Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Development of radamide analogs as Grp94 inhibitors

Aaron Muth, Vincent Crowley, Anuj Khandelwal, Sanket Mishra, Jinbo Zhao, Jessica Hall, Brian S. J. Blagg*

Department of Medicinal Chemistry, The University of Kansas, 1251 Wescoe Hall Drive, Malott 4070, Lawrence, KS 66045-7563, United States

ARTICLE INFO

Article history: Received 10 April 2014 Revised 21 May 2014 Accepted 28 May 2014 Available online 12 June 2014

Keyword: Hsp90 Grp94

ABSTRACT

Hsp90 isoform-selective inhibition is highly desired as it can potentially avoid the toxic side-effects of *pan*-inhibition. The current study developed selective inhibitors of one such isoform, Grp94, predicated on the chimeric and *pan*-Hsp90 inhibitor, radamide (RDA). Replacement of the quinone moiety of RDA with a phenyl ring (**2**) was found to be better suited for Grp94 inhibition as it can fully interact with a unique hydrophobic pocket present in Grp94. An extensive SAR for this scaffold showed that substitutions at the 2- and 4-positions (**8** and **27**, respectively) manifested excellent Grp94 affinity and selectivity. Introduction of heteroatoms into the ring also proved beneficial, with a 2-pyridine derivative (**38**) exhibiting the highest Grp94 affinity (K_d = 820 nM). Subsequent cell-based assays showed that these Grp94 inhibitors inhibit migration of the metastatic breast cancer cell line, MDA-MB-231, as well as exhibit an anti-proliferative affect against the multiple myeloma cell line, RPMI 8226.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Heat shock protein 90 (Hsp90) is a molecular chaperone that plays an important role in cellular activity by maintaining the conformational maturation of approximately 200 client proteins. In normal cells, Hsp90 is associated with proteins responsible for trafficking, cell signaling, protein folding, as well as the maintenance and degradation of client proteins.^{1,2} Hsp90 functions are modulated through three domains; the N- and C-termini and the middle domain. The N-terminus contains an ATP-binding site which upon binding ATP closes to induce ATP hydrolysis and provides the chaperone machinery the energy required for protein folding. The middle domain binds the γ -phosphate of ATP and is responsible for interactions with client proteins. The C-terminus controls Hsp90 homodimerization and also contains a nucleotide-binding site that allosterically regulates the N-terminal domain.^{3–9} In cancer cells, Hsp90 is overexpressed and involved in numerous pathways required for the rapid growth and proliferation of cancer cells. As a consequence of its essential role in so many processes, Hsp90 is a highly sought after therapeutic target, as disruption can simultaneously affect multiple pathways required for cancer cell growth. $^{10\matharpic12,41\matharpic50}$

Hsp90 N-terminal inhibition has been shown to be an effective therapeutic strategy as 17 inhibitors of the ATP-binding site have advanced to clinical trials for the treatment of various cancers. Despite the number of N-terminal inhibitors in clinical trials,

detrimental activities that include cardiovascular, ocular, and/or hepatotoxicities, amongst others have been observed for many of these investigational new drugs.^{13–15} Unfortunately, the N-terminal ATP-binding site is >85% identical across all four Hsp90 isoforms, which includes cytosolic Hsp90 α and β , the endoplasmic reticulum-localized isoform, Grp94, and the mitochondrial chaperone, Trap1.¹⁶ Since *pan*-inhibition of all Hsp90 isoforms can result in the degradation of more than 200 Hsp90-dependent client proteins, it is likely that toxic side effects cannot be overcome by traditional approaches that focus upon pan-Hsp90 inhibition. Recently, it was shown that Hsp90 α is the isoform responsible for maturation of the hERG channel, and is therefore more likely responsible for the clinically observed cardiotoxicity.¹⁷ Consequently, new approaches toward Hsp90 inhibition are needed in an effort to refine the number of Hsp90-dependent proteins as well as to escape the deleterious consequences of pan-Hsp90 inhibition. The development of isoform-selective Hsp90 inhibitors represents an attractive strategy for the discovery of such attributes.

Compared to cytoplasmic Hsp90 α and β , the biological role manifested by glucose regulated protein-94 kDa (Grp94) is far less explored.¹⁶ Preliminary studies on Grp94 indicate that many of its client proteins are involved in intercellular communication and adhesion (i.e., Toll-like receptors, myocilin, integrins, immunoglobulins).¹⁸ These properties suggest that Grp94 inhibition may be useful for the treatment of specific disease states. For example, since Grp94 plays an integral role in the trafficking of integrins to the cell membrane, it represents a potential target for the development of anti-metastatic agents.¹⁹ This is further illustrated by recent work showing that Grp94 inhibition (via siRNA knockdown)





^{*} Corresponding author. Tel.: +1 (785) 864 2288; fax: +1 (785) 864 5326. *E-mail address:* bblagg@ku.edu (B.S.J. Blagg).

led to inhibition of cell proliferation, migration and metastasis in two aggressive breast cancer cell lines (MDA-MB-231 cells and reactive oxygen species resistant (ROS) MCF-7 cells).²⁰ Grp94 has also been ascribed a role in glaucoma due to its involvement in mutant myocilin clearance.²¹ In addition, Grp94 was found to be overexpressed in multiple myeloma as a consequence of endoplasmic reticulum stress,^{22–24} in fact, Grp94 inhibition was shown to decrease the proliferation rate of multiple myeloma cells.²⁵

Despite the highly homologous nature of Hsp90 isoforms, Grp94 possesses a five-amino acid insertion (QEDGQ) between residues 182 and 186, which translates into a small pocket that can be accessed by Grp94 inhibitors.²⁹ This insertion does not exist in other Hsp90 isoforms, and therefore creates a unique hydrophobic environment within the ATP-binding domain that can lead to selective inhibition.^{26–29} In 2009, a novel mode of binding was identified for the radicicol/geldanamycin chimeric inhibitor, radamide (RDA)^{30,31,37-40} bound to Grp94 (Fig. 1). Co-crystal structures of RDA bound to both Hsp90 and Grp94 (Fig. 1)³⁰ indicated two key elements were needed to target the hydrophobic binding pocket and to selectively bind Grp94. The first proposed modification included replacement of the quinone ring with a phenyl substituent that interacts favorably with the hydrophobic pocket exclusive to Grp94. The second requirement for binding to Grp94 was projection of the phenyl group into this hydrophobic environment. Such observations led to the development of BnIm, which demonstrated good Grp94 inhibitory activity in a number of assays.²⁶ However, little effort towards the development of RDA as a Grp94 inhibitor has been pursued, until now. Therefore, the goal of this project was to further evaluate RDA as a Grp94 inhibitor and to establish structure-activity relationships for this class of inhibitors.

2. Results and discussion

Optimization of the RDA scaffold required several modifications to access the unique hydrophobic pocket within Grp94. The first investigation needed to determine the optimal linker length between the amide and the aromatic ring that projects into the

hydrophobic pocket (compounds 2–5) as suggested by Figure 1. This is necessary to determine how deep these molecules can project into the pocket and to provide a scaffold upon which substituents can be readily incorporated for elucidation of structureactivity relationships. Substitutions around the aryl ring would then probe for beneficial interactions with the peptide binding pocket as outlined in Scheme 1. These observations led to the design and synthesis of compounds that would provide an initial set of data to direct subsequent optimization efforts. Since RDA bound to Grp94 (Scheme 1) indicated an extended π -stacking environment with Phe199 and Tyr200, naphthyl, indole, and indoline scaffolds were also pursued to take advantage of potential interactions. These aryl systems could then be modified to contain additional substitutions about the ring for further access into the A and **B** pockets. Due to π - π interactions with Phe199 and Tvr200. probing the electronic nature of the arvl ring via the addition of heteroatoms that could enhance interactions was also investigated and led to the identification of additional compounds. Lastly, since the cis-amide conformation was found important for binding Grp94 (Fig. 1), tertiary amides were prepared to promote the cisconformation.

Projection of the phenyl ring into the hydrophobic binding region (Scheme 1) required optimization of the linker length between the amide and phenyl groups, leading to the synthesis of **2–5** (Fig. 2). Molecular modeling studies indicated that phenyl amide 2 would provide the ideal linker for binding Grp94. In an effort to validate this model and to support this hypothesis, linkers between the amide and the phenyl ring were synthesized and analyzed for inhibitory activity. In the event, TBS-protected acid 1, was coupled with the corresponding aniline, benzylamine, phenethylamine, or phenyl hydrazine, utilizing 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) and pyridine in dichloromethane, to generate the corresponding amides. These compounds were subsequently treated with tetrabutylammonium fluoride (TBAF) to unmask the phenols, 2-5, in good yields. These compounds were evaluated for their ability to bind Grp94 via a competitive binding fluorescence polarization assay (Fig. 2).^{32,33} As suggested by



Figure 1. (A) Structures of natural product Hsp90 inhibitors GDA and RDC, chimeric inhibitor RDA, and Grp94-selective inhibitor BnIm; (B) Co-crystal structure of RDA bound to Hsp90 (PDB: 2FXS); (C) Co-crystal structure of RDA bound to Grp94 (PDB: 2GFD).



Scheme 1. Development of RDA-based Grp94 inhibitors.

computational studies, phenyl amide **2**, was found to contain the optimal linker as compared to benzyl amide, phenethyl amide, and hydrazine amide (**3**, **4**, and **5**, respectively). As described earlier, RDA analogs appear to manifest π – π interactions with Phe199 and Tyr200, therefore, compound **6**, containing a cyclohexyl ring in lieu of the phenyl ring, was synthesized and evaluated. As expected, this compound demonstrated a decrease in binding affinity (compound **2** Grp94 K_d , 20.5 μ M, compound **6** Grp94 K_d , 35.2 μ M), which was in accord with our predictive model that supported π – π stacking interactions between Grp94 and compound **2**. These studies validated compound **2** as our starting point for further development and allowed further interrogation of this scaffold.

Functionalization of phenyl amide **2** at the 2-, 3-, and 4-positions was pursued to explore the space surrounding the phenyl ring and to identify key interactions by the inclusion of electron donating and withdrawing groups. As seen in Scheme 1, there are two main pockets (**A** and **B**) within the hydrophobic binding

region. Molecular modeling indicated that the 2-position of the phenyl ring is directed towards pocket **A**, whereas the 3- and 4-positions point at **B**. Substitutions at these positions could also provide information about the potential for π -stacking and hydrogen bonding interactions.

Functionalization of 2 at the 2-position on the phenyl ring was pursued with the goal of projecting substituents into pocket A and/ or altering the electronic nature for additional π - π interactions. EDCI-mediated coupling with the corresponding anilines, 7a-15a, followed by silyl-deprotection, generated amides 7-15 in good yields. Evaluation of 7-15 by the fluorescence polarization assay demonstrated enhanced binding affinities for these compounds (≤20% tracer bound) as compared to the unsubstituted lead compound. These substitutions illustrated that both electronic and steric effects influence the ability of these amides to bind Grp94, and the 2-chloro exhibited the highest affinity (8, 0.1% tracer bound). A trend is observed in Figure 3 which suggests that substituents larger than chlorine (for example, 2-bromo and 2-trifluoromethyl, compounds 9 and 11, respectively) exhibit a size-dependent decrease in binding affinity. The 2-fluoro derivative, 7, also exhibited a decrease in binding affinity, which may result from its inability to fill this hydrophobic environment. However, this could also be a consequence of the electronic effects manifested by fluorine, which prevents the amide bond from adopting the cis-conformation.³⁴ Electron donating groups, hydrogen bond acceptors and hydrogen bond donors all exhibit a decreased affinity for binding Grp94. Ultimately, it was discovered that a chlorine atom at the 2-position (compound 8) proved optimal for binding to pocket A.

Additional structure-activity relationships were pursued by the incorporation of substitutions at the 3-position of the phenyl ring. As shown in Scheme 1, the 3-position of the phenyl ring provides access to pocket **B** and therefore, substitutions were incorporated to elucidate the size of this pocket, the electronic nature that can enhance $\pi - \pi$ stacking interactions, and potentially, hydrogen bonding interactions with Gly196. This led to the synthesis of 3substituted amides by an EDCI-mediated coupling of acid 1 with the corresponding anilines, 16a-24a, followed by silvl-deprotection to give the desired amides. 16-24, in good overall yields. As seen in Figure 4, these substitutions followed a similar trend for Grp94 binding as observed for the 2-position, as chlorine at the 3-position (17) displayed the highest binding affinity for Grp94. In comparison, larger (compounds 18-20) and smaller (compound 16) substituents resulted in diminished binding. In contrast to the 2-position, incorporation of a hydroxyl or amino group at the



entry	R ₁	R ₂	% Tracer Bound
2	Ph	Н	19.8 (±1.2)
3	CH ₂ Ph	Н	65.8 (±3.5)
4	CH ₂ CH ₂ Ph	Н	33.0 (±2.1)
5	NHNHPh	Н	26.3 (±1.5)
6	cyclohexyl	Н	26.1 (±1.4)

Figure 2. Optimization of Amide Linker and Hydrophobic Moiety.^{a-d} ^aCompounds **2–6** (25 μM) were incubated with cGrp94 and FITC-GDA (tracer) for 24 h before fluorescence polarization values were determined. ^bDMSO (1%) served as a negative control (vehicle), and GDA (50 nM) served as the positive control. ^cAll compounds tested at 25 μM. ^dAll compounds were tested in triplicate.



entry	R	% Tracer Bound
7	F	16.3 (±0.1)
8	Cl	0.1 (±0.1)
9	Br	6.1 (±0.1)
10	Ι	7.9 (±0.1)
11	CF ₃	13.4 (±0.5)
12	OH	18.0 (±0.3)
13	OMe	16.1 (±0.6)
14	NH ₂	20.5 (±1.0)
15	CH ₃	3.4 (±0.1)

Figure 3. Evaluation of 2-phenyl substitutions.^{a-d} ^aCompounds **7–15** (25 μM) were incubated with cGrp94 and FITC-GDA (tracer) for 24 h before fluorescence polarization values were determined. ^bDMSO (1%) served as a negative control (vehicle), and GDA (50 nM) served as the positive control. ^cAll compounds tested at 25 μM. ^dAll compounds were tested in triplicate.



entry	R	% Tracer Bound
16	F	11.0 (±0.1)
17	Cl	0.1 (±0.1)
18	Br	9.3 (±0.3)
19	CF ₃	39.4 (±3.0)
20	Ph	46.8 (±4.6)
21	OH	3.5 (±0.1)
22	OMe	27.4 (±0.9)
23	NH ₂	17.4 (±0.8)
24	NHAc	8.3 (±0.1)

Figure 4. Evaluation of 3-phenyl substitutions.^{a-d a}Compounds **16–24** (25 µM) were incubated with cGrp94 and FITC-GDA (tracer) for 24 h before fluorescence polarization values were determined. ^bDMSO (1%) served as a negative control (vehicle), and GDA (50 nM) served as the positive control. ^cAll compounds tested at 25 µM. ^dAll compounds were tested in triplicate.

