylene)-4-phenylmethyl-1-piperazineacetohydrazide (37): Synthesized according to General Procedure D: **46a** (124 mg, 0.50 mmol, 1.0 equiv.), **47e** (91 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–15% MeOH/EtOAc) yielded **37** (174 mg, 84.6%) as an off-white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.10 (s, 1H), 10.19 (br s, 1H), 8.26 (s, 1H), 7.32-7.30 (m, 4H), 7.28-7.25 (m, 1H), 6.89 (dd, 1H, J = 3.0, 9.0 Hz), 6.71 (dd, 1H, J = 3.0, 8.5 Hz), 3.54 (s, 2H), 3.20 (s, 2H), 2.66-2.59 (m, 6H), 2.53 (br s, 4H), 1.64 (sext, 2H, J = 7.5 Hz), 0.95 (t, 3H, J = 7.5 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 166.1, 155.4 (d, $J_{C\text{-}F}$ = 235.1 Hz), 152.8, 150.0, 137.9, 132.7 (d, $J_{C\text{-}F}$ = 6.6 Hz), 129.2, 128.4, 127.3, 119.1 (d, $J_{C\text{-}F}$ = 22.5 Hz), 116.7 (d, $J_{C\text{-}F}$ = 7.9 Hz), 113.4 (d, $J_{C\text{-}F}$ = 23.4 Hz), 63.0, 61.0, 53.8, 53.1, 31.9, 22.5, 14.0. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −129.1. HRMS (ESI): 413.2345 $(M+H)^+$; calcd. for $C_{23}H_{30}FN_4O_2$: 413.2353. HPLC purity: 99%.

4-{4-[N′-(5-Fluoro-2-hydrxy-3-propylphenylmethylene)hydrazinecarbonylmethyl]-1-piperazinylmethyl}benzenesulfonamide (38): Synthesized according to General Procedure D: **46b** (164 mg, 0.50 mmol, 1.0 equiv.), **47e** (91 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–20% MeOH/EtOAc) yielded **38** (221 mg, 89.9%) as a white solid. ^{1}H-NMR (500 MHz, $(CD_3)_2CO$) δ 11.68 (br s, 1H), 10.92 (br s, 1H), 8.46 (s, 1H), 7.84 (d, 2H, J = 8.0 Hz), 7.49 (d, 2H, J = 8.0 Hz), 6.98 (dd, 1H, J = 3.0, 9.5 Hz), 6.93 (dd, 1H, J = 3.0, 8.5 Hz), 6.59 (br s, 2H), 3.57 (s, 2H), 3.18 (s, 2H), 2.64-2.59 (m, 6H), 2.50 (br s, 4H), 1.63 (sext, 2H, J = 7.5 Hz), 0.93 (t, 3H, J = 7.5 Hz). ^{13}C-NMR (125 MHz, $(CD_3)_2CO$) δ 166.6, 156.1 (d, $J_{C\text{-}F}$ = 233.1 Hz), 153.4 (d, $J_{C\text{-}F}$ = 1.4 Hz), 149.7 (d, $J_{C\text{-}F}$ = 2.9 Hz), 143.9, 143.6, 132.8 (d, $J_{C\text{-}F}$ = 6.9 Hz), 129.9, 126.8, 119.0 (d, $J_{C\text{-}F}$ = 22.8 Hz), 118.3 (d, $J_{C\text{-}F}$ = 8.1 Hz), 114.2 (d, $J_{C\text{-}F}$ = 23.6 Hz), 62.5, 61.6, 54.2, 53.4, 32.3, 23.1, 14.1. ^{19}F-NMR (470 MHz, $(CD_3)_2CO$) δ −127.7. HRMS (ESI): 492.2074 $(M+H)^+$; calcd. for $C_{23}H_{31}FN_5O_4S$: 492.2081. HPLC purity: 96%.

N′-(5-Fluoro-2-hydroxy-3-propylphenylmethylene)-4-benzoyl-1-piperazineacetohydrazide (39): Synthesized according to General Procedure D: **46c** (131 mg, 0.50 mmol, 1.0 equiv.), **47e** (91 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–10% MeOH/EtOAc) yielded **39** (189 mg, 88.9%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.13 (s, 1H), 10.48 (br s, 1H), 8.15 (s, 1H), 7.38-7.32 (m, 5H), 6.83 (dd, 1H, *J* = 3.0, 9.0 Hz), 6.55 (dd, 1H, *J* = 3.0, 8.5 Hz), 3.80 (br s, 2H), 3.46 (br s, 2H), 3.18 (s, 2H), 2.59-2.54 (m, 6H), 1.57 (sext, 2H, *J* = 7.5 Hz), 0.88 (t, 3H, *J* = 7.5 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.5, 165.6, 155.3 (d, $J_{C\text{-}F}$ = 235.1 Hz), 152.6 (d, $J_{C\text{-}F}$ = 1.4 Hz), 150.2 (d, $J_{C\text{-}F}$ = 2.5 Hz), 135.3, 132.5 (d, $J_{C\text{-}F}$ = 6.8 Hz), 130.1, 128.7, 126.9, 119.1 (d, $J_{C\text{-}F}$ = 22.6 Hz), 116.6 (d, $J_{C\text{-}F}$ = 7.9 Hz), 113.4 (d, $J_{C\text{-}F}$ = 23.4 Hz), 60.7, 53.5 (br), 47.5 (br), 42.0 (br), 31.8, 22.4, 13.9. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −129.0. HRMS (ESI): 427.2144 $(M+H)^+$; calcd. for $C_{23}H_{28}FN_4O_3$: 427.2145. HPLC purity: 99%.

