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Structure—activity relationship study of benserazide derivatives as PilB inhibitors†

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Antimicrobial resistance is an imminent health threat worldwide. Development of alternative treatments for drug-resistant microbes is of paramount importance. Targeting virulence factors, such as the type IV pilus construction enzyme PilB, is a strategy of treatment. Recently, we reported the discovery of a potent inhibitor of PilB, the FDA approved drug benserazide ($IC_{50} = 3.68 \, \mu M$). Herein, we report the structure–activity relationship profiling of benserazide analogues and identify key moieties that enable PilB inhibition. We found that bis-hydroxyl groups on the *ortho* position of the aryl ring, a rigid imine, and exchange of the serine for a thiol have resulted in marked improvement in potency. Our studies identified **11c** as a PilB inhibitor with an IC_{50} of 580 nM and selectivity for PilB over an unrelated ATPase, apyrase. These compounds provide the chemical tools to validate virulence factors as antibacterial mechanisms of action.

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Introduction

Infections by antibiotic-resistant pathogens pose a critical public health threat worldwide. 1,2 An estimated 4.71 million deaths are associated with antibiotic resistance globally in 2021, with 1.14 million directly caused by drug resistant pathogens.3 By 2050, deaths associated with and due to drug resistant pathogens will nearly double to 8.22 million and 1.91 million, respectively.3 The overuse of antibiotics during the COVID-19 pandemic has exacerbated this problem, particularly with multidrug-resistant pathogens like Acinetobacter baumannii, Klebsiella pneumoniae, and Pseudomonas aeruginosa.^{1,4} The approval and availability of new antibiotics have inevitably led to the increase in resistance shortly after use in the clinic.⁵ Repeated exposure of bacteria to antibiotics necessitates the development of drug-resistance mechanisms to enable bacterial cell proliferation. This eventually results in the enrichment and even dominance of resistant bacteria in environments where antibiotics are present. Horizontal Gene Transfer (HTG), a process through which bacteria can transfer drug resistance genes, further accelerates the spread of resistance determinants, presenting a severe threat to public health.^{6,7} Consequently, it is imperative to develop strategies to combat disease without pressuring bacteria into resistance mechanisms. An

Well known virulence factors include two-component systems (TCS), quorum sensing (QS), type III secretion system (T3SS), fimbriae, endotoxin like lipopolysaccharide (LPS), and exotoxin like botulinum.^{11–14} The notion of targeting virulence factors is not novel and has been shown to be successful. For example, Raxibacumab, Obiltoxaximab, BabyBIG and Botulism Antitoxin Heptavalent are examples of FDA-approved

emerging strategy is to target virulence factors, the non-vital characteristics of pathogens that enable them to cause disease.⁸⁻¹⁰

Fig. 1 Select T4P inhibitors.

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antivirulence biologics.¹⁵ There is also some promise for the use of small molecules to target virulence factors. Recently, small molecule inhibitors targeting a response regulator in

HO NH₂ H OH OH amino acid

Fig. 2 Focus moieties of benserazide

Staphylococcus aureus, a T3SS, and an inhibitor for biogenesis of a bacterial adhesive pilus have been reported. ¹⁶⁻¹⁸ However, as of yet, there are no FDA-approved antivirulence small molecules for clinical use.

The bacterial type IV pilus (T4P) is another promising druggable virulence factor. ^{2,19–21} The pilus filament is a flexible and polymeric structure composed of thousands of copies of the pilin protein. These pilins are assembled into the T4P by a hexameric ATPase known as PilB or PilF depending on the bacterial species. ^{20,21} This highly conserved T4P assembly ATPase is present in many high-priority pathogens such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Neisseria meningitidis*. ^{22–24} Within pathogens, T4P are dutifully tied to

 9
 9a
 10
 11

 9a: R= H
 10a: R= H
 11a: R= H, E:Z 3:2

 9b: R= CH₃
 9ab: R= CH₃
 10b: R= CH₃
 11b: R= CH₃, E:Z 4:1

 9c: R= CH₂SH
 9ac: R= CH₂SH
 10c: R= CH₂SH
 11c: R= CH₂SH, E:Z 3:1

`Boc

Scheme 1 (a) 2,2 Dimethoxypropane, p-TsOH, CH₂Cl₂, rt, 16 h; (b) H₂NNH₂·H₂O, MeOH, rt, 16 h; (c) HCl (4M in dioxane), rt, 16 h; (d) aldehyde, Na₂SO₄, MeOH, rt or 40 °C, 16 h; (e) NaBH₄, CH₂Cl₂, rt, 12 h.