3-position (compounds **21** and **22**, respectively) led to increased affinity for Grp94 (potentially through a gained hydrogen bonding interaction with Gly196). Both amino and *N*-acetamides manifested decreased binding affinity as compared to the phenol, as they are known to form weaker hydrogen bonds. Ultimately, the 3-chloro derivative proved to bind Grp94 more efficiently than the 3-phenol, indicating a preference for hydrophobic over hydrogen bonding interactions at this location.

The 4-position of the phenyl ring was also explored by the introduction of electron donating and withdrawing groups. As indicated in Scheme 1, the 4-position appears optimal for directing substituents into pocket **B**, however, a potential hydrogen bonding interaction with Gly196 could also be rationalized. Similar to 3-substitutions, the 4-position was investigated for steric constraints

in pocket **B**, as well as elucidation of the electronic nature for π - π interactions with Phe199 and Tyr200. Compounds **25–37** were synthesized via EDCI-mediated coupling of TBS-acid **1** with the corresponding anilines, **25a–37a**, followed by silyl-deprotection to provide amides **25–37** in good overall yields. When evaluated for their ability to bind Grp94, the 4-position followed a very clear SAR trend with particular sensitivity to sterics (as shown in Fig. 5). In contrast to the 2- and 3-positions, the 4-position appears to accommodate larger functionalities, as illustrated by the inclusion of bromine at the 4-position (compound **27**), which displayed excellent affinity for Grp94. The enhanced affinity for **27** was anticipated as the 4-bromine can project well into the hydrophobic pocket of area **B** (Scheme 1). A decrease in binding affinity was observed (as compared to **27**) for both smaller substitutions



Figure 5. Evaluation of 4-phenyl substitutions.^{a-d a}Compounds **25–37** (25 µM) were incubated with cGrp94 and FITC-GDA (tracer) for 24 h before fluorescence polarization values were determined. ^bDMSO (1%) served as a negative control (vehicle), and GDA (50 nM) served as the positive control. ^cAll compounds tested at 25 µM. ^dAll compounds were tested in triplicate.

(4-fluoro and 4-chloro, compounds 25 and 26, respectively) and larger substitutions (4-iodo, 4-trifluoromethyl, and 4-isopropyl, compounds 28, 29, and 37, respectively). Similar to the trends observed for the 2- and 3-positions, electron withdrawing groups at the 4-position also displayed higher binding affinity than electron donating groups. A hydrogen bond donor at the 4-position also displayed good binding affinity (4-hydroxyl, compound 32), and this affinity could be altered by conversion to the methyl ether (34), which manifested decreased affinity. Compound 35, containing a nitrile group at the 4-position, also displayed enhanced Grp94 binding, which may be attributed to its electron withdrawing ability as well as its ability to serve as a hydrogen bond acceptor. As predicted by molecular modeling studies which clearly outlined the A and B pockets as important for binding, both the 2- and 3substituted compounds (8 and 17, respectively) exhibited the greatest influence on Grp94 binding as compared to the 4-substituted derivatives (27).

Preliminary molecular modeling studies (Scheme 1) indicated that one explanation for the enhanced activity of the phenyl amide scaffold was due to its potential to π -stack with the electron-rich rings of Phe199 and Tyr200, suggesting that π -stacking with these residues could be enhanced by alteration of the electron density on the phenyl ring. Structure–activity relationships at the 2-, 3-, and 4-positions supported this hypothesis as decreased electron density (via electron withdrawing groups) led to enhanced binding. Further validation of this hypothesis prompted the introduction of heteroatoms into the aromatic ring to enhance these π -stacking interactions.

Investigation of the influence that electron withdrawing groups exhibit led to a series heterocycles that exhibited decreased electron density within the aromatic ring. Sulfur containing 5-membered heterocycles (**46–48**) were synthesized to represent a ring similar in size as the corresponding 6-membered analogs (**38– 45**), while manifesting some electron deficiency. As described previously, these analogs were generated by an EDCI-mediated

coupling of TBS-protected acid 1 with corresponding amines 38a-48a, followed by silyl-deprotection to afford the desired amides, 38-48. Evaluation of the pyridine analogs showed that a nitrogen atom at the 2-position (38) resulted in the highest binding affinity, followed by the 3- (39) and 4-incorporation (40), respectively (as shown in Fig. 6). Molecular modeling studies suggest this trend was due at least in part by the ability of the nitrogen atom to project towards the open channel, while simultaneously maintaining optimal orientation for π -stacking with Phe199 and Tyr200. However, when the nitrogen was moved to the 3-position (40), nitrogen is shifted towards the channel, resulting in less favorable π -stacking interactions. The 4-pyridine derivative (40) demonstrated the lowest binding affinity due to its inability to access the open channel, and instead was forced into unfavorable interactions with the hydrophobic binding pocket of **B**. Pyridazine **42** was prepared to determine whether the incorporation of 2- and 3-containing nitrogen atoms could simultaneously exist while providing π - π interactions. Unfortunately **42** did not improve binding affinity, suggesting that the inclusion of a nitrogen atom at the meta-position is not favored. Subsequent analogs that contained the 2-pyridine nitrogen and other nitrogen locations (41-45, Fig. 6) were explored, but unfortunately, did not improve affinity. In contrast, thiazole 46 and thiadiazoles 47 and 48 displayed good binding affinity and reinforced the importance of nitrogen incorporation at the 2-position.

An understanding of this scaffold's ability to selectively bind Grp94 required binding affinity evaluation to another isoform, Hsp90 α . As shown in Figure 8, phenyl amide 2 did not demonstrate selectivity for Grp94 (~1:1), illustrating the need to optimize this scaffold for greater selectivity. However, substitution at the 2-, 3-, and 4-positions on the phenyl ring (compounds 8, 17, and 27, respectively) did enhance selectivity for Grp94. Specifically, compound 27 showed the greatest selectivity (~27-fold at 25 μ M), indicating the importance of substituents at the 4-position for selective inhibition, while 8 and 17 manifested 8- and 25-fold



Figure 6. Evaluation of heterocyclic amides.^{a-d} ^aCompounds **38–48** (25 μM) were incubated with cGrp94 and FITC-GDA (tracer) for 24 h before fluorescence polarization values were determined. ^bDMSO (1%) served as a negative control (vehicle), and GDA (50 nM) served as the positive control. ^cAll compounds tested at 25 μM. ^dAll compounds were tested in triplicate.



Figure 7. Evaluation of amides **49–53**.^{a–d} ^aCompounds **49–53** (25 µM) were incubated with cGrp94 and FITC-GDA (tracer) for 24 h before fluorescence polarization values were determined. ^bDMSO (1%) served as a negative control (vehicle), and GDA (50 nM) served as the positive control. ^cAll compounds tested at 25 µM. ^dAll compounds were tested in triplicate.

selectivity, respectively. It was also observed that heterocycles **38**, **46** and **47** exhibited selectivity, albeit to a lesser extent than **8**, **17**, and **27**. In an effort to further improve Grp94-selective binding,

tertiary amides **49–53** were developed. These compounds were designed to enhance isomerization to the *cis*-amide conformation, by making the *cis*-conformation lower in energy, which appears



Figure 8. Inhibition of Grp94 and Hsp90 α at 25 μ M Illustrating Grp94-Selectivity.^{a-} ^e ^aCompounds (25 μ M) were incubated with cGrp94 or Hsp90 α and FITC-GDA (tracer) for 24 h before fluorescence polarization values were determined. ^bDMSO (1%) served as a negative control (vehicle), and GDA (50 nM) served as the positive control. ^cAll compounds tested at 25 μ M. ^dAll compounds were tested in triplicate.

preferred for Grp94-inhibition (Fig. 1). Compounds **49**, **50** and **53** were prepared by an EDCI-mediated coupling of TBS-protected acid **1** with anilines **49a**, **50a**, and **53a**, followed by silyl-deprotection to afford the desired amides, **49**, **50**, and **53**. **51** and **52** required an alternative method for their preparation. Compound **51** was generated by conversion of the benzyl-protected acid **1a** to the corresponding acid chloride, followed by coupling with aniline **51a** and subsequent debenzylation to afford desired amide **51** (Fig. 7). Compound **52** was prepared by catalyst-mediated coupling of aldehyde **1b** with nitrosobenzene, followed by silyl-deprotection to afford amide **52** (Fig. 7).³⁵ Unfortunately, compounds **49**–**51** and **53** exhibited little to no improvement in Grp94-selectivity (Fig. 8), while simultaneously manifesting decreased binding

affinities (Figs. 7 and 8). In contrast, compound **52**, which contains an *N*-hydroxyl, demonstrated a significant increase in selectivity, however, at the consequence of lower affinity (Figs. 7 and 8).

Further optimization of this scaffold was pursued to develop more potent and selective Grp94 inhibitors by combining the features identified above to produce **54–65**, which contain the phenyl amide, an ortho-pyridine, a thiazole, or a thiadiazole ring along with substitution patterns based on earlier studies (Fig. 9). These compounds were also synthesized by the EDCI-mediated coupling of acid 1 with anilines 54a-65a. Subsequent silvl-deprotection of the resulting amides afforded 54-65. Unfortunately, 54-58, 60, 61, and 63–65 were unable to exhibit Grp94 binding affinity similar to 8, 17, 27 or 38. Some of the compounds (60, 61, and 63) exhibited a slight enhancement for Grp94 selectivity, but also demonstrated lesser affinity. However, 58, 59 and 62 demonstrated a high Grp94 binding affinity (≤1% tracer bound), while also manifesting good selectivity (4–18% tracer bound for Hsp 90α). Despite these characteristics, compounds 54-65 did not manifest greater binding affinity or selectivity for Grp94 than previously prepared analogs.

Compounds **8**, **17**, **27**, **38**, **46** and **47** were evaluated for determination of their respective K_d values for Grp94 binding. K_d 's were determined in a manner similar to the 25 μ M fluorescence polarization screen, wherein compounds were incubated with Grp94 and FITC-GDA for 24 h at increasing concentrations. From the data, the K_d values for **8**, **17**, **27**, **38**, **46**, and **47** were determined as shown in Table 1. In accord with earlier studies, substitutions at the 2- and 4-positions (**8** and **27**, respectively) manifested better activity than the 3-position (**17**). The importance of a nitrogen atom at the 2-position was also confirmed as **38** manifested the highest affinity (0.82 μ M), while both **46** and **47** also displayed less affinity



entry	R ₁	R ₂	R ₃	R ₄	X	Y	Z	% Tracer Bound
54	Н	Br	Н	Br	СН			50.7 (±3.3)
55	Cl	Н	Cl	Н	СН			15.6 (±0.4)
56	Cl	Br	Н	Н	СН			71.4 (±13.2)
57	Н	Н	Cl	Br	CH			24.9 (±1.0)
58	Cl	Н	Н	Н	Ν			1.4 (±0.1)
59	Н	Br	Н	Н	Ν			0.1 (±0.1)
60	Н	Н	Cl	Н	Ν			7.7 (±0.3)
61	Н	Н	Н	Cl	Ν			9.8 (±0.4)
62	Н	Н	Н	Br	Ν			0.2 (±0.1)
63	Cl				S	СН	Ν	22.4 (±1.6)
64	Br				S	СН	Ν	27.7 (±1.8)
65	Br				S	Ν	Ν	5.1 (±0.3)

Figure 9. Combinations of key features and their Grp94 inhibition.^{a-d} ^aCompounds **54–65** (25 μM) were incubated with cGrp94 and FITC-GDA (tracer) for 24 h before fluorescence polarization values were determined. ^bDMSO (1%) served as a negative control (vehicle), and GDA (50 nM) served as the positive control. ND = not determined. ^cAll compounds tested at 25 μM. ^dAll compounds were tested in triplicate.

entry	K _d (μM)	³⁵⁰ 1
8	1.85 (±0.22)	300
17	6.42 (±0.92)	
27	1.50 (±0.18)	E 150-
38	0.82 (±0.09)	100-
46	1.08 (±0.13)	50-
47	1.54 (±0.26)	
		loaC

Table 1 *K*_d's of **8**, **17**, **27**, **38**, and **46** for Grp94^{a-d}

^a Compounds 8, 17, 27, 38, 46 and 47 were incubated with cGrp94 and FITC-GDA (tracer) for 24 h before fluorescence polarization values were determined.

^b DMSO (1%) served as a negative control (vehicle), and GDA (50 nM) served as the positive control.

^c All compounds were tested in triplicate.

^d Millipolarization (mP) units were used to determine % tracer bound.

(1.08 μ M and 1.54 μ M, respectively). Compounds **38** and **46** manifested the highest affinity for Grp94, and therefore were used in subsequent cell-based assays.

2.1. Inhibition of migration and proliferation of MDA-MB-231 cells

Inhibition of Grp94 has been linked to decreased metastasis, migration and proliferation in the highly metastatic breast cancer cell lines, MDA-MB-231 and ROS-resistant MCF-7.²⁰ Specifically, silencing Grp94 expression caused almost complete inhibition of migration as well as a significant decrease in proliferation of the highly aggressive metastatic breast cancer cell line, MDA-MB-231.²⁰ While compounds **38** and **46** manifested modest anti-proliferative activities against MDA-MB-231 cells (**38**: IC₅₀ = 12 μ M, **46**: IC₅₀ = 16 μ M), they did demonstrate potent anti-migratory properties against the same cell line. This was determined by employing a wound healing assay where a monolayer of cells was grown in a 12-well plate format. In each well, two wounds were created in

which cells can migrate and heal the wound. Migration was recorded at 0 h, 16 h, and 24 h using a digital camera mounted microscope. In addition, each compound was tested at $2.5 \,\mu$ M (Fig. 10) up to $25 \,\mu$ M (Supporting information, Figs. S1–S2) where they demonstrated no cytotoxicity. The results at $2.5 \,\mu$ M are shown in Figure 10.

Compounds **38** and **46** were evaluated in this assay as they manifested the most potent (K_d values, Table 1) Grp94 inhibition while also manifesting good selectivity for Grp94 binding (Fig. 8). As shown in Figure 10, they inhibited cell migration consistent with Grp94 inhibition, which was also in accord with prior studies by Verrax et al.²⁰, wherein they showed that Grp94 knockdown led to migratory inhibition of MDA-MB-231 cells. The ability of these compounds to inhibit migration mirrored the trend observed in the fluorescence polarization assay, where compound **38** manifested a greater anti-migratory effect than compound **46**. The anti-migratory effect of these compounds was determined to be dose-dependent (Supporting information, Figs. S1 and S2). Ultimately, the wound-healing assay illustrates the potential for



Figure 10. ^{a,b}Inhibition of MDA-MB-231 Cell Migration by Compounds **38** and **46**^{a,b} ^aMDA-MB-231 cells were incubated for 24 h prior to compound addition. ^bCells were incubated for 16 h and 24 h at 37 °C with the respective conjugate indicated above (2.5 μM).

Grp94-inhibitors to be used in the treatment of highly metastatic breast cancers, while manifesting minimal toxicity.