N′-(5-Fluoro-2-hydroxy-3-propylphenylmethylene)-4-(4-cyanopheylmehtyl)-1-piperazineacetohydrazide (40): Synthesized according to General Procedure D: **46d** (137 mg, 0.50 mmol, 1.0 equiv.), **47e** (91 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–15% MeOH/EtOAc) yielded **40** (180 mg, 82.1%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.07 (br s, 1H), 10.16 (br s, 1H), 8.25 (s, 1H), 7.57 (d, 2H, *J* = 8.5 Hz), 7.42 (d, 2H, *J* = 8.0 Hz), 6.86 (dd, 1H, *J* = 3.0, 9.0 Hz), 6.68 (dd, 1H, *J* = 3.0, 8.0 Hz), 3.55 (s, 2H), 3.18 (s, 2H), 2.70-2.57 (m, 6H), 2.51 (br s, 4H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 166.0, 155.4 (d, $J_{C\text{-}F}$ = 235.1 Hz), 152.7 (d, $J_{C\text{-}F}$ = 1.4 Hz), 150.0 (d, $J_{C\text{-}F}$ = 2.5 Hz), 144.0, 132.6 (d, $J_{C\text{-}F}$ = 6.8 Hz), 132.2, 129.5, 119.1 (d, $J_{C\text{-}F}$ = 22.6 Hz), 119.0, 116.6 (d, $J_{C\text{-}F}$ = 8.0 Hz), 113.4 (d, $J_{C\text{-}F}$ = 23.5 Hz), 110.9, 62.3, 60.9, 53.7, 53.1, 31.9, 22.4, 14.0. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −129.0. HRMS (ESI): 438.2301 $(M+H)^+$; calcd. for $C_{24}H_{29}FN_5O_2$: 438.2305. HPLC purity: 96%.

N′-(5-Fluoro-2-hydroxy-3-propylphenylmethylene)-4-(4-cyanobenzoyl)-1-piperazineacetohydrazide (41): Synthesized according to General Procedure D: **46e** (144 mg, 0.50 mmol, 1.0 equiv.), **47e** (91 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–10% MeOH/EtOAc) yielded **41** (196 mg, 86.5%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.03 (br s, 1H), 10.17 (br s, 1H), 8.19 (s, 1H), 7.66 (d, 2H, *J* = 8.0 Hz), 7.46 (d, 2H, *J* = 8.0 Hz), 6.84 (dd, 1H, *J* = 3.0, 9.0 Hz), 6.59 (dd, 1H, *J* = 3.0, 8.5 Hz), 3.82 (br s, 2H), 3.41 (br s, 2H), 3.22 (s, 2H), 2.66 (br s, 2H), 2.57-2.52 (m, 2H), 1.55 (sext, 2H, *J* = 7.5 Hz), 0.87 (t, 3H, *J* = 7.5 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 168.3, 165.4, 155.3 (d, $J_{C\text{-}F}$ = 235.5 Hz), 152.6 (d, $J_{C\text{-}F}$ = 1.1 Hz), 150.3 (d, $J_{C\text{-}F}$ = 2.0 Hz), 139.7, 132.6 (d, $J_{C\text{-}F}$ = 6.8 Hz), 132.5, 127.7, 119.2 (d, $J_{C\text{-}F}$ = 22.6 Hz), 118.0, 116.4 (d, $J_{C\text{-}F}$ = 7.9 Hz), 113.6, 113.4 (d, $J_{C\text{-}F}$ = 23.3 Hz), 60.7, 53.4 (br), 47.4 (br), 42.0 (br), 31.7, 22.3, 13.9. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −128.7. HRMS (ESI): 452.2098 $(M+H)^+$; calcd. for $C_{24}H_{27}FN_5O_3$: 452.2098. HPLC purity: 99%.

N′-(5-Fluoro-2-hydroxy-3-propylphenylmethylene)-4-(4-fluoropheylmethyl)-1-piperazineacetohydrazide (42): Synthesized according to General Procedure D: **46f** (133 mg, 0.50 mmol, 1.0 equiv.), **47e** (91 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035

mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–20% MeOH/EtOAc) yielded **42** (202 mg, 93.8%) as a yellow solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.07 (br s, 1H), 10.16 (br s, 1H), 8.26 (s, 1H), 7.26 (dd, 2H, *J* = 5.5, 8.5 Hz), 6.99 (t, 2H, *J* = 8.5 Hz), 6.88 (dd, 1H, *J* = 3.0, 9.0 Hz), 6.70 (dd, 1H, *J* = 3.0, 8.0 Hz), 3.48 (s, 2H), 3.18 (s, 2H), 2.66-2.58 (m, 6H), 2.50 (br s, 4H), 1.62 (sext, 2H, *J* = 7.5 Hz), 0.94 (t, 3H, *J* = 7.5 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 166.1, 162.1 (d, $J_{C\text{-}F}$ = 243.8 Hz), 155.5 (d, $J_{C\text{-}F}$ = 235.0 Hz), 152.8 (d, $J_{C\text{-}F}$ = 0.9 Hz), 150.1 (d, $J_{C\text{-}F}$ = 2.0 Hz), 133.7, 132.7 (d, $J_{C\text{-}F}$ = 6.8 Hz), 130.7 (d, $J_{C\text{-}F}$ = 7.9 Hz), 119.2 (d, $J_{C\text{-}F}$ = 22.5 Hz), 116.7 (d, $J_{C\text{-}F}$ = 7.9 Hz), 115.2 (d, $J_{C\text{-}F}$ = 21.0 Hz), 113.5 (d, $J_{C\text{-}F}$ = 23.4 Hz), 62.1, 61.0, 53.8, 53.0, 31.9, 22.5, 14.1. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −118.8, −129.0. HRMS (ESI): 431.2250 $(M+H)^+$; calcd. for $C_{23}H_{29}F_2N_4O_2$: 431.2259. HPLC purity: 98%.