Boc

HCI

many cell functions, such as bacterial adhesion, biofilm formation, twitching motility and virulence. 21,22,24-26 Due to its high conservation and critical role in pathogenesis, T4P has been explored as a possible drug target. Some pre-clinical studies demonstrated anti-T4P compounds have therapeutic potential either alone or as an additive to antibiotics. 77,28 One study described that phenothazines, T4P targeting small molecules, reduce meningococcal colonization in human vessels, improve humanized mouse survival, and reduce vascular dysfunction and inflammation when used in combination with antibiotics, demonstrating potential benefit beyond antibiotic treatment.

As the T4P assembly ATPase, PilB/PilF is essential for pilus biogenesis. Its absence in bacteria has been shown to eliminate T4P biogenesis, making this ATPase a promising target for the development of antivirulence chemotherapeutics.25,29 A few inhibitors of PilB/F have been reported. 19,27,30 A high throughput screening (HTS) based on the inhibition of bacterial attachment to cultured human cells led to the discovery of P4MP4 as a PilB/ F inhibitor (Fig. 1).27 It was shown that this compound reduced bacterial adhesion to infected cells by targeting PilF with an IC50 of 175 µM.27 Our previous studies used Chloracidobacterium thermophilum PilB (CtPilB) as a model enzyme in HTS for the discovery of PilB inhibitors. These efforts led to the discovery of quercetin, levodopa and benserazide (Fig. 1), all of which demonstrated to inhibit CtPilB activities in vitro and T4P biogenesis in bacteria.19,30 Among these PilB inhibitors, benserazide was identified as one of the most promising with an IC₅₀ of 3.69 μM against CtPilB.

However, little is known about the structure-activity relationship profile of benserazide against PilB. In this study, we performed a medicinal chemistry campaign to develop the pharmacophore and identify key features that promote PilB inhibition. To develop the structure activity relationship, we divided benserazide into two key regions: the amino acid and benzylamine groups linked by a hydrazine and investigated the amino acid composition as well as hydroxy substitution pattern in the aryl ring (Fig. 2). Furthermore, we probed the effect of rigidity by introducing an imine moiety.

Our studies show that changing the substitution pattern in the aryl ring and amino acid composition of benserazide lead to improved activity. In particular, 11c was identified as a compound with significantly improved potency (0.58 μ M ν s. 3.69 μ M) and selectivity against an unrelated ATPase, apyrase.

Results and discussion

Design and synthesis

These compounds were synthesized as shown in Scheme 1. *N*-Boc-L-serine methyl ester was first protected using 2,2-dimethoxypropane and subsequently reacted with hydrazine to afford intermediates 2 and 3, respectively. Hydrazide 3 was treated with excess hydrochloric acid to afford compound 4 in 99% yield. Alternatively, 3 was condensed with various aldehydes to yield the hydrazone 5, which was isolated *via* column chromatography. Reduction of the imine was conducted in the presence of sodium borohydride to generate intermediate 6.

Subsequent Boc and dimethyl aminal deprotection in acid afforded compounds 7a-g. Alternatively, intermediates 5 can be deprotected under acidic conditions to afford compounds 8a-l with E: Z ratios ranging from 3:7 to 5:1. For compounds 11a-c,

Table 1 Inhibition of PilB with benzylamine derivatives^a

$HO \longrightarrow NH_2 \xrightarrow{N} H$						
Entry	R	% Inhib. 3 μM	% Inhib. 30 μM			
Benserazide	OH 2 3 OH 5 4 OH	34 ± 10	80 ± 8			
4	H	NA	27 ± 19			
7a	ОН	NA	32 ± 4			
7 b	ОН	NA	NA			
7 c	ОН	NA	NA			
7 d	ОН	19 ± 12	78.2 ± 4			
7e	ОН	NA	40 ± 2			
7 f	НО	NA	45 ± 6			
7g	НО	32 ± 12	74 ± 5			

 $^{^{}a}$ NA = no activity.