Compounds **38** and **46** demonstrated the ability to bind Grp94 in MDA-MB-231 cells. The anti-Grp94 (9G10) antibody recognizes the open conformation of Grp94, and when an inhibitor is bound to this region, Grp94 switches to the closed conformation. Therefore, the 9G10 antibody does not recognize Grp94 bound to an inhibitor.^{26,37} Compounds **38** and **46** demonstrated a dose-dependent effect by inducing a conformational change in Grp94, and thus, prevented the 9G10 antibody from recognizing and immunoprecipitating Grp94 from MDA-MB-231 cells (as shown in Fig. 11). These findings correspond well with the observed anti-proliferative and anti-migratory concentrations, indicating these effects parallel Grp94-inhibition.

Previous studies have shown that Grp94-inhibition decreases insulin-like growth factor-II (IGF-II) secretion, which also plays an important role in the proliferation and migration of certain cancers.^{26,51,52} Compounds **38** and **46** decreased secretion of the Grp94-dependent client protein, IGF-II, as shown in Fig. 12. The dramatic decrease in IGF-II secretion was observed at concentrations that parallel the concentrations needed to exhibit anti-migratory activity.

Compounds **38** and **46** also demonstrated selective Grp94-inhibition by Western blot analysis of Hsp90 client proteins Akt and Cyclin D1 from MDA-MB-231 cell lystates (Fig. 13). Degradation of Hsp90-dependent cytosolic clients, Akt and Cyclin D1, was not observed at a high concentration of compound **38** (60 μ M), indicating other Hsp90 isoforms were not affected at the concentration needed to exhibit anti-migratory activity (2.5–25 μ M). Compound **46** demonstrated modest client-protein degradation at 80 μ M, however, this effect was observed at concentrations significantly higher than the concentration needed for anti-migratory activity. This finding along with the observed effects of utilizing the 9G10 antibody (Fig. 11) and decreased IGF-II secretion (Fig. 12) indicate these compounds inhibit Grp94 at concentrations relevant to the observed anti-migratory activity.



Figure 11. Effect of compounds **38** and **46** on Grp94 conformation. MDA-MB-231 cells were treated with the indicated concentrations of **38** and **46** (concentrations listed are μ M) or radicicol (**R** 15 μ M) overnight, and cell lysates were immunoprecipitated with the conformational-specific antibody 9G10 and subsequently were immunoblotted for Grp94. Lower panel, immunoblot of whole cell lysates with 9G10.



Figure 12. Western blot analysis of IGF-II secretion into MDA-MB-231 breast cancer cell media upon treatment with amides **38** and **46**. H (high) represents a concentration equal to 10 μ M. L (low) represents a concentration equal to 1 μ M. Radicicol (RDC, 15 μ M) and dimethylsulfoxide (DMSO, 100%) were employed as positive and negative controls, respectively.



Figure 13. Western blot analyses of Hsp90-dependent client proteins from MDA-MB-231 breast cancer cell lysate upon treatment with amides **38** and **46**. H (high) represents a concentration equal to 5-fold the anti-proliferative IC₅₀ value. L (low) represents a concentration equal to 0.5-fold the anti-proliferative IC₅₀ value. Radicicol (RDC, 15 μ M) and dimethylsulfoxide (DMSO, 100%) were employed as positive and negative controls, respectively.

2.2. Anti-proliferative effect against RPMI 8226 cells

Recently, elevated levels of Grp94 were linked to the accelerated proliferation of multiple myeloma cells,^{22–24} identifying Grp94 as a potential therapeutic target for the treatment of myeloma.²⁵ The RPMI 8226 multiple myeloma cell line was shown to be particularly sensitive to Grp94 inhibition, which prompted investigation of compounds **38** and **46** for their anti-proliferative activity against this cell line.

As anticipated, the two most potent compounds for binding Grp94 (Table 1) also exhibited potent anti-proliferative properties against the RPMI 8226 cell line (IC₅₀ <10 μ M, Table 2). Interestingly, **38** displayed a slightly lower anti-proliferative effect (9.12 μ M) than **46** despite its superior binding affinity. These compounds were then evaluated for their ability to selectively inhibit Grp94 in RPMI 8226 cells in a similar fashion seen for MDA-MB-231 cells.

Compounds **38** and **46** did demonstrate an ability to bind Grp94 in RPMI 8226 cells in a dose-dependent manner. This was first determined by use of the 9G10 antibody to recognize Grp94 in immunoprecipitated RPMI8226 cell lysates treated with compounds **38** and **46** (Fig. 14). These data indicate that compounds **38** and **46** bind Grp94 at concentrations needed to inhibit the proliferation of RPMI 8226 cells.

Compounds **38** and **46** also decreased secretion of the Grp94dependent client protein, IGF-II; at concentrations similar to the observed anti-proliferative activity manifested by both compounds

 Table 2

 a--CAnti-proliferative evaluation of 38 and 46 in RPMI 8226 cells

Entry	$IC_{50}\left(\mu M\right)$
38 46	9.12 (±0.4) 7.83 (±2.2)

^a RPMI 8226 cells were incubated for 24 h prior to drug addition.

^b Cells were incubated for 72 h at 37 °C in 5% CO₂ with the respective conjugate. ^c All experiments were performed in triplicate.

D R 38 46 15 4.5 9 45 46 bound Grp94 unbound Grp94

Figure 14. Effect of compounds **38** and **46** on Grp94 conformation. RPMI 8226 cells were treated with the indicated concentrations of **38** and **46** (concentrations listed are μ M) or radicicol (**R** 15 μ M) overnight, and cell lysates were immunoprecipitated with the conformational-specific antibody 9G10 and subsequently were immunoblotted for Grp94. Lower panel, immunoblot of whole cell lysates with 9G10.



Figure 15. Western blot analysis of IGF-II secretion into RPMI 8226 cell media upon treatment with amides **38** and **46**. H (high) represents a concentration equal to 2-fold the anti-proliferative IC₅₀ value. L (low) represents a concentration equal to 0.25-fold the anti-proliferative IC₅₀ value. Radicicol (RDC, 15 μ M) and dimethyl-sulfoxide (DMSO, 100%) were employed as positive and negative controls, respectively.



Figure 16. Western blot analyses of Hsp90-dependent client proteins from RPMI 8226 multiple myeloma cell lysate upon treatment with amides **38** and **46**. H (high) represents a concentration equal to 5-fold the anti-proliferative IC₅₀ value. L (low) represents a concentration equal to 0.5-fold the anti-proliferative IC₅₀ value. Radicicol (RDC, 15 μ M) and dimethylsulfoxide (DMSO, 100%) were employed as positive and negative controls, respectively.

(Fig. 15). Along with the use of the 9G10 antibody (Fig. 14), this data also suggests that the observed anti-proliferative activity of compounds **38** and **46** against RPMI 8226 cells is attributed to the inhibition of Grp94 in RPMI 8226 cells.

Compounds **38** and **46** also demonstrated that their anti-proliferative activities are likely due to selective Grp94 inhibition. This was illustrated by the inability of compound **38** to degrade Hsp90 client-proteins Akt and cyclin D1 (Fig. 16) at concentrations above and below its respective anti-proliferative IC_{50} value. Compound **46** demonstrated modest client-protein degradation at concentrations five-fold higher than its IC_{50} , indicating a slightly lower selectivity for Grp94. However, this data does indicate that compounds **38** and **46** inhibit Grp94 in RPMI 8226 cells, which results in their anti-proliferative activity. The ability of this scaffold to selectively inhibit Grp94 and manifest anti-proliferative activity against the RPMI 8226 multiple myeloma cell line demonstrates the therapeutic potential of Grp94 inhibition for the treatment of these cancers.

3. Conclusion

Using the previously reported co-crystal structure of RDA bound to Grp94, a series of RDA analogs were designed,



Figure 17. Summary of SAR for Grp94 binding of RDA based derivatives.

synthesized, and evaluated for their ability to inhibit Grp94. A comprehensive SAR study of this scaffold indicated several key features necessary for selective inhibition of Grp94 (Fig. 17). Initial optimization of the linker led to the identification of phenyl amide, 2, manifesting the highest Grp94 affinity (blue, Fig. 17). This finding demonstrated both the importance of a short linker, as well as the presence of an aromatic ring for π -stacking interactions. Substituents at the 2- and 4-positions (pink and green, Fig. 17) of the phenyl amide demonstrated increased Grp94 binding affinity and selectivity, with chlorine and bromine substituents identified as optimal (Table 1, compounds 8 and 27, respectively). Substitution at the 3-position (orange, Fig. 17) also proved important; however, both potency and selectivity were decreased relative to substitutions at the 2- and 4-positions (Table 1, compound 17). In contrast, modifications to the 4-position resulted in the greatest effect on selectivity, as most substituents at this position manifested enhanced selectivity. Decreasing the electron density of the aromatic ring via the introduction of heteroatoms (38, 46, and 47) not only increased binding affinity, but also increased selectivity. However, this finding was specific to the incorporation of a nitrogen atom at the 2-position (Fig. 6). The 2-pyridine ring was successfully incorporated with the 4-bromine or 3-chlorine derivative to provide potent and selective Grp94-inhibitors. Subsequent in vitro analysis of 38 and 46 further validated the therapeutic potential for Grp94 inhibitors as well as validated selective Grp94-inhibition. The ability of these compounds to inhibit Grp94 was correlative with their anti-migratory activity against the highly metastatic breast cancer cell line, MDA-MB-231, as well as anti-proliferative activity against the multiple myeloma cell line, RPMI 8226.

4. General experimental methods

4.1. Fluorescence polarization

Assay buffer (25 μ L, 20 mM HEPES pH 7.3, 50 mM KCl, 5 mM MgCl₂, 1 mM DTT, 20 mM Na₂MoO₄, 0.01% NP-40, and 0.5 mg/mL BGG) containing desired compound or GDA were plated in 96-well plates (black well, black bottom) at the indicated final concentrations in DMSO (1% DMSO final concentration).³² Recombinant cGrp94 and FITC-GDA were then added (10 and 6 nM, respectively). Plates were incubated with rocking for 24 h at 4 °C. Fluorescence was read using excitation and emission filters of 485 and 528 nm, respectively. Percent FITC-GDA bound was determined by using the DMSO millipolarization unit (mP) as the 100% bound value and the mP value of free FITC-GDA as the 0% bound value. *K*_d values were calculated from separate experiments performed in triplicate using GraphPad Prism.

4.2. Anti-proliferation assays

RPMI 8226 and MDA-MB-231 cells were maintained in RPMI1640 (Cellgro) and DMEM (Cellgro), respectively, supplemented with nonessential amino acids, L-glutamine (2 mM), streptomycin (500 μ g/mL), penicillin (100 units/mL) and 10% FBS. Cells were grown to confluence in a humidified atmosphere (37 °C, 5% CO₂), seeded (2000/well, 100 μ L) in 96-well plates, and allowed to attach overnight. Compound at varying concentrations in DMSO (1% DMSO final concentration) was added, and cells were returned to the incubator for 72 h. After 72 h, the number of viable cells was determined using an MTS/PMS cell proliferation kit (Promega) per the manufacturer's instructions. Cells incubated in 1% DMSO were use as 100% proliferation, and values were adjusted accordingly. IC₅₀ values were calculated from separate experiments performed in triplicate using GraphPad Prism.

4.3. Wound-healing assay

Cells were grown to confluence in a humidified atmosphere (37 °C, 5% CO₂), seeded (200,000/well, 1 mL) in 12-well plates, and allowed to grow to a confluent monolayer (24 h) at 37 °C and 5% CO₂. Each well was then scratched with a 200 μ L sterile pipet tip, photographed with an Olympus IX-71 microscope (60× objective, time = 0 h), the respective compound solutions (2.5 μ L, final DMSO concentration is 0.25%) were added, and the plates were placed back in the incubator. Cell migration was recorded after 16 and 24 h of incubation with compound via photographs taken by the Olympus IX-71 microscope. All experiments were run in quadruplicate on two different days.

4.4. Western blotting

MDA-MB-231 and RPMI 8226 cells were plated in 6-well plates and treated with high and low concentrations of compounds 38 and 46, as well as 15 µM RDC in DMSO (0.25% DMSO final concentration), or DMSO only for 24 h. Cells were harvested in cold PBS and lysed in mammalian protein extraction reagent (MPER, Pierce) and protease inhibitors (Roche) on ice for 15 min. Lysates were clarified at 15,000 rpm for 15 min at 4 °C. Protein concentrations were determined with the Pierce BCA assay kit per the manufacturer's instructions. Equal amounts of protein (20 µg) were electrophoresed under reducing conditions, transferred to a PVDF membrane, and immunoblotted with the corresponding specific antibodies. The corresponding amount of media necessary to maintain equal actin levels was also electrophoresed under reducing conditions, transferred to a PVDF membrane, and immunoblotted with the IGF-II antibody. Membranes were incubated with an appropriate horseradish peroxidase-labeled secondary antibody, developed with chemiluminescent substrate, and visualized.

4.5. Grp94 immunoprecipitation

Detergent lysates of the indicated cells were immunoprecipitated with 9G10 monoclonal anti-Grp94 (Enzo Life Sciences) followed by protein G-MagBeads (GenScript) as previously described.³⁶

4.6. Molecular modeling

Surflex-Dock in Sybyl v8.0 was used for molecular modeling and docking studies. The co-crystal structure of RDA bound to Grp94 was utilized for all docking experiments.³⁰ The docked molecules were locked into the *cis*-amide conformation and utilized with 10 different starting conformations while rotation of the rotatable bonds was unrestricted. Visual interpretation and figure preparation were then carried out in Pymol (Scheme 1).

4.7. Chemistry general

¹H NMR were recorded at 400 or 500 MHz (Bruker DRX-400 Bruker with a H/C/P/F QNP gradient probe) spectrometer and ¹³C NMR spectra were recorded at 125 MHz (Bruker DRX 500 with broadband, inverse triple resonance, and high resolution magic angle spinning, HR-MA probe spectrometer); chemical shifts are reported in δ (ppm) relative to the internal chloroform-*d* (CDCl₃, 7.27 ppm). FAB (HRMS) spectra were recorded with a LCT Premier (Waters Corp., Milford, MA). The purity of all compounds was determined to be >95% purity as determined by ¹H NMR and ¹³C NMR spectra, unless otherwise noted. The most active five compounds were verified for >95% purity by HPLC analyses. TLC was performed on glass backed silica gel plates (Uniplate) with spots visualized by UV light. All solvents were reagent grade and, when

necessary, were purified and dried by standard methods. Concentration of solutions after reactions and extractions involved the use of a rotary evaporator operating at reduced pressure.

4.8. General amide formation for compounds 2-50 and 53-65

To a solution of acid **1** (0.05 mmol), EDCI-HCl (0.12 mmol), and pyridine (0.13 mmol) in CH₂Cl₂ (1 mL) was added the corresponding aniline (0.1 mmol) and stirred at room temperature under Ar overnight. Upon complete consumption of acid **1**, the solvent was removed in vacuo and redissolved in THF (1 mL). The reaction mixture was then treated with TBAF (0.2 mmol) and stirred for 30 min, and upon completion saturated aqueous NH₄Cl was added and extracted $3 \times$ with EtOAc. The combined organic layers were then dried over Na₂SO₄, filtered, and concentrated in vacuo to give a crude oil. The residue was purified via flash chromatography (SiO₂, 49:1, CH₂Cl₂/MeOH) to afford the desired amide.