N′-(5-Fluoro-2-hydroxy-3-propylphenylmethylene)-4-(4-fluorobenzoyl)-1-piperazineacetohydrazide (43): Synthesized according to General Procedure D: **46g** (140 mg, 0.50 mmol, 1.0 equiv.), **47e** (91 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–10% MeOH/EtOAc) yielded **43** (195 mg, 87.7%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.07 (br s, 1H), 10.32 (br s, 1H), 8.18 (s, 1H), 7.36 (dd, 2H, *J* = 5.0, 8.5 Hz), 7.05 (t, 2H, *J* = 8.5 Hz), 6.84 (dd, 1H, *J* = 3.0, 9.0 Hz), 6.57 (dd, 1H, *J* = 3.0, 8.5 Hz), 3.81 (br s, 2H), 3.48 (br s, 2H), 3.20 (s, 2H), 2.62-2.50 (m, 6H), 1.56 (sext, 2H, *J* = 7.5 Hz), 0.88 (t, 3H, *J* = 7.5 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.6, 165.6, 163.5 (d, $J_{C\text{-}F}$ = 249.3 Hz), 155.4 (d, $J_{C\text{-}F}$ = 235.4 Hz), 152.7 (d, $J_{C\text{-}F}$ = 0.9 Hz), 150.3, 132.6 (d, $J_{C\text{-}F}$ = 6.8 Hz), 131.3 (d, $J_{C\text{-}F}$ = 3.4 Hz), 129.4 (d, $J_{C\text{-}F}$ = 8.4 Hz), 119.2 (d, $J_{C\text{-}F}$ = 22.6 Hz), 116.5 (d, $J_{C\text{-}F}$ = 7.9 Hz), 115.8 (d, $J_{C\text{-}F}$ = 21.6 Hz), 113.4 (d, $J_{C\text{-}F}$ = 23.4 Hz), 60.7, 53.5 (br), 47.7 (br), 42.1 (br), 31.8, 22.4, 13.9. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −112.6, −128.8. HRMS (ESI): 445.2049 $(M+H)^+$; calcd. for $C_{23}H_{27}F_2N_4O_3$: 445.2051. HPLC purity: 98%.

N′-(5-Fluoro-2-hydroxy-3-propylphenylmethylene)-4-[4-(trifluoromethyl)phenylmethyl]-1-piperazineacetohydrazide (44): Synthesized according to General Procedure D: **46h** (158 mg, 0.50 mmol, 1.0 equiv.), **47e** (91 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–20% MeOH/EtOAc) yielded **44** (184 mg, 76.5%) as a light yellow solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.08 (br s, 1H), 10.18 (br s, 1H), 8.25 (s, 1H), 7.56 (d, 2H, *J* = 8.0 Hz), 7.43 (d, 2H, *J* = 8.0 Hz), 6.88 (dd, 1H, *J* = 3.0, 9.0 Hz), 6.69 (dd, 2H, *J* = 3.0, 8.5 Hz), 3.56 (s, 2H), 3.20 (s, 2H), 2.64-2.58 (m, 6H), 2.52 (br s, 4H), 1.62 (sext, 2H, *J* = 7.5 Hz), 0.93 (t, 3H, *J* = 7.5 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 166.1, 155.5 (d, $J_{C\text{-}F}$ = 235.1 Hz), 152.8 (d, $J_{C\text{-}F}$ = 1.2 Hz), 150.0 (d, $J_{C\text{-}F}$ = 2.6 Hz), 142.4, 132.7 (d, $J_{C\text{-}F}$ = 6.8 Hz), 129.5 (q, $J_{C\text{-}F}$ = 32.1 Hz), 129.3, 125.3 (q, $J_{C\text{-}F}$ = 3.8 Hz), 124.4 (q, $J_{C\text{-}F}$ = 270.5 Hz), 119.2 (d, $J_{C\text{-}F}$ = 22.6 Hz), 116.7 (d, $J_{C\text{-}F}$ = 7.9 Hz), 113.5 (d, $J_{C\text{-}F}$ = 23.5 Hz), 62.3, 61.0, 53.8, 53.1, 31.9, 22.5, 14.0. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −65.4, −128.9. HRMS (ESI): 481.2216 $(M+H)^+$; calcd. for $C_{24}H_{29}F_4N_4O_2$: 481.2227. HPLC purity: 97%.

N′-(5-Fluoro-2-hydroxy-3-propylphenylmethylene)-4-[4-(trifluoromethyl)benzoyl]-1-piperazineacetohydrazide (45): Synthesized according to General Procedure D: **46i** (165

mg, 0.50 mmol, 1.0 equiv.), **47e** (91 mg, 0.50 mmol, 1.0 equiv.), 1.2 M HCl (29 μL, 0.035 mmol, 0.070 equiv.), EtOH (3 mL, 0.15 M). Purification by silica gel column chromatography (gradient, 0–10% MeOH/EtOAc) yielded **45** (140 mg, 56.7%) as a white solid. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 11.04 (br s, 1H), 10.19 (br s, 1H), 8.21 (s, 1H), 7.65 (d, 2H, *J* = 8.5 Hz), 7.48 (d, 2H, *J* = 8.0 Hz), 6.86 (dd, 1H, *J* = 3.0, 9.0 Hz), 6.60 (dd, 1H, *J* = 3.0, 8.0 Hz), 3.85 (br s, 2H), 3.44 (br s, 2H), 3.22 (s, 2H), 2.67 (br s, 2H), 2.60-2.51 (m, 4H), 1.58 (sext, 2H, *J* = 7.5 Hz), 0.90 (t, 3H, *J* = 7.5 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 169.0, 165.5, 155.4 (d, $J_{C\text{-}F}$ = 235.4 Hz), 152.7 (d, $J_{C\text{-}F}$ = 0.9 Hz), 150.4 (d, $J_{C\text{-}F}$ = 2.4 Hz), 138.9, 132.7 (d, $J_{C\text{-}F}$ = 6.8 Hz), 132.0 (q, $J_{C\text{-}F}$ = 32.6 Hz), 127.5, 125.8 (q, $J_{C\text{-}F}$ = 3.6 Hz), 123.7 (q, $J_{C\text{-}F}$ = 271.0 Hz), 119.3 (d, $J_{C\text{-}F}$ = 22.6 Hz), 116.5 (d, $J_{C\text{-}F}$ = 7.9 Hz), 113.4 (d, $J_{C\text{-}F}$ = 23.4 Hz), 60.8, 53.5 (br), 47.5 (br), 42.0 (br), 31.8, 22.4, 14.0. ^{19}F-NMR (470 MHz, $CDCl_3$) δ −66.0, −128.7. HRMS (ESI): 495.2008 $(M+H)^+$; calcd. for $C_{24}H_{27}F_4N_4O_3$: 495.2019. HPLC purity: 98%.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviation Used