8f

Table 2 Inhibition of PilB with aldimine derivatives

$HO \longrightarrow NH_2$ $N = R$	
% Inhib. 3 μM	% I

NH_2 H					
Entry	R	% Inhib. 3 μM	% Inhib. 30		
8a ^a	HO N=sort	NA	23 ± 21		
8b	HO N=orod	NA	18 ± 9		
8c	N=sort	NA	2 ± 3		
8d	HO HO	NA	84 ± 4		
8e	HO OH	NA	69 ± 10		

8e NA
$$69 \pm 10$$

 40 ± 26

 80 ± 16

HO
$$65 \pm 5$$

$$86 \pm 3$$

8h N
$$\rightarrow$$
 NA 67 \pm 4

8i NA
$$14 \pm 4$$

8j HO
$$35 \pm 9$$
 84 ± 2
8k 71 ± 4 91 ± 8

Table 2 (Contd.)

R

μΜ

Entry

their respective *N*-Boc amino acid methyl ester **9a–c** were treated with hydrazine to provide hydrazide intermediates **9aa–ac** and subsequently condensed with 2,6-dihydroxybenzaldehyde to generate intermediates **10a–c** after purification by column chromatography. Deprotection using hydrochloric acid afforded final compounds **11a–c** with E: Z ratios ranging from 3: 2 to 4: 1.

In vitro screen of benserazide analogues

With a library of compounds in hand, we surveyed their activity using a malachite green assay to probe the activity of CtPilB. In this assay, PilB activity is measured by the increase in absorbance at 620 nm as a function of phosphate concentration. Thus, a PilB inhibitor would result in decreases in absorbance. Briefly, CtPilB (3.3 nM), in the presence of ATP (1 mM), and putative inhibitor was incubated at 54 °C for 30 minutes in 96 well plates. The reaction was quenched using 30% TCA, with 10 mM malachite green then added, and absorbance at 620 nm was measured using a SpectraMax M5 microplate reader at room temperature. The results are shown in Table 1. In this assay, compounds were assayed at 3 and 30 µM to provide a dose-dependent assessment of inhibition using benserazide as a benchmark. Removal of the aryl ring in 4 led to a drastic loss in activity. Thus, we investigated mono- and di-hydroxy phenyl substitutions. Ortho, meta, and para analogues 7a-c were essentially inactive although 7a had slight activity at 30 μM. Keeping the ortho-hydroxy group constant and adding a second hydroxyl around the ring (7d-g) revealed that the 2,3and 2,6-dihydroxy substitutions had equal potency at 3 and 30 μM. Our initial investigations indicated the preference for the 2 and 6 positions of the phenyl ring.

^a Compound dissolved in 50% glycerol solution. NA = no activity.

11c

Table 3 Amino acid scope

	R N	O HO OH	
Entry	R	% Inhib.3 μM	% Inhib.30 μM
11a	H	55 ± 8	92 ± 5

 26 ± 11

 90 ± 1

 87 ± 4

 97 ± 11

To improve the activity of the benserazide analogues, we investigated the effect of conformational restriction by introduction of a rigid double bond between the hydrazine and benzylic carbon of these derivatives (8a-I, Table 2). Monosubstitutions (8a-c) exhibited a general lack of activity against PilB. Comparing disubstituted compounds 8d-i, an increase in overall inhibitory activity was observed. Among the disubstituted hydroxy analogies, compounds 8f and 8g are more potent than the rest. Due to 8g trending in a more potent direction than 8f, we investigated the effect of the H-donor and steric effect in 8h with a 6-methoxy group; unfortunately, this was less potent. However, the 2-OH substituent appears to be important as a drastic decrease in activity was observed with compound 8i.