Methyl 3-*chloro*-4,6-*dihydroxy*-2-(3-*oxo*-3-(*phenylamino*)*propyl*) *benzoate* (**2**). 15 mg, 86% yield, white solid. ¹H NMR (400 MHz, CDCl₃): δ 11.23 (s, 1H), 7.53 (d, *J* = 7.91 Hz, 2H), 7.34 (t, *J* = 7.66 Hz, 2H), 7.12 (t, *J* = 7.36 Hz, 1H), 6.59 (s, 1H), 6.33 (s, 1H), 3.97 (s, 3H), 3.50 (dd, *J* = 5.76, 10.15 Hz, 2H), 2.66–2.59 (m, 2H). ¹³C NMR (125 MHz, CDCl₃): δ = 170.43, 169.99, 162.91, 156.28, 141.66, 137.73, 129.10 (2C), 124.40, 119.72 (2C), 113.92, 106.81, 102.94, 52.75, 36.73, 28.81. HRMS (FAB) *m*/*z* [M+Na⁺] for C₁₇H₁₆CINO₅Na: 372.0609, found 372.0609.

Methyl 2-(3-(*benzylamino*)-3-*oxopropyl*)-3-*chloro*-4,6-*dihydroxybenzoate* (**3**). 30 mg, 83% yield, white solid. ¹H NMR (500 MHz, CDCl₃): δ 7.32–7.21 (m, 5H), 6.44 (s, 1H), 4.40 (d, *J* = 4.7 Hz, 2H), 3.86 (s, 3H), 3.44–3.29 (m, 2H), 2.50–2.33 (m, 2H). ¹³C NMR (125 MHz, CDCl₃) δ 171.98, 170.68, 162.51, 157.03, 141.87, 138.12, 127.81, 127.60, 114.22, 106.34, 102.63, 52.53, 49.78, 43.73, 35.65, 28.92. HRMS (FAB) *m/z* [M+Na⁺] for C₁₈H₁₈CINO₅Na: 386.0874; found, 386.0877.

Methyl 3-chloro-4,6-dihydroxy-2-(3-oxo-3-(phenethylamino)propyl)benzoate (**4**). 17 mg, 90% yield, white solid. ¹H NMR (400 MHz, CDCl₃): δ 11.29 (s, 1H), 7.32 (dd, *J* = 6.63, 8.10 Hz, 2H), 7.26–7.23 (m, 1H), 7.22–7.17 (m, 2H), 6.57 (s, 1H), 5.48 (t, *J* = 5.95 Hz, 1H), 3.91 (s, 3H), 3.56 (q, *J* = 6.58 Hz, 2H), 3.40–3.34 (m, 2H), 2.84 (t, *J* = 6.84 Hz, 2H), 2.41–2.35 (m, 2H). ¹³C NMR (125 MHz, CDCl₃): δ 171.92, 170.64, 162.90, 156.43, 141.74, 138.72, 128.77, 128.71, 126.63, 113.90, 106.64, 102.80, 52.58, 40.63, 35.81, 35.61, 29.05. HRMS (FAB) *m/z* [M+Na⁺] for C₁₉H₂₀ClNO₅Na: 400.0922, found 400.0931.

Methyl 3-chloro-4,6-dihydroxy-2-(3-oxo-3-(2-phenylhydrazinyl) propyl)benzoate (**5**). 17 mg, 94% yield, orange solid. ¹H NMR (400 MHz, CD₃OD, major conformer): δ 7.23–7.13 (m, 2H), 6.83– 6.74 (m, 3H), 6.41 (s, 1H), 3.92 (s, 3H), 3.36–3.28 (m, 2H), 2.59– 2.53 (m, 2H); ¹³C NMR (125 MHz, CD₃OD, major conformer): δ 174.8, 171.6, 161.5, 159.0, 150.0, 142.1, 130.2, 130.0, 121.0, 115.0, 114.1, 113.3, 109.5, 103.2, 52.9, 34.3, 29.1. HRMS (FAB) *m/z* [M+Na⁺] for C₁₇H₁₇ClN₂O₅Na: 387.0724; found, 387.0723.

Methyl 3-chloro-2-(3-(cyclohexylamino)-3-oxopropyl)-4,6-dihydroxybenzoate (**6**). 33 mg, 88% yield, white solid. ¹H NMR (500 MHz, CDCl₃) δ 6.41 (s, 1H), 3.89 (s, 3H), 3.68 (s, 1H), 3.40– 3.24 (m, 2H), 2.40–2.26 (m, 2H), 1.91–1.76 (m, 2H), 1.64 (dt, *J* = 13.8, 3.8 Hz, 2H), 1.61–1.52 (m, 1H), 1.39–1.25 (m, 2H), 1.07 (ddd, *J* = 19.2, 9.8, 3.9 Hz, 3H). ¹³C NMR (125 MHz, CDCl₃) δ 171.44, 170.78, 162.49, 157.48, 142.07, 114.43, 106.04, 102.51, 52.46, 49.40, 48.22, 35.90, 33.07, 28.92, 25.46, 24.83. HRMS (FAB) *m/z* [M+Na⁺] for C₁₇H₂₂ClNO₅Na: 378.1187; found, 378.1167.

Methyl 3-fluoro-2-(3-((2-chlorophenyl)amino)-3-oxopropyl)-4,6dihydroxybenzoate (7). 15 mg, 81% yield, white solid. ¹H NMR (500 MHz, CDCl₃) δ 11.19 (s, 1H), 8.49–8.00 (m, 1H), 7.34 (s, 1H), 7.12–6.97 (m, 3H), 6.53 (s, 1H), 6.05 (s, 1H), 3.91 (s, 3H), 3.49– 3.37 (m, 2H), 2.67–2.57 (m, 2H).¹³C NMR (125 MHz, CDCl₃) δ 170.45, 169.98, 163.02, 156.23, 141.37, 124.71, 124.68, 124.37, 124.31, 121.60, 114.87, 114.72, 113.86, 106.81, 102.99, 52.73, 36.80, 28.83. HRMS (FAB) m/z [M+Na⁺] for C₁₇H₁₅ClFNO₅Na: 390.0521; found, 390.0547.

Methyl 3-*chloro-2-(3-((2-chlorophenyl)amino)-3-oxopropyl)-4,6dihydroxybenzoate* (**8**). 14 mg, 73% yield, white solid. ¹H NMR (500 MHz, CDCl₃) δ 11.22 (s, 1H), 8.36 (d, *J* = 8.3 Hz, 1H), 7.61 (s, 1H), 7.31 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.26–7.21 (m, 1H), 7.02–6.97 (m, 1H), 6.53 (s, 1H), 3.91 (s, 4H), 3.54–3.32 (m, 2H), 2.76–2.47 (m, 3H).¹³C NMR (125 MHz, CDCl₃) δ 170.49, 163.08, 156.23, 141.30, 134.49, 129.04, 127.85, 124.68, 121.47, 113.86, 106.83, 103.00, 52.75, 37.03, 28.90. HRMS (FAB) *m/z* [M+Na⁺] for C₁₇H₁₅Cl₂ NO₅Na: 406.0225; found, 406.0246.

Methyl 2-(3-((2-bromophenyl)amino)-3-oxopropyl)-3-chloro-4,6dihydroxybenzoate (**9**). 16.3 mg, 76% yield, pale yellow solid. ¹H NMR (500 MHz, CDCl₃) δ 11.25 (s, 1H), 8.49–7.19 (m, 7H), 6.93 (td, *J* = 7.7, 1.4 Hz, 1H), 6.53 (s, 1H), 6.13 (s, 1H), 3.91 (s, 3H), 3.53–3.36 (m, 2H), 2.64 (t, *J* = 8.3 Hz, 2H).¹³C NMR (125 MHz, CDCl₃) δ 170.54, 170.04, 163.07, 156.29, 141.26, 135.54, 132.27, 128.50, 125.26, 121.80, 113.89, 113.18, 106.80, 103.01, 52.79, 37.05, 27.08. HRMS (FAB) *m*/*z* [M+Na⁺] for C₁₇H₁₅BrClNO₅Na: 449.9720; found, 449.9767.

Methyl 3-chloro-4,6-dihydroxy-2-(3-((2-iodophenyl)amino)-3-oxopropyl)benzoate (**10**). 18.2 mg, 77% yield, pale yellow solid. ¹H NMR (500 MHz, CDCl₃) δ 11.27 (s, 1H), 8.21 (d, *J* = 8.2 Hz, 1H), 7.77–7.23 (m, 3H), 6.82–6.78 (m, 1H), 6.53 (s, 1H), 6.03 (s, 1H), 3.92 (s, 3H), 3.51–3.37 (m, 3H), 2.72–2.52 (m, 3H). ¹³C NMR (125 MHz, CDCl₃) δ 170.58, 163.13, 156.24, 141.26, 138.84, 138.04, 129.38, 126.07, 121.88, 113.88, 106.85, 103.01, 52.84, 37.05, 28.97. HRMS (FAB) *m*/*z* [M+Na⁺] for C₁₇H₁₅ClINO₅Na: 497.9581; found, 497.9593.

Methyl 3-*chloro*-4,6-*dihydroxy*-2-(3-*oxo*-3-((2-(*trifluoromethyl*) *phenyl*)*amino*)*propyl*)*benzoate* (**11**). 33 mg, 79% yield, white amorphous solid: ¹H NMR (400 MHz, CDCl₃-MeOD) δ 6.87 (d, *J* = 1.4 Hz, 1H), 6.84 (d, *J* = 1.4 Hz, 1H), 6.41 (s, 1H), 6.08 (d, *J* = 3.1 Hz, 1H), 5.84 (dd, *J* = 3.0, 1.3 Hz, 1H), 4.88 (s, 2H), 3.84 (s, 3H), 3.49–3.41 (m, 2H), 3.02–2.92 (m, 2H); ¹³C NMR (CDCl₃+CH₃OH, 125 MHz): δ 171.0, 162.2, 158.3, 153.1, 147.3, 147.1, 141.9, 126.7, 119.7, 114.9, 109.7, 106.5, 102.6, 52.6, 42.7, 30.9, 26.2. (FAB) *m/z* [M+H⁺] for C₁₈H₁₆ClF₃NO₅: 418.0669; found, 418.0658.

Methyl 3-*chloro*-4,6-*dihydroxy*-2-(3-((2-*hydroxyphenyl*)*amino*)-3-*oxopropyl*)*benzoate* (**12**). 23 mg, 63% yield, white amorphous solid: ¹H NMR (500 MHz, (CD₃)₂CO) δ 9.21 (br s, 1H), 7.49 (dd, J = 8.1, 1.6 Hz, 1H), 7.03 (td, J = 7.8, 1.6 Hz, 1H), 6.91 (dd, J = 8.1, 1.5 Hz, 1H), 6.82 (td, J = 7.7, 1.5 Hz, 1H), 6.52 (s, 1H), 3.96 (s, 3H), 3.54–3.37 (m, 2H), 2.87–2.74 (m, 2H); ¹³C NMR (125 MHz, (CD₃)₂CO): δ 172.9, 171.3, 162.4, 158.7, 149.1, 142.8, 127.6, 126.5, 122.7, 120.6, 118.7, 114.9, 108.1, 103.2, 53.0, 36.3, 29.4; (FAB) *m*/*z* [M+H⁺] for C₁₇H₁₆ClNO₆: 388.0563; found, 388.0563.

Methyl 3-chloro-4,6-dihydroxy-2-(3-((2-methoxyphenyl)amino)-3-oxopropyl)benzoate (**13**). 14.5 mg, 77% yield, white solid. ¹H NMR (500 MHz, CDCl₃) δ 11.40 (s, 1H), 8.41 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.77 (s, 1H), 7.10–6.85 (m, 3H), 6.59 (s, 1H), 3.96 (s, 3H), 3.87 (s, 3H), 3.56–3.46 (m, 2H), 2.71–2.62 (m, 2H).¹³C NMR (125 MHz, CD₃OD) δ 173.39, 171.67, 161.61, 158.99, 151.43, 142.28, 128.21, 126.10, 123.37, 121.46, 114.98, 111.74, 109.46, 103.16, 56.19, 52.83, 37.23, 29.40. HRMS (FAB) *m/z* [M+H] for C18H18CINO6: 380.0901; found, 380.0920.

Methyl 2-(3-((2-aminophenyl)amino)-3-oxopropyl)-3-chloro-4,6dihydroxybenzoate (**14**). 7.5 mg, 41% yield, pale yellow solid. ¹H NMR (400 MHz, CD₃OD) δ 7.05–6.92 (m, 2H), 6.79–6.61 (m, 1H), 6.32 (d, *J* = 4.7 Hz, 1H), 3.84 (d, *J* = 5.2 Hz, 3H), 3.31–3.25 (m, 2H), 2.66–2.60 (m, 2H). ¹³C NMR (125 MHz, CD₃OD) δ 173.77, 171.72, 161.58, 159.34, 143.21, 142.29, 128.24, 127.13, 125.21, 119.62, 118.61, 115.21, 109.28, 103.28, 52.91, 36.45, 29.33. HRMS (FAB) *m/z* [M+Na⁺] for C₁₇H₁₇ClN₂O₅Na: 387.0724; found, 387.0744. *Methyl* 3-*chloro*-4,6-*dihydroxy*-2-(3-*oxo*-3-(*o*-*tolylamino*)*propyl*) *benzoate* (**15**). 10 mg, 25% yield, white powder. ¹H NMR (400 MHz, CDCl₃): δ 11.19 (s, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.28– 7.19 (m, 2H), 7.19–7.07 (m, 2H), 6.67 (bs, 1H), 6.60 (s, 1H), 4.00 (s, 3H), 3.60–3.48 (m, 2H), 2.78–2.62 (m, 2H), 2.29 (s, 3H); ¹³C NMR (125 MHz, CDCl₃ and CD₃OD): δ 170.8, 170.5, 161.9, 157.8, 141.9, 135.3, 130.4, 129.5, 126.6, 125.3, 123.4, 114.6, 106.0, 102.4, 52.4, 36.3, 28.9, 17.6. HRMS (FAB) *m*/*z* [M+Na⁺] for C₁₈H₁₈ClNO₅Na: 386.0771; found, 386.0769.

Methyl 3-*chloro*-2-(3-((3-*fluorophenyl*)*amino*)-3-*oxopropyl*)-4,6*dihydroxybenzoate* (**16**). 27 mg, 74% yield, white amorphous solid: ¹H NMR (500 MHz, (CD₃)₂CO) δ 9.38 (br s, 1H), 7.86–7.64 (m, 1H), 7.37–7.22 (m, 2H), 6.81 (ddt, *J* = 9.1, 6.3, 2.6 Hz, 1H), 6.51 (s, 1H), 3.95 (s, 3H), 3.57–3.20 (m, 2H), 2.84–2.52 (m, 2H); ¹³C NMR(125 MHz, (CD₃)₂CO): δ 171.3, 164.7, 162.8, 162.3, 158.6, 143.0, 142.1, 131.0, 130.9, 115.5, 110.4, 108.2, 106.9, 103.2, 52.9, 36.8, 28.8; HRMS (FAB) *m*/*z* [M+H⁺] for C₁₇H₁₅CIFNO₅: 368.0701; found, 368.0701.