PAC-1 Procaspase-Activating Compound 1

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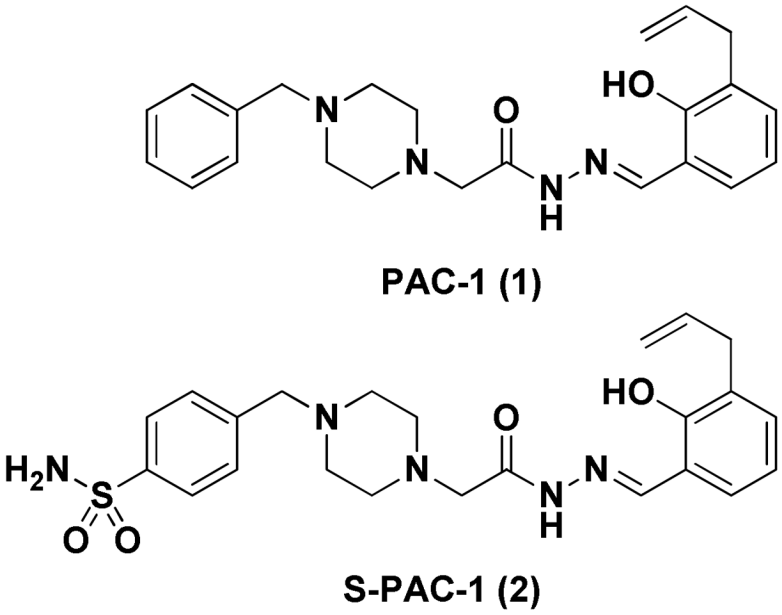


Figure 1.
Structures of **PAC-1** (**1**) and **S-PAC-1** (**2**).

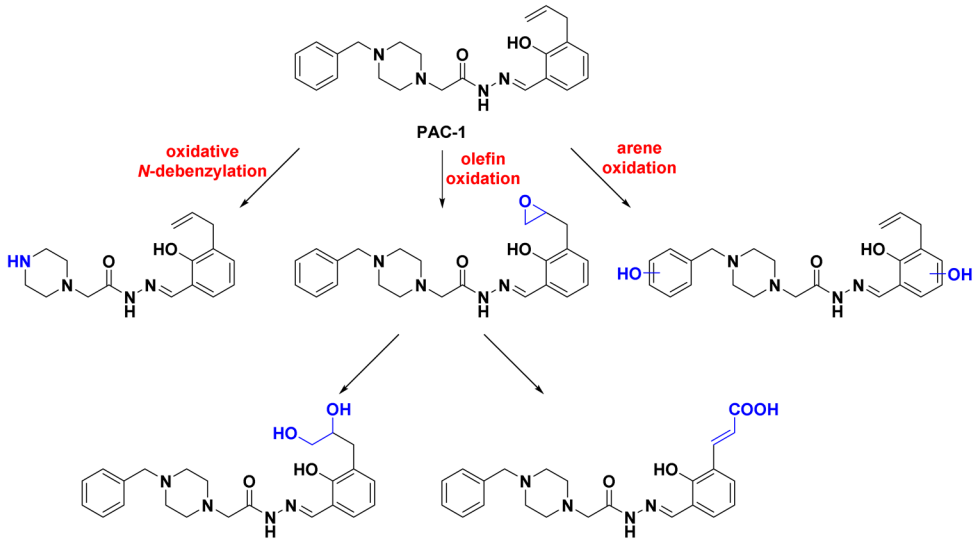


Figure 2.

PAC-1 is susceptible to enzymatic oxidation in vitro and in vivo, giving metabolites that result from *N*-debenzylation, olefin oxidation, and arene oxidation.[75]

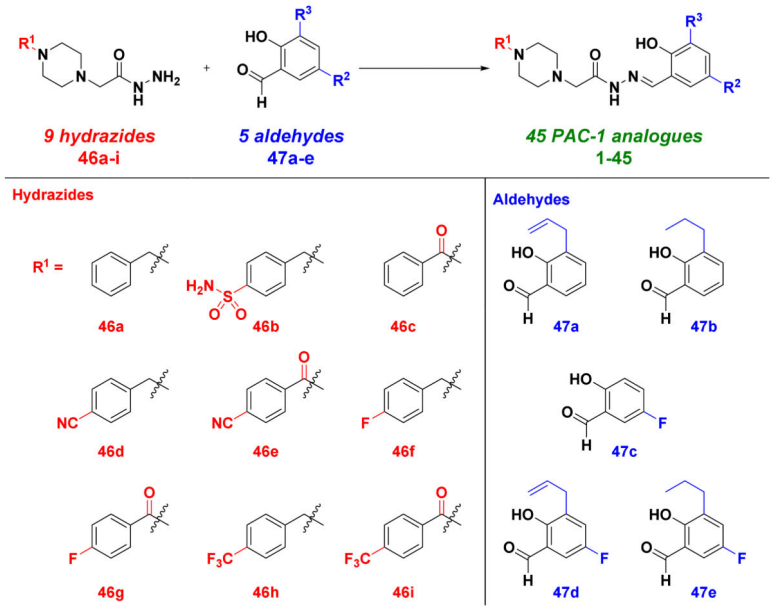


Figure 3.