We next investigated the effect of a third hydroxyl group (8j-k). Compound 8j, which is a direct benserazide analogue, exhibited similar inhibitory activity, with 35% and 84% inhibition at 3 and 30 μ M, respectively. Exchange of the *para* hydroxy for a second *ortho* hydroxy (8k) exhibited high potency against PilB with an inhibition at 3 and 30 μ M of 71% and 91%, respectively. To confirm the deleterious effect of methylating the hydroxy moieties, analog 8l was synthesized and essentially had no activity against PilB.

With an understanding of the effect of hydroxy substitutions on the aryl ring, we next investigated the amino acid moiety and synthesized compounds 11a–c (Table 3). Substitution of glycine for serine (11a) had similar activity compared to 8g. Interestingly, addition of a methyl group in alanine had a negative impact on the activity. Exchange of the serine moiety for cysteine, which exchanges an alcohol for a thiol (8g to 11c) caused significant increase in with 90% inhibition at $3~\mu M$.

With the results in hand, we selected the most potent compounds from the series, *i.e.*, **8g**, **8k**, and **11c** for a doseresponse curve at concentrations ranging from 0.01–50 μ M using benserazide as a control (Fig. 3). Under these conditions, benserazide had an IC₅₀ of 3.68 μ M whereas **8g**, **8k**, and **11c** had 1.32, 1.06, and 0.58 μ M, respectively. Our results confirm that these analogues are more potent than benserazide in our screen. Indeed, the rank order of potency is **11c** > **8k** > **8g**, with **11c** being 6-fold more potent than benserazide.

We next performed an apyrase assay to evaluate the selectivity of our most potent compounds for CtPilB ATPase activity. Each compound was tested at varying concentrations up to 64 μ M (approximately 110 fold higher concentration relative to IC₅₀ of **11c**) to determine inhibitory effect on apyrase, which serves as a non-specific ATPase control. As shown in Fig. 4, no

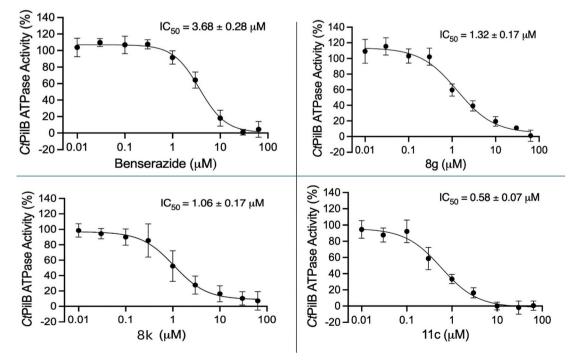


Fig. 3 IC_{50} graphs of selected compounds.

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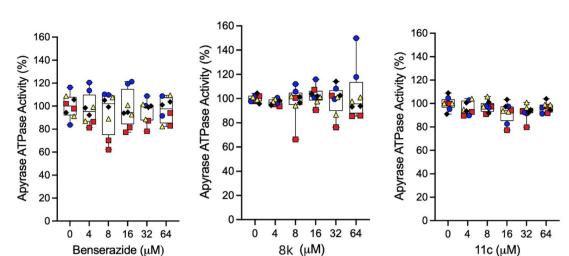


Fig. 4 Inhibition of ATPase apyrase by select compounds.

significant inhibition of apyrase activity at any tested concentration of the compound (p-value > 0.05) was observed. These results indicate that the compounds do not have non-selective ATPase activity, thereby supporting selectivity for CtPilB ATPase.

Each color set represents a biological experiment performed in duplicate. There is no significant difference in apyrase ATPase activity across varying concentrations of (a) benserazide, (b) 8k, and (c) 11c. (p-Values > 0.05 for all comparisons).

Conclusion

This study investigated the underlying structural motifs in benserazide that lead to its potency for PilB inhibition. Our structure-activity relationship study identified key functionalities that led to improved inhibitory activity. In general, we found that trihydroxy > dihydroxy > mono hydroxy substitution in the aryl ring with preference for bis-ortho hydroxy substitutions. In addition, the introduction of a conformationally restricted hydrazone double bond led to significant increase in the activity of compounds. On the amino acid moiety, we found that an exchange of alcohol to a thiol group led to improved potency as exemplified by 11c (IC₅₀ = $0.58 \mu M$). We further demonstrate the lack of activity against an unrelated ATPase apyrase, suggesting selectivity toward PilB. Our studies highlight the potential of targeting PilB and related enzymes as potential anti-virulence factors. These studies are on-going and will be disclosed in due course.