Methyl 3-*chloro*-2-(3-((3-*chlorophenyl*)*amino*)-3-*oxopropyl*)-4,6*dihydroxybenzoate* (**17**). 30 mg, 78% yield, white amorphous solid: ¹H NMR (500 MHz, (CD₃)₂CO) δ 9.33 (s, 1H), 8.06–7.85 (m, 1H), 7.49 (ddd, *J* = 8.2, 2.1, 1.0 Hz, 1H), 7.31 (t, *J* = 8.1 Hz, 1H), 7.08 (ddd, *J* = 7.9, 2.1, 0.9 Hz, 1H), 6.51 (s, 1H), 3.95 (s, 3H), 3.69–3.33 (m, 2H), 2.85–2.53 (m, 2H); ¹³C NMR(125 MHz. (CD₃)₂CO): δ 171.3, 171.3, 162.3, 158.6, 143.0, 141.8, 134.7, 131.0, 123.8, 119.8, 118.1, 114.8, 108.2, 103.2, 52.9, 36.8, 28.8; HRMS (FAB) *m*/ *z* [M–H⁻] for C₁₇H₁₄Cl₂NO₅: 382.0249; found, 382.0237.

Methyl 2-(3-((3-bromophenyl)amino)-3-oxopropyl)-3-chloro-4,6dihydroxybenzoate (**18**). 27 mg, 74% yield, white amorphous solid: ¹H NMR (500 MHz, (CD₃)₂CO) δ 9.32 (s, 1H), 8.10 (q, *J* = 1.6 Hz, 1H), 7.66–7.46 (m, 1H), 7.38–7.15 (m, 2H), 6.51 (s, 1H), 3.95 (s, 3H), 3.60–3.33 (m, 2H), 2.73–2.61 (m, 2H). ¹³C NMR (125 MHz, (CD₃)₂CO) δ 171.32, 171.26, 158.63, 142.99, 141.93, 131.31, 126.77, 122.77, 122.70, 122.62, 118.63, 118.54, 114.81, 108.21, 103.18, 52.93, 36.81, 36.76, 28.84; HRMS (FAB) *m/z* [M+Na⁺] for C₁₇H₁₅ClBrNO₅Na: 449.9720; found, 449.9720.

Methyl 3-*chloro*-4,6-*dihydroxy*-2-(3-*oxo*-3-((3-(*trifluoromethyl*) *phenyl*)*amino*)*propyl*)*benzoate* (**19**). 34 mg, 81% yield, white amorphous solid: ¹H NMR (400 MHz, CDCl₃) δ 7.85 (s, 1H), 7.77–7.65 (m, 1H), 7.39 (t, *J* = 7.9 Hz, 1H), 7.32–7.28 (m, 1H), 6.41 (d, *J* = 0.7 Hz, 1H), 5.27 (d, *J* = 0.7 Hz, 1H), 3.91 (d, *J* = 0.7 Hz, 3H), 3.50–3.39 (m, 2H), 2.67–2.45 (m, 2H); ¹³C NMR (125 MHz, CDCl₃): δ 170.7, 161.8, 158.0, 147.6, 141.6, 136.8, 136.5, 129.4, 126.4, 126.0, 125.1, 124.5, 120.1, 106.0, 102.4, 52.3, 30.7, 26.1; HRMS (FAB) *m/z* [M+H⁺] for C₁₈H₁₆CIF₃NO₅: 418.0669; found, 418.0661.

Methyl 2-(3-([1,1'-biphenyl]-3-ylamino)-3-oxopropyl)-3-chloro-4,6-dihydroxybenzoate (**20**). 35 mg, 72% yield, white amorphous solid: ¹H NMR (500 MHz, (CD₃)₂CO) δ 8.10–8.01 (m, 1H), 7.66 (tdd, *J* = 6.3, 2.1, 1.0 Hz, 3H), 7.52–7.44 (m, 2H), 7.44–7.33 (m, 3H), 6.52 (s, 1H), 3.97 (s, 3H), 3.63–3.36 (m, 2H), 2.73–2.56 (m, 2H); ¹³C NMR(125 MHz, (CD₃)₂CO): δ 143.2, 130.1, 129.7, 128.3, 127.7, 122.6, 118.9, 118.5, 103.1, 52.9, 36.8, 29.0;. HRMS (FAB) *m/z* [M+Na⁺] for C₂₃H₂₀ClNO₅Na: 448.0928; found, 448.0927.

Methyl 3-*chloro*-4,6-*dihydroxy*-2-(3-((3-*hydroxyphenyl*)*amino*)-3-*oxopropyl*)*benzoate* (**21**). 16 mg, 88% yield, off-white solid. ¹H NMR (400 MHz, CD₃OD): δ 7.16 (t, J = 2.27 Hz, 1H), 7.06 (td, J = 2.36, 8.01 Hz, 1H), 6.92 (dd, J = 2.70, 7.62 Hz, 1H), 6.50 (dd, J = 2.47, 8.35 Hz, 1H), 6.37 (s, 1H), 3.86 (s, 3H), 3.91 (s, 3H), 3.32– 3.25 (m, 2H), 2.60 (ddt, J = 3.13, 7.38 Hz, 10.18, 2H). ¹³C NMR (125 MHz, CD₃OD): δ 173.26, 171.66, 161.48, 158.88, 142.34, 140.93, 130.50, 114.95, 114.93, 112.36, 112.09, 109.54, 108.40, 103.14, 52.87, 37.06, 29.09. HRMS (FAB) m/z [M+Na⁺] for C₁₇H₁₆ClNO₆Na: 388.0558, found 388.0555.

Methyl 3-chloro-4,6-dihydroxy-2-(3-((3-methoxyphenyl)amino)-3-oxopropyl)benzoate (**22**). 14.6 mg, 76% yield, white solid. ¹H NMR (500 MHz, CDCl₃) δ 11.15 (s, 1H), 7.30–7.11 (m, 3H), 6.92–6.58 (m, 2H), 6.52 (s, 1H), 6.25 (s, 1H), 3.90 (s, 3H), 3.75 (s, 3H), 3.46–3.37 (m, 2H), 2.58–2.51 (m, 2H).¹³C NMR (126 MHz, CDCl₃) δ 170.82, 170.71, 162.10, 160.03, 157.67, 142.01, 139.24, 129.61, 114.59, 111.83, 109.92, 106.09, 105.39, 102.47, 55.27, 52.54, 36.41, 28.58. HRMS (FAB) m/z [M+Na⁺] for C₁₈H₁₈ClNO₆: 402.0720; found, 402.0722.

Methyl 2-(3-((3-aminophenyl)amino)-3-oxopropyl)-3-chloro-4,6dihydroxybenzoate (**23**). 9 mg, 49% yield, pale yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 7.12–7.05 (m, 1H), 7.01 (t, *J* = 8.0 Hz, 1H), 6.68 (dd, *J* = 7.8, 2.1 Hz, 1H), 6.44–6.26 (m, 2H), 3.87 (s, 3H), 3.38 (t, *J* = 8.4 Hz, 2H), 2.61–2.47 (m, 2H). ¹³C NMR (125 MHz, CDCl₃) δ 170.50, 162.27, 157.40, 146.36, 141.98, 129.72, 114.46, 111.35, 110.11, 106.84, 106.20, 102.55, 52.57, 36.57, 29.69, 28.69. HRMS (FAB) *m*/*z* [M+Na⁺] for C₁₇H₁₇ClN₂O₅Na: 387.0724; found, 387.0736.

Methyl 2-(3-((3-acetamidophenyl)amino)-3-oxopropyl)-3-chloro-4,6-dihydroxybenzoate (**24**). 18 mg, 89% yield white solid. ¹H NMR (400 MHz, CD₃OD): δ 7.86 (t, *J* = 1.91 Hz, 1H), 7.34–7.21 (m, 3H), 6.40 (s, 1H), 3.91 (s, 3H), 3.36–3.32 (m, 2H), 2.69–2.62 (m, 2H), 2.12 (d, *J* = 1.77 Hz, 3H). ¹³C NMR (125 MHz, CD₃OD): δ 173.33, 171.69, 171.65, 161.43, 159.00, 142.26, 140.37, 140.33, 130.02, 116.94, 116.83, 114.97, 113.06, 109.58, 103.15, 52.84, 37.01, 29.04, 23.81. HRMS (FAB) *m*/*z* [M+Na⁺] for C₁₉H₁₉ClN₂O₆Na: 428.0829, found 429.0824.

Methyl 3-chloro-2-(3-((4-fluorophenyl)amino)-3-oxopropyl)-4,6dihydroxybenzoate (**25**). 14.5 mg, 80% yield, white solid. ¹H NMR (500 MHz, CDCl₃) δ 11.28 (s, 1H), 7.89 (s, 1H), 7.50 (ddd, J = 9.4, 4.8, 2.4 Hz, 2H), 7.02 (t, J = 8.7 Hz, 2H), 6.50 (s, 1H), 3.96 (s, 3H), 3.52–3.44 (m, 2H), 2.65–2.56 (m, 2H). ¹³C NMR (125 MHz, CDCl₃) δ 170.34, 169.85, 162.91, 160.34, 156.21, 141.58, 121.57, 121.50, 115.82, 115.64, 113.88, 106.86, 102.98, 52.73, 36.57, 28.79.HRMS (FAB) m/z [M+Na⁺] for C₁₇H₁₅CIFNO₅Na: 390.0521; found, 390.0553.

Methyl 3-chloro-2-(3-((4-chlorophenyl)amino)-3-oxopropyl)-4,6dihydroxybenzoate (**26**). 13.8 mg, 72% yield, white solid. ¹H NMR (500 MHz, CDCl₃) δ 11.16 (s, 1H), 7.52–7.45 (m, 2H), 7.32–7.28 (m, 2H), 6.60 (s, 1H), 6.14 (s, 1H), 3.97 (s, 3H), 3.55–3.45 (m, 2H), 2.65–2.59 (m, 2H). ¹³C NMR (125 MHz, CDCl₃) δ 170.45, 170.35, 162.39, 157.07, 141.80, 136.48, 129.09, 128.99, 120.94, 120.84, 114.29, 106.38, 102.70, 52.62, 36.48, 28.64.HRMS (FAB) *m*/*z* [M+Na⁺] for C₁₇H₁₅Cl₂NO₅Na: 406.0225; found, 406.0234.

Methyl 2-(3-((4-bromophenyl)amino)-3-oxopropyl)-3-chloro-4,6dihydroxybenzoate (**27**). 15 mg, 70% yield, white solid. ¹H NMR (500 MHz, CDCl₃) δ 7.38 (s, 4H), 6.49 (s, 1H), 3.90 (s, 3H), 3.45–3.39 (m, 2H), 2.58–2.52 (m, 2H). ¹³C NMR (125 MHz, CDCl₃) δ 170.27, 169.88, 162.91, 156.18, 141.50, 136.83, 132.05, 121.18, 116.89, 113.864, 106.87, 103.01, 52.74, 36.71, 28.71.HRMS (FAB) *m*/*z* [M+Na⁺] for C₁₇H₁₅BrClNO₅Na: 449.9720; found, 449.9740.

Methyl 3-chloro-4,6-dihydroxy-2-(3-((4-iodophenyl)amino)-3oxopropyl)benzoate (**28**). 22 mg, 76% yield, white solid. ¹H NMR (500 MHz, CDCl₃ and CD₃OD) δ 7.55 (dd, 2H), 7.28 (dd, 2H), 6.43 (s, 1H), 3.89 (s, 3H), 3.41–3.39 (m, 2H), 2.56–2.53 (m, 2H). ¹³C NMR (125 MHz, CDCl₃ and CD₃OD) δ 169.93, 162.88, 156.22, 149.99, 141.51, 137.99, 136.22, 129.72, 128.84, 126.65, 126.34, 121.46, 103.01, 52.74, 36.74, 28.69. HRMS (FAB) *m/z* [M+Na⁺] for C₁₇H₁₅ClINO₅Na: 497.9683; found, 497.9656.

Methyl 3-*chloro*-4,6-*dihydroxy*-2-(3-*oxo*-3-((4-(*trifluoromethyl*) *phenyl*)*amino*)*propyl*)*benzoate* (**29**). 31 mg, 74% yield, white amorphous solid: ¹H NMR (500 MHz, (CD₃)₂CO) δ 8.13–7.78 (m, 2H), 7.65 (dd, *J* = 8.8, 2.5 Hz, 2H), 6.51 (t, *J* = 1.7 Hz, 1H), 3.95 (t, *J* = 1.8 Hz, 3H), 3.44 (ddt, *J* = 10.2, 6.1, 1.8 Hz, 2H), 2.72 (ddt, *J* = 10.2, 6.3, 1.8 Hz, 2H); ¹³C NMR (125 MHz, (CD₃)₂CO) δ (171.5, 171.4, 171.3, 162.2, 158.6, 142.9, 126.9, 126.9, 126.8, 126.8, 119.8, 119.8, 114.8, 108.2, 103.2, 52.9, 36.8, 28.8. HRMS (FAB) *m/z* [M+H⁺] for C₁₈H₁₆ClF₃NO₅: 418.0669; found, 418.0669.

Methyl 2-(3-([1,1'-biphenyl]-4-ylamino)-3-oxopropyl)-3-chloro-4,6-dihydroxybenzoate (**30**). 12.5 mg, 60% yield, white solid. ¹H NMR (400 MHz, CDCl₃) δ 7.57–7.48 (m, 6H), 7.41–7.23 (m, 3H), 7.15 (d, *J* = 5.6 Hz, 1H), 6.54 (s, 1H), 6.02 (s, 1H), 3.92 (s, 3H), 3.49–3.41 (m, 2H), 2.62–2.55 (m, 2H). ¹³C NMR (125 MHz, CDCl₃) δ 170.41, 169.88, 162.96, 156.19, 141.64, 140.41, 137.25, 128.81, 127.70, 127.17, 126.84, 119.96, 113.88, 106.87, 102.96, 52.75, 36.75, 28.82. HRMS (FAB) *m*/*z* [M+Na⁺] for C₂₃H₂₀ClNO₅Na: 448.0928; found, 448.0946.

Methyl 3-*chloro*-4,6-*dihydroxy*-2-(3-*oxo*-3-(*p*-*tolylamino*)*propyl*) *benzoate* (**31**). 14 mg, 76% yield, white solid. ¹H NMR (500 MHz, CD₃OD) δ 7.32 (dd, *J* = 8.5, 2.0 Hz, 2H), 7.08–7.02 (m, 2H), 6.47 (s, 1H), 3.88 (s, 3H), 3.45–3.37 (m, 2H), 2.56–2.49 (m, 2H), 2.24 (s, 3H). ¹³C NMR (125 MHz, CD₃OD) δ 168.00, 167.60, 167.51, 154.20, 139.34, 132.64, 131.47, 131.44, 127.00, 117.33, 117.21, 111.59, 104.03, 100.23, 50.14, 34.05, 26.29, 18.34.HRMS (FAB) *m/z* [M+Na⁺] for C₁₈H₁₈ClNO₅Na: 386.0771; found, 386.1007.