Nine hydrazides and five aldehydes were used to construct a library of 45 **PAC-1** derivatives designed to display enhanced metabolic stability by blocking oxidative *N*-debenzylation, olefin oxidation, and/or arene oxidation.

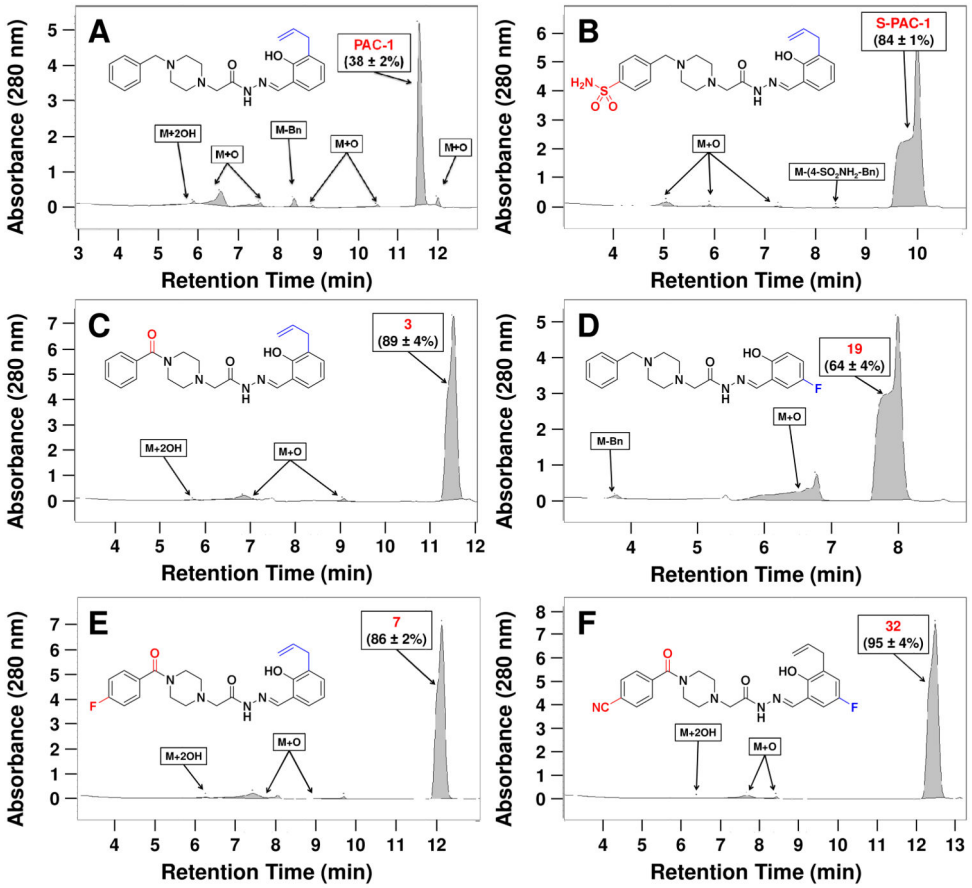


Figure 4.

Metabolic stability of **PAC-1** and derivatives was evaluated in rat liver microsomes at 10 μM for 3 hours. LC/MS results of liver microsome experiments for **A. PAC-1**, **B. S-PAC-1**, **C. 3**, **D. 19**, **E. 7**, and **F. 32** are shown. Data shown are representative of three independent experiments.

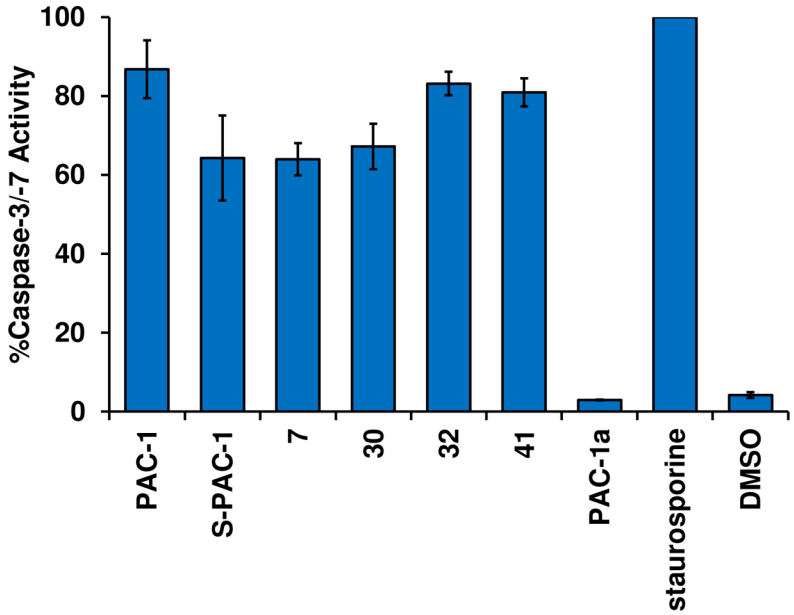


Figure 5.

PAC-1 and active derivatives activate executioner caspases in cells. U-937 cells were treated with compounds (30 μM for **PAC-1** derivatives, 1 μM for staurosporine) for 16 hours, and then lysed. Caspase-3/7 activity was assessed by cleavage of the fluorogenic substrate Ac-DEVD-AFC. Cells treated with vehicle alone or inactive derivative **PAC-1a** show minimal caspase activity after 16 hours. Values shown are mean ± s.e.m. (n = 3).