Experimental

General chemistry procedures

All chemicals, reagents, and solvents were purchased from commercially available sources as reagent-grade products and used without further purification. Yields reported are for purified products and might not be optimized. Flash silica gel chromatography was performed using SiliaFlash P60 40-63 µm, 60 Å. Thin-layer chromatography was performed to determine the reaction progress utilizing Silicycle aluminum backed silica gel F-

254 plates. An Agilent 400 MR 400 MHz or a Varian Inova 400 MHz were used for ¹H spectroscopic experiments. A Bruker Avance II 500 MHz was predominately utilized for 13C NMR spectroscopic experiments unless stated otherwise. ¹H and ¹³C NMR spectra are referenced to an internal standard (methanol d_4), and all chemical shifts are reported in δ ppm. NMR spectra characterizations are presented as follows: chemical shift, multiplicity (brs = broad singlet, s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets, dq = doublet of quartets, tt = triplet of triplets, qd = quartet of doublets, and m = multiplet), coupling constants (Hz), and integration. High-resolution mass spectroscopy (HRMS) was performed on a Thermo Electron TSQ triple quadrupole mass spectrometer equipped with an ESI source. Characterization of 8d, 8f, and 8i matches previously reported spectra and E: Z ratios of all compounds were determined by analogy to these compounds in ¹H NMR.³² All compounds tested are >95% pure by ¹H NMR/¹³C NMR or HPLC, unless otherwise noted.

ATPase assays and IC₅₀ determination

Two ATPases were used in this study. For the T4P assembly ATPase, the N-terminally truncated CtPilB19,31 was used to analyze the inhibitory activity of newly synthesized compounds in this study. Apyrase,33 an alternative ATPase purchased from Sigma, was used to examine the specificity of PilB inhibitors. The ATPase activity of CtPilB was analyzed as previously described^{19,31} in a volume of 60 µl with 1 mM of ATP, 0.034% malachite green, 10 mM ammonium molybdate, 1 N HCl, 3.4% ethanol and 3.3 nM of the N-terminally truncated CtPilB hexamer for 30 minutes at 54 °C. The activity of apyrase was analyzed in 20 mM MES (2-(N-morpholino) ethanesulfonic acid), 50 mM NaCl, 5 mM CaCl₂, 1 mM DTT (dithiothreitol).³³ The reactions were conducted with 6.7×10^{-7} U μl^{-1} of apyrase and 1 mM of ATP in 60 µl volume at 37 °C for 30 minutes. 30% TCA was used to terminate all enzymatic reactions, which were then followed by the malachite green-based assays as described previously.19,31 To perform this assay, data was analyzed by GraphPad Prism for the calculation of IC50.

With the exception of compound 8a, all the compounds in this study were dissolved in DMSO (Thermo fisher) and all enzymatic reactions containing 2% DMSO with or without a test compound. 8a was dissolved in 50% glycerol and all enzymatic reactions with this compound and its controls had a final concentration of glycerol at 2%. Benserazide used in this study was from Tokyo Chemical Industry Co.

Protein expression and purification

The truncated *CtP*ilB variant (without the first 139 residues at the N terminus) were purified as previously described.^{19,31}

General procedure 1. To a well stirred solution of N-Boc-L-serine methyl ester (1) (5.60 g, 25.5 mmol) in CH_2Cl_2 (20 mL) were added 2,2-dimethoxypropane (16 mL, 127 mmol) and p-toluenesulfonic acid monohydrate (485 mg, 2.5 mmol) at room temperature. The resulting mixture was subsequently stirred for 12 h. After the reaction was completed (as monitored by TLC analysis), the reaction mixture was quenched with saturated aqueous solution NaHCO₃ and extracted with CH_2Cl_2 (3 \times 20 mL). The combined organic layers were dried over magnesium sulfate, and then concentrated *in vacuo* to afford 3-(*tert*-butyl) 4-methyl 2,2-dimethyloxazolidine-3,4-dicarboxylate (2, 6.10 g, 92% yield) as colorless oil and was used without further purification.