Methyl 3-chloro-4,6-dihydroxy-2-(3-((4-hydroxyphenyl)amino)-3-oxopropyl)benzoate (**32**). 8.8 mg, 48% yield, white solid. ¹H NMR (500 MHz, CD₃OD) δ 7.36–7.27 (m, 2H), 6.74–6.63 (m, 2H), 6.38 (s, 1H), 3.88 (s, 3H), 3.30–3.28 (m, 2H), 2.64–2.55 (m, 2H). ¹³C NMR (125 MHz, CD₃OD) δ 172.98, 171.64, 161.37, 158.85, 155.34, 142.31, 131.72, 123.38, 116.18, 114.89, 109.69, 103.11, 52.84, 36.88, 29.19.HRMS (FAB) *m*/*z* [M+Na⁺] for C₁₇H₁₆ClNO₆Na: 388.0564; found, 388.0556.

Methyl 3-chloro-4,6-dihydroxy-2-(3-((4-methoxyphenyl)amino)-3-oxopropyl)benzoate (**33**). 32 mg, 73% yield, white powder. ¹H NMR (500 MHz, CD₃OD): δ 7.42–7.37 (m, 2H), 6.84–6.79 (m, 2H), 6.41 (s, 1H), 3.90 (s, 3H), 3.75 (s, 3H), 3.45–3.39 (m, 2H), 2.58–2.52 (m, 2H). ¹³C NMR (125 MHz, CD₃OD): δ 170.74, 170.72, 161.9, 157.8, 156.1, 142.0, 131.1, 121.6, 114.6, 114.0, 106.0, 102.3, 55.4, 52.4, 36.1, 28.6. HRMS (FAB) *m*/*z* [M+Na⁺] for C₁₈H₁₈ClNO₆Na: 402.0720; found, 402.0725.

Methyl 2-(3-((4-aminophenyl)amino)-3-oxopropyl)-3-chloro-4,6dihydroxybenzoate (**34**). 8 mg, 33% yield, dark brown solid. ¹H NMR (400 MHz, CD₃OD) δ 7.45–7.27 (m, 2H), 6.90–6.74 (m, 2H), 6.50 (s, 1H), 4.00 (s, 3H), 3.41 (dt, *J* = 3.2, 1.7 Hz, 13H), 2.77–2.65 (m, 2H). ¹³C NMR (125 MHz, CDCl₃ and CD₃OD) δ 170.59, 169.91, 162.49, 156.88, 143.10, 141.99, 129.23, 121.91, 115.51, 106.47, 102.65, 52.63, 49.80, 36.43, 29.70, 28.90. HRMS (FAB) *m*/*z* [M+Na⁺] for C₁₇H₁₇ClN₂O₅Na: 387.0826; found, 387.0836.

Methyl 3-*chloro*-2-(3-((4-*cyanophenyl*)*amino*)-3-*oxopropyl*)-4,6*dihydroxybenzoate* (**35**). 13.6 mg, 72% yield, white solid. ¹H NMR (500 MHz, CDCl₃) δ 7.71–7.60 (m, 4H), 6.56 (s, 1H), 3.98 (s, 3H), 3.53–3.46 (m, 2H), 2.70–2.64 (m, 2H). ¹³C NMR (125 MHz, CDCl₃) δ 170.97, 170.41, 162.19, 157.38, 142.28, 141.72, 133.26, 119.32, 118.94, 114.44, 106.70, 106.26, 102.66, 52.59, 36.46, 28.41.HRMS (FAB) *m*/*z* [M+H] for C₁₈H₁₅ClN₂O₅: 375.0748; found, 375.2276.

Methyl 3-chloro-2-(3-((4-ethynylphenyl)amino)-3-oxopropyl)-4, 6-dihydroxybenzoate (**36**). 18 mg, 95% yield, off-white solid. ¹H NMR (400 MHz, CDCl₃): δ 7.51–7.46 (m, 2H), 7.39–7.34 (m, 2H), 6.38 (s, 1H), 3.87 (s, 3H), 3.48–3.32 (m, 5 H), 3.01 (s, 1H), 2.60– 2.53 (m, 2H); ¹³C NMR (125 MHz, CDCl₃ and CD₃OD): δ 171.2, 170.7, 161.8, 157.8, 141.9, 138.7, 132.7, 119.2, 117.1, 114.6, 106.0, 102.3, 83.3, 76.5, 52.3, 36.0, 28.3. HRMS (FAB) *m*/*z* [M+Na⁺] for C₁₉H₁₆CINO₅Na: 396.0615; found, 396.0608.

Methyl 3-*chloro*-4,6-*dihydroxy*-2-(3-((*4-isopropylphenyl*)*amino*)-3-*oxopropyl*)*benzoate* (**37**). 26 mg, 87% yield, white solid. ¹H NMR (500 MHz, (CD₃)₂CO) δ 9.13 (s, 1H), 7.74–7.52 (m, 2H), 7.27–7.13 (m, 2H), 6.54 (t, *J* = 1.5 Hz, 1H), 4.08–3.89 (m, 3H), 3.54–3.36 (m, 2H), 2.90 (td, *J* = 7.0, 2.1 Hz, 1H), 2.69 (ddt, *J* = 8.1, 6.5, 1.6 Hz, 2H), 2.15–2.11 (m, 1H), 1.25 (dt, *J* = 6.9, 1.4 Hz, 7H). ¹³C NMR (125 MHz, (CD₃)₂CO) δ 171.36, 170.68, 162.29, 158.56, 144.45, 143.28, 138.23, 138.15, 127.23, 120.07, 114.76, 108.22, 103.09, 52.91, 36.73, 34.28, 29.03, 24.41. HRMS (FAB) *m/z* [M+Na⁺] for C₂₀H₂₂ClNO₅Na: 414.1187; found, 414.1165. *Methyl* 3-*chloro-4*,6-*dihydroxy-2-(3-oxo-3-(pyridin-2-ylamino)* propyl)benzoate (**38**). ¹H NMR (500 MHz, (CD₃)₂CO) δ 8.39–8.07 (m, 2H), 7.80 (dd, *J* = 8.7, 7.4, 1.8 Hz, 1H), 7.10 (dd, *J* = 7.3, 4.8, 1.1 Hz, 1H), 6.55 (s, 1H), 3.99 (s, 3H), 3.57–3.42 (m, 2H), 2.98– 2.77 (m, 2H). ¹³C NMR (125 MHz, (CD₃)₂CO) δ 171.60, 162.34, 158.67, 153.19, 148.85, 143.11, 138.72, 120.02, 114.86, 114.29, 108.11, 103.12, 52.92, 36.62, 28.77. HRMS (FAB) *m/z* [M+Na⁺] for C₁₆H₁₅ClN₂O₅Na: 373.0669; found, 373.0630.

Methyl 3-chloro-4,6-dihydroxy-2-(3-oxo-3-(pyridin-3-ylamino) propyl)benzoate (**39**). 23 mg, 65% yield, white amorphous solid: ¹H NMR (500 MHz, (CD₃)₂CO) δ 8.80 (dd, *J* = 4.7, 1.5 Hz, 1H), 8.35 (dd, *J* = 9.0, 1.5 Hz, 1H), 7.51 (dd, *J* = 9.0, 4.7 Hz, 1H), 6.40 (s, 1H), 3.82 (s, 3H), 3.48–3.21 (m, 2H), 2.80–2.75 (m, 2H); ¹³C NMR(125 MHz, (CD₃)₂CO): δ 158.6, 145.2, 143.0, 141.9, 126.7, 124.2, 103.1, 52.9, 36.7, 36.6, 28.8; HRMS (FAB) *m*/*z* [M+H⁺] for C₁₆H₆ClN₂O₅: 351.0748; found, 351.0755.

Methyl 3-*chloro-4*,6-*dihydroxy-2-(3-oxo-3-(pyridin-4-ylamino)* propyl)benzoate (**40**). 10 mg, 51% yield, white solid. ¹H NMR (500 MHz, (CD₃)₂CO and CD₃OD) δ 9.55 (s, 1H), 8.53–8.40 (m, 2H), 7.73–7.61 (m, 2H), 6.52 (s, 1H), 3.95 (s, 3H), 3.52–3.40 (m, 2H), 2.75–2.71 (m, 2H). ¹³C NMR (125 MHz, (CD₃)₂CO) δ 172.20, 171.54, 165.56, 162.82, 151.33 (2C), 146.82, 142.44, 113.98 (2C), 103.33, 54.98, 52.68, 36.96, 28.73. HRMS (FAB) *m/z* [M+Na⁺] for C₁₆H₁₅ClN₂O₅Na: 373.0669; found, 373.0695.

Methyl 3-*chloro*-4,6-*dihydroxy*-2-(3-*oxo*-3-(*pyrazin*-2-*ylamino*) *propyl*)*benzoate* (**41**). 9 mg, 26% yield, white powder. ¹H NMR (400 MHz, CDCl₃ and CD₃OD): δ 11.22 (s, 1H), 9.58 (s, 1H), 8.38– 8.35 (m, 1H), 8.27–8.23 (m, 1H), 8.02 (br s, 1H), 6.58 (s, 1H), 3.98 (s, 3H), 3.55–3.48 (m, 2H), 2.76–2.69 (m, 2H); ¹³C NMR (125 MHz, CDCl₃ and CD₃OD): δ 171.5, 170.6, 161.7, 158.0, 148.5, 142.2, 141.3, 139.3, 136.7, 114.6, 105.9, 102.3, 52.3, 35.7, 27.9. HRMS (FAB) *m*/*z* [M+Na⁺] for C₁₅H₁₄ClN₃O₅Na: 374.0520; found, 374.0524.

Methyl 3-*chloro*-4,6-*dihydroxy*-2-(3-*oxo*-3-(*pyridazin*-3-*ylamino*) *propyl*)*benzoate* (**42**). 26 mg, yield 74%, white amorphous solid: ¹H NMR (500 MHz, (CD₃)₂CO) δ 10.04 (s, 1H), 8.92 (dd, *J* = 4.7, 1.5 Hz, 1H), 8.47 (dd, *J* = 9.0, 1.5 Hz, 1H), 7.63 (dd, *J* = 9.0, 4.7 Hz, 1H), 6.52 (s, 1H), 3.94 (s, 3H), 3.54–3.38 (m, 2H), 2.99–2.84 (m, 2H); ¹³C NMR (125 MHz, (CD₃)₂CO) δ 158.78, 149.10, 142.85, 128.79, 118.60, 118.54, 103.20, 52.93, 36.60, 28.60; HRMS (FAB) *m/z* [M+Na⁺] for C₁₅H₁₄ClN₃O₅Na: 374.0520; found, 374.0534.

Methyl 3-*chloro*-4,6-*dihydroxy*-2-(3-*oxo*-3-(*pyrimidin*-5-*ylamino*) *propyl*)*benzoate* (**43**). 23 mg, 66% yield, white amorphous solid: ¹H NMR (400 MHz, CDCl₃–MeOD) δ 9.01 (s, 2H), 8.84 (s, 1H), 6.42 (s, 1H), 3.91 (s, 3H), 3.59–3.36 (m, 2H), 2.70–2.51 (m, 2H); ¹³C NMR (CDCl₃, 125 MHz): δ 172.1, 170.9, 162.0, 158.1, 152.9, 147.6 (2), 141.8, 114.8 (2), 106.2, 102.6, 52.6, 35.7, 28.2; (FAB) *m*/*z* [M+H⁺] for C₁₅H₁₅ClN₃O₅: 352.0712; found, 352.0712.

Methyl 3-*chloro*-4,6-*dihydroxy*-2-(3-*oxo*-3-(*pyrimidin*-2-*ylamino*) *propyl*)*benzoate* (**44**). 21 mg, yield 60%, white amorphous solid: ¹H NMR (500 MHz, (CD₃)₂CO) δ 9.04 (d, *J* = 1.3 Hz, 2H), 8.83 (s, 1H), 6.51 (s, 1H), 3.94 (s, 3H), 3.57–3.31 (m, 2H), 2.82–2.69 (m, 2H); ¹³C NMR(125 MHz, (CD₃)₂CO): δ 171.7, 171.2, 158.6, 154.2, 148.0, 147.9, 142.7, 135.6, 135.5, 114.8, 108.3, 103.2, 52.9, 36.4, 28.7; HRMS (FAB) *m/z* [M+Na⁺] for C₁₅H₁₄ClN₃O₅Na: 374.0520; found, 374.0527.

Methyl 2-(3-((1,2,4-triazin-5-yl)amino)-3-oxopropyl)-3-chloro-4, 6-dihydroxybenzoate (**45**). 8.7 mg, 12% yield, yellow powder. ¹H NMR (500 MHz, CDCl₃ and CD₃OD): δ 8.89 (d, *J* = 2.5 Hz, 1H), 8.47 (d, *J* = 2.5 Hz, 1H), 6.37 (s, 1H), 3.80 (s, 3H), 3.45–3.38 (m, 2H), 2.89–2.80 (m, 2H). ¹³C NMR (125 MHz, CDCl₃ and CD₃OD): δ 171.8, 170.7, 161.8, 158.8, 157.9, 149.7, 145.1, 141.5, 114.6, 105.9, 102.3, 52.3, 36.2, 27.6. HRMS (FAB) *m*/*z* [M+Na⁺] for C₁₄H₁₃ClN₄O₅Na: 375.0472; found, 375.0468.

Methyl 3-chloro-4,6-dihydroxy-2-(3-oxo-3-(thiazol-2-ylamino) propyl)benzoate (**46**). 30 mg, 84% yield, white amorphous solid:

¹H NMR (500 MHz, (CD₃)₂CO) δ 7.40 (d, J = 3.6 Hz, 1H), 7.11 (d, J = 3.6 Hz, 1H), 6.52 (s, 1H), 3.94 (s, 3H), 3.57–3.34 (m, 2H), 2.98–2.78 (m, 2H); ¹³C NMR ((CD₃)₂CO, 125 MHz) δ 171.3, 170.7, 162.4, 158.9, 158.7, 142.7, 138.5, 114.9, 113.9, 108.2, 103.3, 53.0, 35.4, 28.6. (FAB) *m*/*z* [M+H⁺] calcd for C₁₄H₁₄ClN₂O₅S: 357.0312; found, 357.0323.

Methyl 2-(3-((1,3,4-thiadiazol-2-yl)amino)-3-oxopropyl)-3-chloro-4,6-dihydroxybenzoate (**47**). 28 mg, 78% yield, white amorphous solid: ¹H NMR (500 MHz, CDCl₃-MeOD,) δ 8.79 (d, J = 0.9 Hz, 1H), 6.44 (d, J = 0.9 Hz, 1H), 3.89 (d, J = 0.9 Hz, 3H), 3.62–3.30 (m, 2H), 2.93–2.40 (m, 4H); ¹³C NMR(CDCl₃-MeOD, 125 MHz): 171.0, 170.9, 162.3, 159.5, 158.1, 148.0, 141.3, 114.9, 106.1, 102.8, 52.6, 34.9, 28.0. (FAB) *m*/*z* [M+Na⁺] for C₁₃H₁₂ClN₃O₅ SNa: 380.0084; found, 380.0090.