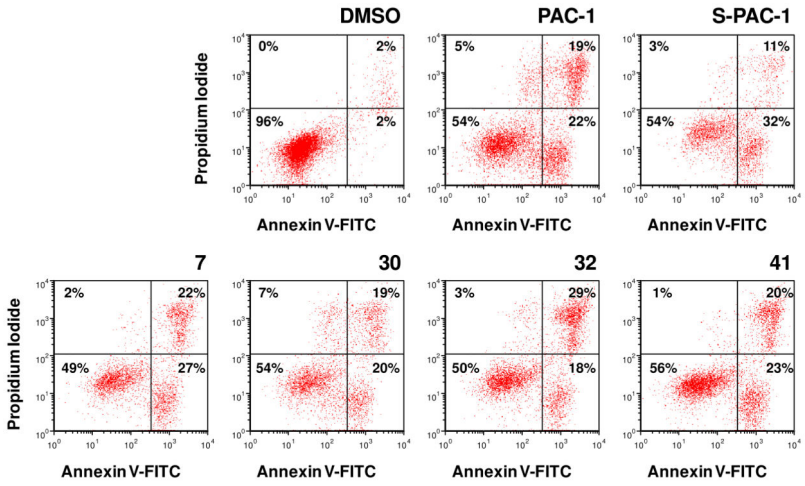


Figure 6.

PAC-1 and derivatives induce apoptosis in U-937 cells. Cells were treated for 12 hours at 50 μM, and viability was assessed by Annexin V-FITC/propidium iodide staining. Data shown are representative of three independent experiments.

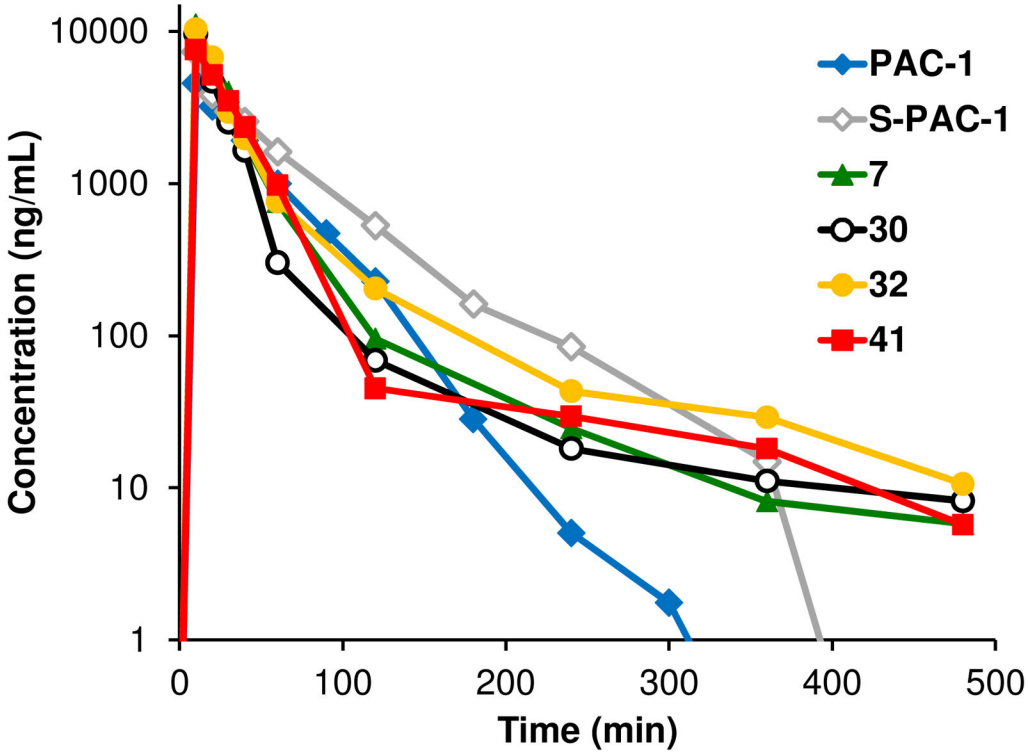
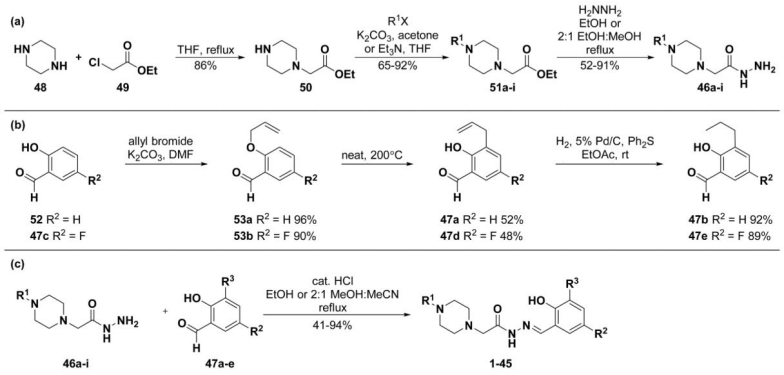


Figure 7.

Pharmacokinetic profiles of **PAC-1** and selected derivatives following 25 mg/kg intravenous dose (n = 2). Detectable levels of the novel derivatives are present in serum for at least 8 hours post-treatment, while **PAC-1** and **S-PAC-1** are no longer detectable after 5 and 6 hours post-treatment, respectively.



Scheme 1.

Synthesis of PAC-1 analogues. (a) Synthesis of hydrazides **46a-i**. (b) Synthesis of aldehydes **47a-e**. (c) Condensation of hydrazides and aldehydes to form PAC-1 analogues **1–45**.