General procedure 2. Intermediate 2 (5.33 g, 20.6 mmol) was dissolved in 30 mL methanol in a round bottom flask. To the stirring solution was added hydrazine monohydrate (10 mL, 206 mmol) and the solution was allowed to stir at room temperature for 12 h. After the reaction was completed as monitored by TLC with ninhydrin stain, the solvent was removed under reduced pressure and the resulting oil was resuspended in CH₂Cl₂ (20 mL). The solution was washed with 20 mL water and the organic layer was dried over sodium sulphate. The solvent was then removed under reduced pressure to afford *tert*-butyl 4-(hydrazinecarbonyl)-2,2-dimethyloxazolidine-3-carboxylate (3, 5.298 g, 99% yield) as a colorless semisolid and was subsequently used without further purification.

General procedure 3. Intermediate 3 (500 mg, 1.93 mmol) was dissolved in 10 mL methanol in a 6 dram vial. Sodium sulphate (232 mg, 1.93 mmol) was added and the heterogenous mixture was set to stir at room temperature. To the stirring mixture was added aldehyde (2.12 mmol) and the mixture was capped and allowed to stir for 16 h. After the reaction was deemed complete by TLC, the mixture was loaded onto Celite and subjected to column chromatography to afford intermediates 4 as solids.

General procedure 4. Intermediates 4 (150 mg, 0.413 mmol) was dissolved in anhydrous methylene chloride (10 mL) in a flame-dried 6-dram vial. Sodium borohydride (156 mg, 4.13 mmol) and then added and the heterogenous mixture was vigorously stirred for 16 hours. Upon completion of reaction as monitored by TLC, water (5 mL) was added and the organic washed. The organic layer was extracted and the remaining aqueous layer was washed with methylene chloride (3 \times 5 mL). The combined organic layers were dried with sodium sulphate and the solvent removed. The resulting crude mixture was

subjected to column chromatography (0-80% ethyl acetate in hexanes) to afford intermediates 6 as solids.

General procedure 5. Intermediates 3, 5, 6, or 10 (100 mg) were added to a 2-dram vial and dissolved in 1 mL of methylene chloride. The solution was set to stir, and HCl (1.5 mL, 4 M in dioxane) was subsequently added. The solution was allowed to stir for 12 hours upon which a precipitate forms. Methanol (2 mL) is added to the resulting heterogenous solution to form a homogenous solution. The solution is then transferred to a 6-dram vial and diethyl ether (15 mL) is then added causing a precipitate to form. The solvent is then removed and the precipitate is then triturated in fresh diethyl ether 3 times. The solvent is then removed *via* rotary evaporation to yield compounds 4, 7, 8, or 11 as hydrochloride salts.

3-(*Tert***-butyl) 4-methyl (***R***)-2,2-dimethyloxazolidine-3,4-dicarboxylate (2).** Synthesized by general procedure **1.** Colorless oil, 92% yield.

¹H NMR (400 MHz, CD₃OD) δ 4.15–4.12 (m, 1H), 3.86–3.78 (m, 1H), 3.71–3.63 (M, 1H), 3.42 (brs, 3H), 1.32 (brs, 3H), 1.19 (brs, 6H), 1.08 (brs, 6H). ¹³C NMR (126 MHz, MeOD) δ 171.7, 151.6, 94.6, 80.2, 65.9, 59.2, 51.5, 27.1, 24.0, 23.1.

Tert-butyl (*R*)-4-(hydrazinecarbonyl)-2,2-dimethyloxazolidine-3-carboxylate (3). Synthesized by general procedure 2. Colorless oil, 99% yield.

 1 H NMR (400 MHz, CD₃OD) δ 4.33–4.21 (m, 1H), 4.18–4.12 (m, 1H), 3.98–3.86 (m, 1H). 1.63 (s, 3H), 1.52–1.38 (m, 12H). 13 C NMR (126 MHz, MeOD) δ 172.4, 153.2, 96.1, 81.7, 67.8, 60.3, 28.6, 25.2, 24.6.