Methyl 2-(3-((1,2,4-thiadiazol-5-yl)amino)-3-oxopropyl)-3-chloro-4,6-dihydroxybenzoate (**48**). 19 mg, 53% yield, white amorphous solid: ¹H NMR (400 MHz, CDCl₃-MeOD) δ 8.19 (s, 1H), 7.72 (s, 1H), 6.36 (s, 1H), 3.83 (s, 3H), 3.50-3.32 (m, 2H), 2.91-2.69 (m, 2H); ¹³C NMR (CDCl₃-MeOD, 125 MHz): δ 179.3, 176.3, 174.5, 163.0, 162.2.161.6, 144.8, 118.6, 110.2, 106.4, 56.3, 38.1, 33.5. (FAB) *m*/*z* [M+Na⁺] for C₁₃H₁₂ClN₃O₅SNa: 380.0084; found, 380.0078.

Methyl 3-*chloro*-4,6-*dihydroxy*-2-(3-(*methyl*(*phenyl*)*amino*)-3*oxopropyl*)*benzoate* (**49**). 28 mg, 77% yield, white amorphous solid: ¹H NMR (400 MHz, CDCl₃–MeOD) δ 7.34 (dd, *J* = 8.4, 6.8 Hz, 2H), 7.31–7.23 (m, 2H), 7.15–7.08 (m, 2H), 6.33 (s, 1H), 3.87 (s, 3H), 3.25 (d, *J* = 7.5 Hz, 5H), 2.35–2.27 (m, 2H); ¹³C NMR (CDCl₃–CH₃OH, 125 MHz) δ 172.9, 171.0, 162.1, 157.9, 143.7, 142.3, 130.0 (2), 128.1, 127.2 (2), 114.8, 106.0, 102.3, 52.4, 37.6, 33.4, 28.9; (FAB) *m/z* [M+H⁺]for C₁₈H₁₉CINO₅: 364.0952; found, 364.0943.

Methyl 3-*chloro*-2-(3-(*ethyl*(*phenyl*)*amino*)-3-*oxopropyl*)-4,6-*dihydroxybenzoate* (**50**). 30 mg, 79% yield, white amorphous solid: ¹H NMR (500 MHz, CDCl₃-MeOD) δ 7.34 (t, *J* = 7.7 Hz, 2H), 7.31–7.24 (m, 2H), 7.06 (dd, *J* = 7.3, 1.7 Hz, 2H), 6.33 (d, *J* = 4.0 Hz, 1H), 3.87 (d, *J* = 3.9 Hz, 3H), 3.70 (q, *J* = 7.1 Hz, 3H), 3.27–3.19 (m, 2H), 2.34–2.16 (m, 2H), 1.07 (td, *J* = 7.2, 3.4 Hz, 3H); ¹³C NMR (CDCl₃-MeOD, 125 MHz) δ 172.3, 171.0, 162.0, 157.9, 142.3, 141.9, 129.9 (2), 128.3 (2), 128.2, 114.7, 106.1, 102.2, 52.4, 44.3, 33.7, 28.8, 13.0; (FAB) *m/z* [M+H⁺] for C₁₉H₂₁ClNO₅: 378.1108; found, 378.1108.

Methyl 3-chloro-4,6-dihydroxy-2-(3-(isopropyl(phenyl)amino)-3oxopropyl)benzoate (51). A solution of benzyl protected acid 1a (100 mg, 0.22 mmol) in dry CH₂Cl₂ was treated with oxalyl chloride (23 µL, 0.33 mmol) under Ar atmosphere and then stirred at room temperature for 2 h. The solvent was then removed in vacuo and the residue was placed under high vacuum for 30 min to remove any unreacted oxalyl chloride. The residue was then redissolved in dry CH₂Cl₂ (3 mL), cooled to 0 °C, and was treated sequentially with diisopropylethyl amine (57 µL, 0.33 mmol) and *N*-isopropylaniline (72 µL, 0.33 mmol). The resulting mixture was stirred at room temperature overnight under Ar. The solvent was removed in vacuo and the residue was passed through a short pad of silica (1:1, EtOAc/Hexanes) and was concentrated to afford the amide crude product, which was dissolved in MeOH (5 mL) and treated with Pd/C (10 mol %, 23 mg). The resulting suspension was charged with a hydrogen balloon and stirred under H₂ at room temperature. When debenzylation was found to be complete, the mixture was filtered through celite, concentrated, and purified on silica gel chromatography (1:1, EtOAc/Hexanes) to afford compound **51** (30 mg, 35% yield over 2 steps) as a white solid. ¹H NMR (400 MHz, CDCl₃): δ 7.45–7.27 (m, 13H), 7.06–7.12 (m, 2H), 6.41 (s, 1H), 5.07 (hept, J = 6.8 Hz, 1H), 5.04 (s, 2H), 4.98 (s, 2H), 3.88 (s, 3H), 3.03-2.93 (m, 2H), 2.30-2.21 (m, 2H), 1.08 (d, I = 6.8 Hz, 6 H). ¹³C NMR (125 MHz, CDCl₃): δ 171.1, 167.5, 155.4, 154.5, 138.6, 138.3, 136.2, 135.9, 130.3, 129.2, 128.6, 128.5, 128.1, 127.9, 126.9, 126.8, 118.4, 115.5, 98.2, 70.9, 70.8, 52.4,

45.8, 34.4, 27.9, 21.0. HRMS (FAB) *m*/*z* [M+Na⁺] for C₂₀H₂₂CINO₅Na: 414.1084; found, 414.1076.

Methyl 3-chloro-4,6-dihydroxy-2-(3-(hydroxy(phenyl)amino)-3oxopropyl)benzoate (52). A round bottom flask was charged with aldehyde 1b (100 mg, 0.2 mmol), nitrosobenzene (22 mg, 0.2 mmol), and 6,7-dihydro-2-pentafluorophenyl-5H-pyrrolo[2,1*c*]-1,2,4-trazolium tetrafluoroborate (catalyst, 7 mg, 0.02 mmol) and purged with argon. The mixture was then dissolved in CH_2Cl_2 (1.5 mL) followed by addition of DBU (3 µL, 0.02 mmol) and stirred at room temperature for 30 min. Upon completion, the solvent removed in vacuo. The resulting residue was dissolved in THF (2 mL) and treated with TBAF (0.5 mL of 1 M solution). After complete silyl-deprotection, saturated aqueous NH₄Cl was added and extracted $3 \times$ with EtOAc (15 mL). The crude material was purified via flash chromatography (SiO₂, 2:3 EtOAc/Hexanes) to yield 52 as an off white solid (16 mg, 94%). ¹H NMR (400 MHz, CDCl₃): δ 11.30 (s, 1H), 7.45–7.32 (m, 5 H), 6.52 (s, 1H), 3.88 (s, 3H), 3.42–3.36 (m, 2H), 2.73-2.48 (m, 2H). ¹³C NMR (125 MHz, CDCl₃): δ 170.74, 163.10, 156.32 (2C), 141.18, 137.80, 129.57, 126.54, 119.88, 114.00, 106.92, 103.03 (2C), 52.71, 31.51, 28.84, 28.82. HRMS (FAB) m/z [M+Li⁺] for C₁₇H₁₆ClNO₆Li: 372.0826, found 372.0823.

Methyl 3-chloro-4,6-dihydroxy-2-(3-(indolin-1-yl)-3-oxopropyl) benzoate (**53**). 15 mg, 25% yield, white solid. ¹H NMR (400 MHz, CDCl₃): δ ¹H NMR (400 MHz, CDCl₃ and CD₃OD) δ 8.19 (d, J = 8.0 Hz, 1H), 7.24–7.10 (m, 2H), 6.99 (d, J = 1.2 Hz, 1H), 6.42 (d, J = 1.5 Hz, 1H), 4.00 (dd, J = 9.1, 7.8 Hz, 2H), 3.88 (d, J = 2.8 Hz, 3H), 3.52–3.40 (m, 2H), 3.16 (t, J = 8.4 Hz, 2H), 2.75–2.60 (m, 2H). ¹³C NMR (125 MHz, CDCl₃ and CD₃OD) δ 170.88, 170.71, 162.06, 157.89, 142.74, 142.21, 131.21, 127.50, 124.61, 123.81, 116.90, 114.64, 106.04, 102.42, 102.35, 52.51, 50.07, 49.72, 49.55, 49.38, 49.21, 49.03, 48.86, 48.69, 47.97, 35.08, 27.95, 27.84. HRMS (FAB) m/z [M+Na⁺] for C₁₉H₁₈ClNO₅Na: 398.0771; found, 398.0773.

Methyl 3-*chloro-2-(3-((2,4-dibromophenyl)amino)-3-oxopropyl)*-4,6-*dihydroxybenzoate* (**54**). 21 mg, 83%, white solid. ¹H NMR (500 MHz, CDCl₃ and CD₃OD) δ 8.22 (d, *J* = 8.9 Hz, 1H), 7.63 (dd, *J* = 2.2, 1.2 Hz, 1H), 7.49–7.33 (m, 1H), 6.44 (d, *J* = 1.0 Hz, 1H), 3.90 (d, *J* = 1.2 Hz, 3H), 3.47–3.39 (m, 2H), 2.72–2.54 (m, 2H). ¹³C NMR (125 MHz, CDCl₃ and CD₃OD) δ 170.52, 162.40, 157.48, 141.32, 134.74, 134.42, 131.43, 122.78, 116.73, 114.47, 113.56, 106.14, 102.72, 52.64, 36.99, 28.77. HRMS (FAB) *m/z* [M+Na⁺] for C₁₇H₁₄Br₂ClNO₅Na: 527.8927, found 527.8935.

Methyl 3-*chloro*-2-(3-((3,5-*dichlorophenyl*)*amino*)-3-*oxopropyl*)-4,6-*dihydroxybenzoate* (**55**). 19 mg, 91% yield, white solid. ¹H NMR (400 MHz, CDCl₃): δ 11.07 (s, 1H), 7.51 (d, *J* = 1.88 Hz, 2H), 7.29 (s, 1H), 7.11 (t, *J* = 1.7 Hz, 1H), 6.60 (s, 1H), 6.13 (s, 1H), 3.98 (s, 1H), 3.52–3.43 (m, 2H), 2.75–2.49 (m, 2H). ¹³C NMR (125 MHz, CDCl₃ and CD₃OD): δ 170.73, 170.50, 162.48, 157.22, 141.80, 139.98, 135.30, 124.08, 117.85, 114.43, 106.49, 102.87, 52.77, 36.52, 28.62. HRMS (FAB) *m*/*z* [M+Na⁺] for C₁₇H₁₄Cl₃NO₅Na: 439.9830, found 439.9840.

Methyl 2-(3-((4-bromo-3-chlorophenyl)amino)-3-oxopropyl)-3chloro-4,6-dihydroxybenzoate (**56**). 24 mg, 89% yield, white solid. ¹H NMR (500 MHz, (CD₃)₂CO) δ 8.12 (d, *J* = 2.5 Hz, 1H), 7.64 (d, *J* = 8.8 Hz, 1H), 7.48 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.52 (s, 1H), 3.96 (s, 3H), 3.50–3.37 (m, 2H), 2.80–2.63 (m, 2H). ¹³C NMR (125 MHz, (CD₃)₂CO) δ 170.45, 170.32, 161.23, 157.73, 141.96, 140.00, 133.72, 120.46, 119.04, 114.46, 113.91, 107.27, 102.20, 52.01, 35.87, 27.84. HRMS (FAB) *m*/*z* [M+Na⁺] for C₁₇H₁₄BrCl₂NO₅Na: 483.9432; found, 398.9333.

Methyl 2-(3-((2-bromo-3-chlorophenyl)amino)-3-oxopropyl)-3chloro-4,6-dihydroxybenzoate (**57**). 23 mg, 72% yield, white amorphous solid: ¹H NMR (500 MHz, CDCl₃–MeOD) δ 8.14 (d, *J* = 7.9 Hz, 1H), 7.25–7.15 (m, 2H), 6.40 (d, *J* = 1.2 Hz, 1H), 3.90 (d, *J* = 1.2 Hz, 3H), 3.46–3.37 (m, 3H), 2.72–2.62 (m, 2H); ¹³C NMR (CDCl₃–MeOD, 125 MHz): δ 170.9, 170.6, 162.0, 158.1, 141.3, 137.3, 134.8, 128.5 (2), 125.6, 120.2, 114.7, 105.9, 102.5, 52.5, 36.8, 28.7; (FAB) *m*/*z* [M+Na⁺] for C₁₇H₁₄BrCl₂NO₅Na: 483.9330; found, 483.9327.

Methyl 3-*chloro-2-(3-((6-chloropyridin-2-yl)amino)-3-oxopropyl)-4,6-dihydroxybenzoate* (**58**). 27 mg, 70% yield, white amorphous solid: ¹H NMR (500 MHz, (CD₃)₂CO) δ 10.88 (1H, br s), 9.48 (1H, br s)8.09 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.80–7.58 (m, 1H), 7.00 (dd, *J* = 7.9, 1.7 Hz, 1H), 6.63–6.10 (m, 1H), 3.93 (s, 3H), 3.46–3.10 (m, 2H), 2.91–2.55 (m, 2H); ¹³C NMR (125 MHz, (CD₃)₂CO) δ 172.1, 171.5, 158.8, 153.3, 149.6, 143.1, 142.2, 119.9 (2), 115.0, 112.8, 108.3, 103.3, 53.1, 36.7, 28.8; (FAB) *m/z* [M+Na⁺] for C₁₆H₁₄Cl₂N₂O₅Na: 407.0174; found, 407.0177.

Methyl 2-(3-((5-bromopyridin-2-yl)amino)-3-oxopropyl)-3chloro-4,6-dihydroxybenzoate (**59**). 29 mg, 68% yield, white amorphous solid: ¹H NMR (500 MHz, CDCl₃–MeOD) δ 8.31–8.19 (m, 1H), 8.19–8.05 (m, 1H), 7.84–7.71 (m, 1H), 6.54–6.28 (m, 1H), 3.94 (s, 3H), 3.42–3.35 (m, 2H), 2.63 (dq, *J* = 12.7, 3.8, 2.1 Hz, 2H); ¹³C NMR (CDCl₃–MeOD, 125 MHz): δ 171.5, 170.9, 162.1, 158.2, 150.2, 148.6, 141.6, 141.0, 115.5, 114.8, 114.5, 106.1, 102.6, 52.6, 36.4, 28.3. (FAB) *m*/*z* [M+Na⁺] for C₁₆H₁₄BrClN₂O₅Na: 450.9672; found, 450.9681.