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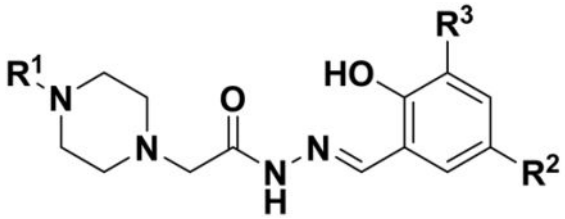
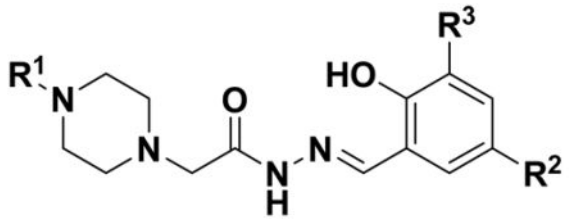
Table 1

Cytotoxicity,[a] metabolic stability,[b] and mouse toxicity[c] of **PAC-1** analogues.



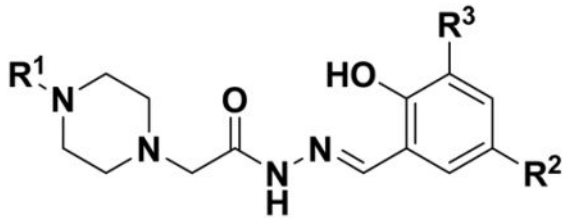
1-45

compound	R^1	R^2	R^3	U-937 72h IC_{50} (μM)	RLM 3h % Stability	mouse toxicity IP 200 mg/kg (* = 100 mg/kg)
1 (PAC-1)	Bn	H	All	10.2 ± 0.3	38 ± 2	severe
2 (S-PAC-1)	4-SO_2NH_2-Bn	H	All	8.9 ± 0.6	84 ± 1	none
3	Bz	H	All	12.1 ± 1.3	89 ± 4	-
4	4-CN-Bn	H	All	13.7 ± 0.9	48 ± 2	-
5	4-CN-Bz	H	All	13.1 ± 3.7	90 ± 4	-
6	4-F-Bn	H	All	11.1 ± 2.1	31 ± 1	-
7	4-F-Bz	H	All	10.2 ± 1.7	86 ± 2	moderate
8	4-CF_3-Bn	H	All	15.3 ± 6.7	16 ± 1	-
9	4-CF_3-Bz	H	All	6.6 ± 1.9	85 ± 6	lethal*
10	Bn	H	*n*-Pr	9.6 ± 2.1	30 ± 1	-
11	4-SO_2NH_2-Bn	H	*n*-Pr	4.9 ± 0.4	61 ± 2	-
12	Bz	H	*n*-Pr	9.4 ± 1.3	71 ± 3	-
13	4-CN-Bn	H	*n*-Pr	9.0 ± 1.2	30 ± 2	-
14	4-CN-Bz	H	*n*-Pr	12.8 ± 2.7	61 ± 3	-
15	4-F-Bn	H	*n*-Pr	10.0 ± 1.7	24 ± 2	-
16	4-F-Bz	H	*n*-Pr	7.3 ± 0.9	69 ± 4	-
17	4-CF_3-Bn	H	*n*-Pr	4.1 ± 0.4	15 ± 2	-
18	4-CF_3-Bz	H	*n*-Pr	4.8 ± 1.2	64 ± 1	lethal*
19	Bn	F	H	17.0 ± 1.4	64 ± 4	-



1-45

compound	R^1	R^2	R^3	U-937 72h IC_{50} (μM)	RLM 3h % Stability	mouse toxicity IP 200 mg/kg (* = 100 mg/kg)
20	4-SO_2NH_2-Bn	F	H	-	-	-
21	Bz	F	H	15.7 ± 2.6	88 ± 1	-
22	4-CN-Bn	F	H	-	-	-
23	4-CN-Bz	F	H	15.3 ± 1.3	88 ± 4	-
24	4-F-Bn	F	H	-	-	-
25	4-F-Bz	F	H	15.3 ± 0.8	86 ± 2	-
26	4-CF_3-Bn	F	H	4.7 ± 0.3	30 ± 5	-
27	4-CF_3-Bz	F	H	8.7 ± 0.5	87 ± 3	moderate
28	Bn	F	All	9.5 ± 0.9	56 ± 1	-
29	4-SO_2NH_2-Bn	F	All	9.8 ± 1.3	89 ± 3	lethal
30	Bz	F	All	8.6 ± 2.0	93 ± 7	moderate
31	4-CN-Bn	F	All	12.7 ± 2.0	65 ± 2	-
32	4-CN-Bz	F	All	10.1 ± 2.0	95 ± 4	mild
33	4-F-Bn	F	All	10.3 ± 4.1	57 ± 1	-
34	4-F-Bz	F	All	8.5 ± 1.4	92 ± 3	severe
35	4-CF_3-Bn	F	All	3.4 ± 0.6	49 ± 3	-
36	4-CF_3-Bz	F	All	6.5 ± 0.6	90 ± 2	moderate
37	Bn	F	*n*-Pr	8.9 ± 1.2	49 ± 6	-
38	4-SO_2NH_2-Bn	F	*n*-Pr	8.7 ± 0.4	62 ± 3	-
39	Bz	F	*n*-Pr	12.3 ± 1.0	86 ± 5	-
40	4-CN-Bn	F	*n*-Pr	11.2 ± 0.9	49 ± 5	-
41	4-CN-Bz	F	*n*-Pr	9.4 ± 1.2	66 ± 3	mild