Methyl 3-*chloro-2-(3-((4-chloropyridin-2-yl)amino)-3-oxopro-pyl)-4,6-dihydroxybenzoate* (**60**). 16 mg, 64% yield, white solid. ¹H NMR (500 MHz, (CD₃)₂CO) δ 8.41 (dd, *J* = 2.0, 0.6 Hz, 1H), 8.28 (dd, *J* = 5.3, 0.6 Hz, 1H), 7.19 (dd, *J* = 5.3, 1.9 Hz, 1H), 6.55 (s, 1H), 3.99 (s, 3H), 3.56–3.42 (m, 2H), 2.98–2.81 (m, 2H). ¹³C NMR (125 MHz, (CD₃)₂CO) δ 172.16, 171.34, 162.41, 158.69, 154.30, 150.12, 145.43, 142.93, 120.15, 113.98, 108.12, 103.20, 52.93, 36.62, 28.62. HRMS (FAB) *m/z* [M+Na⁺] for C₁₆H₁₄Cl₂N₂O₅Na: 407.0280; found, 407.0766.

Methyl 3-*chloro-2-(3-((3-chloropyridin-2-yl)amino)-3-oxopropyl)*-4,6-*dihydroxybenzoate* (**61**). 21 mg, 55% yield, white amorphous solid: ¹H NMR (500 MHz, (CD₃)₂CO) δ 8.36 (dd, J = 4.7, 1.6 Hz, 1H), 7.92 (dd, J = 8.0, 1.6 Hz, 1H), 7.27 (ddd, J = 8.0, 4.7, 0.6 Hz, 1H), 6.52 (s, 1H), 3.97 (s, 3H), 3.55–3.39 (m, 2H), 3.00– 2.76 (m, 2H). ¹³C NMR (125 MHz, (CD₃)₂CO) δ 171.4, 171.28, 158.6, 149.3, 147.5, 143.2 (2), 139.3, 122.9 (2), 114.8, 108.14, 103.1, 52.9, 35.9, 28.8; (FAB) *m/z* [M+Na⁺] for C₁₆H₁₄Cl₂N₂O₅Na: 407.0174; found 407.0174.

Methyl 2-(3-((3-bromopyridin-2-yl)amino)-3-oxopropyl)-3chloro-4,6-dihydroxybenzoate (**62**). 31 mg, 71% yield, white amorphous solid: ¹H NMR (400 MHz, CDCl₃–MeOD) δ 8.47 (dd, *J* = 4.7, 1.6 Hz, 1H), 7.98 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.27–7.22(m, 1H), 6.34 (s, 1H), 3.85 (s, 3H), 3.35–3.29 (m, 2H), 2.91–2.65 (m, 2H). ¹³C NMR (125 MHz, CDCl₃): δ 172.8, 170.0, 161.2, 156.9, 149.7, 147.8, 141.7, 140.6, 124.9, 120.3, 113.8, 105.0, 101.4, 51.6, 36.3, 26.6. (FAB) *m*/*z* [M+Na⁺] for C₁₆H₁₄BrClN₂O₅Na: 450.9672; found, 450.9677.

Methyl 3-*chloro-2-(3-((5-chlorothiazol-2-yl)amino)-3-oxopropyl)-4,6-dihydroxybenzoate* (**63**). 17 mg, 44.7% yield, pale yellow amorphous solid: ¹H NMR (400 MHz, (CD₃)₂CO) δ 7.34 (s, 1H), 6.52 (s, 1H), 5.63 (s, 1H), 3.94 (s, 3H), 3.55–3.31 (m, 2H), 2.93– 2.71 (m, 2H). ¹³C NMR (125 MHz, CDCl₃–MeOD): δ 170.8 (2), 162.0, 158.1, 147.8, 141.8, 136.9, 129.5, 126.6, 125.3, 124.6, 120.9, 114.8, 106.2, 52.5, 30.8, 26.2;. HRMS (FAB) *m/z* [M+H⁺] for C₁₄H₁₃Cl₂N₂O₅S: 390.9922; found, 390.9913.

Methyl 2-(3-((5-bromothiazol-2-yl)amino)-3-oxopropyl)-3chloro-4,6-dihydroxybenzoate (**64**). 36 mg, 83% yield, gray amorphous solid: ¹H NMR (500 MHz, (CD₃)₂CO) δ 7.33 (s, 1H), 6.52 (s, 1H), 3.94 (s, 2H), 3.60–3.28 (m, 2H), 2.93–2.78 (m, 2H). ¹³C NMR(125 MHz, (CD₃)₂CO): δ 170.9, 170.9, 162.2, 159.6, 158.1, 141.4, 137.7, 114.9, 106.1, 102.7 (2), 52.7, 34.7, 28.1; HRMS (FAB) m/z [M+Na⁺] for C₁₄H₁₂BrClN₂O₅SNa: 456.9237; found, 456.9238.

Methyl 2-(3-((5-bromo-1,3,4-thiadiazol-2-yl)amino)-3-oxopropyl)-3-chloro-4,6-dihydroxybenzoate (**65**). 12 mg, 28% yield, gray amorphous solid: ¹H NMR (500 MHz, (CD₃)₂CO) δ 6.53 (s, 1H), 3.94 (s, 4H), 3.59–3.43 (m, 2H), 3.05–2.84 (m, 2H). ¹³C NMR (125 MHz, (CD₃)₂CO): δ 171.7, 161.6, 158.7, 142.2, 134.8, 130.6, 114.9, 108.1, 103.3, 79.3, 53.0, 35.2, 21.1. HRMS (FAB) m/z [M+H⁺] for C₁₃H₁₂BrClN₃O₅S: 435.9370; found, 435.9377.

Acknowledgment

The authors gratefully acknowledge the support of this Project by NIH (CA109265).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.05.075.

References and notes

- 1. Hartl, F. U. Nature 1996, 381, 571.
- 2. Hartl, F. U. Nature 2011, 475, 324.
- 3. Pearl, L. H.; Prodromou, C. Curr. Opin. Struct. Biol. 2000, 10, 46.
- 4. Prodromou, C.; Pearl, L. H. Curr. Cancer Drug Targets 2003, 3, 301.
- Pearl, L. H.; Prodromou, C. Adv. Protein Chem. 2001, 59, 157.
- 6. Meyer, P.; Prodromou, C.; Hu, B.; Vaughan, C.; Roe, S. M.; Panaretou, B.; Piper, P. W.; Pearl, L. H. Mol. Cell 2003, 11, 647.
- Panaretou, B.; Siligardi, G.; Meyer, P.; Maloney, A.; Sullivan, J. K.; Singh, S.; Millson, S. H.; Clarke, P. A.; Naaby-Hansen, S.; Stein, R.; Cramer, R.; Mollapour, M.; Workman, P.; Piper, P. W.; Pearl, L. H.; Prodromou, C. Mol. Cell 2002, 10, 1307.
- 8. Marcu, M. G.; Chadli, A.; Bouhouche, I.; Catelli, M.; Neckers, L. M. J. Biol. Chem. 2000, 275, 37181.
- Soti, C.; Racz, A.; Csermely, P. J. Biol. Chem. 2002, 277, 7066.
- Whitesell, L.; Bagatell, R.; Falsey, R. Curr. Cancer Drug Targets 2003, 3, 349. 10.
- 11. Whitesell, L.; Lindquist, S. L. Nat. Rev. Cancer 2005, 5, 761.
- 12. Zhang, H.; Burrows, F. J. Mol. Med. 2004, 82, 488.
- 13. Kim, Y. S.; Alarcon, S. V.; Lee, S.; Lee, M. J.; Giaccone, G.; Neckers, L.; Trepel, J. B. Curr. Top. Med. Chem. 2009, 9, 1479.
- 14. Biamonte, M. A.; Van de Water, R.; Arndt, J. W.; Scannevin, R. H.; Perret, D.; Lee, W. J. Med. Chem. 2010, 53, 3.
- 15. Holzbeierlein, J.; Windsperger, A.; Vielhauer, G. Curr. Oncol. Rep. 2010, 12, 95.
- 16.
- Sreedhar, A. S.; Kalmar, E.; Csermely, P.; Shen, Y. F. *FEBS Lett.* **2004**, *562*, 11. Peterson, L. B.; Esker, J. D.; Vielhauer, G. A.; Blagg, B. S. *Mol. Pharm.* **2012**, *9*, 17. 1841.
- 18. McLaughlin, M.; Vandenbroeck, K. Br. J. Pharmacol. 2011, 162, 328.
- Goodman, S. L.; Picard, M. Trends Pharmacol. Sci. 2012, 33, 405. 19
- Dejeans, N.; Glorieux, C.; Guenin, S.; Beck, R.; Sid, B.; Rousseau, R.; Bisig, B.; 20. Delvenne, P.; Buc Calderon, P.; Verrax, J. Free Radic. Biol. Med. 2012, 52, 993.
- Suntharalingam, A.; Abisambra, J. F.; O'Leary, J. C. I. I. I.; Koren, J. I. I.; Zhang, 21. B.; Joe, M. K.; Blair, L. J.; Hill, S. E.; Jinwal, U. K.; Cockman, M.; Duerfeldt, A. S.; Tomarev, S.; Blagg, B. S.; Lieberman, R. L.; Dickey, C. A. J. Biol. Chem. 2012, 287, 40661

- 22. Gullo, C. A.; Teoh, G. Immunol. Lett. 2004, 94, 1.
- 23. Obeng, E. A.; Carlson, L. M.; Gutman, D. M.; Harrington, W. J.; Lee, K. P.; Boise, L. H. Blood 2006, 107, 4907.
- 24. Davenport, E. L.; Moore, H. E.; Dunlop, A. S.; Sharp, S. Y.; Workman, P.; Morgan, G. J.; Davies, F. E. Blood 2007, 110, 2641.
- 25. Hua, Y.; White-Gilbertson, S.; Kellner, J.; Rachidi, S.; Usmani, S. Z.; Chiosis, G.; DePinho, R.; Li, Z.; Liu, B. Clin. Cancer Res. 2013, 19, 6242.
- Duerfeldt, A. S.; Peterson, L. B.; Maynard, J. C.; Ng, C. L.; Eletto, D.; Ostrovsky, O.; 26. Shinogle, H. E.; Moore, D. S.; Argon, Y.; Nicchitta, C. V.; Blagg, B. S. J. J. Am. Chem. Soc. 2012, 134, 9796.
- 27. Rosser, M. F.; Nicchitta, C. V. J. Biol. Chem. 2000, 275, 22798.
- 28. Patel, P. D.; Yan, P.; Seidler, P. M.; Patel, H. J.; Sun, W.; Yang, C.; Que, N. S.; Taldone, T.; Finotti, P.; Stephani, R. A.; Gewirth, D. T.; Chiosis, G. Nat. Chem. Biol. 2013. 9. 677.
- 29. Soldano, K. L.; Jivan, A.; Nicchitta, C. V.; Gewirth, D. T. J. Biol. Chem. 2003, 278, 48330
- 30. Immormino, R. M.; Metzger, L. E.; Reardon, P. N.; Dollins, D. E.; Blagg, B. S.; Gewirth, D. T. J. Mol. Biol. 2009, 388, 1033.
- 31 Clevenger, R. C.; Blagg, B. S. J. Org. Lett. 2004, 6, 4459.
- Kim, J.; Felts, S.; Llauger, L.; He, H.; Huezo, H.; Rosen, N.; Chiosis, G. J. Biomol. 32. Screen. 2004, 9, 375.
- Taldone, T.; Patel, P. D.; Patel, M.; Patel, H. J.; Evans, C. E.; Rodina, A.; Ochiana, 33. S.; Shah, S. K.; Uddin, M.; Gewirth, D.; Chiosis, G. J. Med. Chem. 2013, 56, 6803.
- 34. Hunter, L. Beilstein J. Org. Chem. 2010, 6.
- Wong, F. T.; Patra, P. K.; Seayad, J.; Zhang, Y.; Ying, J. Y. Org. Lett. 2008, 10, 2333. 35.
- Melnick, J.; Dul, J. L.; Argon, Y. Nature 1994, 370, 373 36.
- 37. Hadden, K. M.; Blagg, B. S. J. J. Org. Chem. 2009, 74, 4697.
- 38. Duerfeldt, A. S.; Brandt, G. E. L.; Blagg, B. S. J. Org. Lett. 2009, 11, 2353.
- Shen, G.; Wang, M.; Welch, T. R.; Blagg, B. S. J. *J. Org. Chem.* **2006**, *71*, 7618.
 Shen, G.; Blagg, B. S. J. Org. Lett. **2005**, *7*, 2157.
- 41. Grad, I.; Cederroth, C. R.; Walicki, J.; Grey, C.; Barluenga, S.; Winssinger, N.; De Massy, B.; Nef, S.; Picard, D. PLoS One 2010, 5, e15770.
- 42. Yu, X. M.; Shen, G.; Neckers, L.; Blake, H.; Holzbeierlein, J.; Cronk, B.; Blagg, B. S. J. J. Am. Chem. Soc. 2005, 127, 12778.
- 43. Burlison, J. A.; Blagg, B. S. J. Org. Lett. 2006, 8, 4855.
- Burlison, J. A.; Neckers, L.; Smith, A. B.; Maxwell, A.; Blagg, B. S. J. JACS 2006, 44. 128, 15529; Burlison, J. A.; Avila, C.; Vielhauer, G.; Lubbers, D. J.; Holzbeierlein, J.; Blagg, B. S. J. J. Org. Chem. 2008, 73, 2130.
- 45. Donnelly, A. C.; Mays, J. R.; Burlison, J. A.; Nelson, J. T.; Vielhauer, G.; Holzbeierlein, J.; Blagg, B. S. J. J. Org. Chem. 2008, 73, 8901.
- 46. Zhao, H.; Kusuma, B. R.; Blagg, B. S. J. ACS Med. Chem. Lett. 2010, 1, 311.
- 47. Matts, R. L.; Dixit, A.; Peterson, L. B.; Sun, L.; Voruganti, S.; Kalyanaraman, P.; Hartson, D., Verkhivker, G. M.; Blagg, B. S. J. ACS Chem. Biol. 2011, 6, 800. Zhao, H.; Donnelly, A. C.; Kusuma, B. R.; Brandt, G. E. L.; Brown, D.; Rajewski, R.
- 48. A.; Vielhauer, G.; Holzbeierlein, J.; Cohen, M. S.; Blagg, B. S. J. J. Med. Chem. 2011, 54, 3839.
- 49. Kusuma, B. R.; Zhang, L.; Sundstrom, T.; Peterson, L. B.; Dobrowsky, R. T.; Blagg, B. S. J. J. Med. Chem. 2012, 55, 5797.
- 50. Khandelwal, A.; Hall, J. A.; Blagg, B. S. J. J. Org. Chem. 2013, 78, 7859.
- Ostrovsky, O.; Ahmed, N. T.; Argon, Y. Mol. Biol. Cell **2009**, 20, 1855. 51
- Wang, J. S.; Wang, F. B.; Zhang, Q. G.; Shen, Z. Z.; Shao, Z. M. Mol. Cancer Res. 52. 2008, 6, 372.