1-45

compound	R^1	R^2	R^3	U-937 72h IC_{50} (μM)	RLM 3h % Stability	mouse toxicity IP 200 mg/kg (* = 100 mg/kg)
42	4-F-Bn	F	*n*-Pr	7.5 ± 0.7	48 ± 1	-
43	4-F-Bz	F	*n*-Pr	7.5 ± 1.4	67 ± 3	severe
44	4-CF_3-Bn	F	*n*-Pr	3.9 ± 0.6	40 ± 1	-
45	4-CF_3-Bz	F	*n*-Pr	5.2 ± 0.6	64 ± 5	lethal

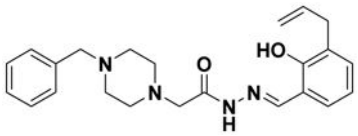
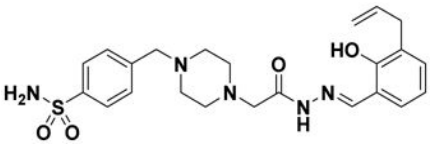
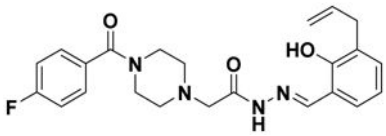
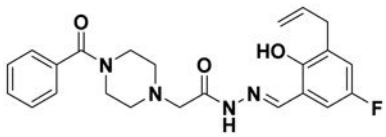
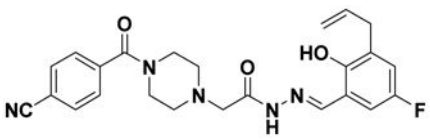
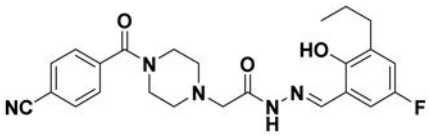
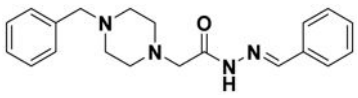
[a] Cells treated with compounds for 72 hours. Biomass quantified by sulforhodamine B assay. IC_{50} values shown are mean ± s.e.m. (n = 3).

[b] Rat liver microsomes treated with compounds (10 μM) for 3 hours. Percent stability values shown are mean ± s.e.m. (n = 3).

[c] Mice dosed with compound via i.p. injection at 200 mg/kg (except *100 mg/kg). Dash indicates that the compound was not evaluated.

Table 2

Zinc chelation[a] and caspase activation[b] by **PAC-1** derivatives.

	Zn^{2+} K_d (nM)	% Caspase-3/-7 Activity
 **PAC-1**	1.28 ± 0.03	87 ± 7
 **S-PAC-1**	2.72 ± 0.13	64 ± 11
 **7**	1.46 ± 0.07	64 ± 4
 **30**	1.07 ± 0.09	67 ± 6
 **32**	1.37 ± 0.10	83 ± 3
 **41**	1.37 ± 0.03	81 ± 4
 **PAC-1a**	$>10^6$	3 ± 0.1

[a] Increasing amounts of $Zn(OTf)_2$ added to a buffered solution of EGTA (7.3 mM) and **PAC-1** derivative (100 μM). K_d was determined by comparing fluorescence intensity (ex. 410 nm, em. 475 nm) and free zinc concentration.

[b] U-937 cells treated with compounds (30 μM) for 16 hours, then lysed. Caspase-3/7 activity assessed by cleavage of fluorogenic substrate Ac-DEVD-AFC.

Table 3

PAC-1and derivatives are cytotoxic to white blood cell cancer lines in culture.[a]

			72-hour IC_{50} (μM)					
Cell line	**Species**	**Origin**	**PAC-1**	**S-PAC-1**	**7**	**30**	**32**	**41**
U-937	human	lymphoma	10.2 ± 0.3	8.9 ± 0.6	10.2 ± 1.7	8.6 ± 2.0	10.1 ± 2.0	9.4 ± 1.2
Jurkat	human	leukemia	4.4 ± 0.6	4.5 ± 1.2	4.0 ± 0.5	4.1 ± 0.7	3.5 ± 0.2	3.4 ± 0.6
GL-1	dog	lymphoma	3.0 ± 0.1	3.2 ± 0.2	3.0 ± 0.1	3.4 ± 0.2	2.4 ± 0.4	2.2 ± 0.3
OSW	dog	lymphoma	10.0 ± 0.8	9.8 ± 0.1	9.3 ± 0.2	10.0 ± 0.6	9.5 ± 0.7	8.5 ± 0.7
EL4	mouse	lymphoma	6.5 ± 0.5	7.9 ± 0.5	6.5 ± 0.8	7.3 ± 1.2	5.1 ± 0.4	4.7 ± 0.7

[a]Cells treated with compounds for 72 hours. Biomass quantified by sulforhodamine B assay. IC_{50} values shown are mean ± s.e.m. (n = 3).

Table 4

Pharmacokinetic parameters for **PAC-1** and selected derivatives.[a]

compound	$t_{1/2}$ (min)	AUC (IV) (min*μg/mL)	AUC (PO) (min*μg/mL)	$\%F_{oral}$
PAC-1	24.6 ± 0.9	210.3 ± 9.3	31.6 ± 1.6	15.1 ± 1.4
S-PAC-1	38.1 ± 3.3	446.0 ± 114.1	54.0 ± 12.4	12.9 ± 6.1
7	89.5 ± 19.3	362.3 ± 55.8	92.2 ± 6.0	25.6 ± 2.3
30	120.5 ± 16.3	291.0 ± 40.6	25.7 ± 18.3	8.5 ± 5.1
32	88.7 ± 3.3	364.9 ± 5.6	105.2 ± 24.2	28.8 ± 6.2
41	122.3 ± 1.4	313.4 ± 5.5	113.0 ± 3.7	36.1 ± 0.5

[a] 25 mg/kg dose was administered via intravenous injection or oral gavage. Values shown are mean ± standard deviation (n = 2).