(*S*)-2-Amino-3-hydroxypropanehydrazide dihydrochloride (4). Synthesized by general procedure 5. White solid, 99% yield.

¹H NMR (400 MHz, DMSO-d₆) δ 9.91 (brs, 2H), 8.57 (brs, 3H), 5.63 (brs, 1H), 3.97 (t, J = 4.0 Hz, 1H), 3.86–3.78 (m, 2H). ¹³C NMR (126 MHz, DMSO) δ 166.3, 60.2, 53.3. HRMS: (ESI) [M + H]⁺ calc. for C₃H₁₀N₃O₂⁺ 120.0768, observed 120.0769.

Tert-butyl (R,E)-4-(2-(2-hydroxybenzylidene)hydrazine-1-carbonyl)-2,2-dimethyloxazolidine-3-carboxylate (5a). Synthesized by general procedure 3. White solid, 74% yield. Described as a 4:1 E:Z mixture of isomers. Major isomer peak denoted with *.

¹H NMR (400 MHz, CD₃OD) δ 8.32* (s, 1H), 8.22 (s, 1H), 7.55–7.48 (m, 1H), 7.45–7.34* (m, 1H), 7.33–7.21* (m, 1H), 6.94–6.83 (m, 2H), 5.21–5.15 (m, 1H), 4.46–4.39 (m, 1H), 4.39–4.32* (m, 1H), 4.25* (t, J = 8.9 Hz, 1H), 4.08–3.95* (m, 1H), 1.66* (brs, 3H), 1.55* (brs, 3H), 1.50* (brs, 3H), 1.40 (brs, 6H). ¹³C NMR (126 MHz, CD₃OD) δ 169.1*, 168.8, 159.4*, 158.4, 153.9*, 153.0*, 151.4, 151.2*,133.0*, 132.9, 132.7, 131.6, 131.5, 129.53, 129.3, 120.8, 120.6, 119.3, 117.7*, 117.2, 96.2*, 95.8, 82.4, 81.9*, 81.4, 67.9*, 67.6, 67.4, 67.3, 60.5*, 59.6, 59.2, 30.7, 28.6*, 26.4, 26.1, 25.7, 25.4, 25.1*, 24.8*, 24.7, 24.6. HRMS: (ESI) [M – H][–] calc. for C₁₈H₂₄N₃O₅[–] 362.1721, observed 362.1721.

(R,E)-1-(3-(Tert-butoxycarbonyl)-2,2-dimethyloxazolidine-4-carbonyl)-2-(3-hydroxybenzylidene)hydrazin-1-ide (5b). Synthesized by general procedure 3. White solid, 66% yield. Compound is reported as a 1:1 E:Z mixture of isomers. Major isomer peaks denoted with *.

¹H NMR (500 MHz, (CD₃)₂CO) δ 8.06 (s, 1H)*, 7.87 (brs, 1H), 7.34–7.07 (m, 3H)*, 6.88–6.83 (m, 1H)*, 5.33–5.27 (m, 1H), 4.46–

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4.35 (m, 1H)*, 4.28–4.22 (m, 1H)*, 4.07–3.96 (m, 1H)* 1.68 (brs, 1H)*, 1.67 (brs, 1H), 1.56 (brs, 3H), 1.55 (brs, 3H)*, 1.51 (brs, 3H)*, 1.40 (s, 6H)*. 13 C NMR (126 MHz, MeOD) δ 173.5*, 172.9, 169.8, 163.4, 159.0*, 153.9, 153.3*, 153.0, 150.0*, 146.1*, 136.8*, 136.5, 130.9*, 120.7*, 120.1, 119.0, 118.5*, 114.2, 113.7*, 96.2, 96.2*, 95.9, 82.1, 81.8, 81.4*, 67.9, 67.8*, 67.6, 67.5, 61.5, 60.6*, 59.8, 59.4, 28.7, 28.6*, 26.3, 26.1, 25.7, 25.5, 25.2*, 25.1, 24.8, 24.8*. HRMS: (ESI) [M - H]^- calc. for $C_{18}H_{24}N_3O_5^-$ 362.1721, observed 362.1717.

(R,E)-1-(3-(Tert-butoxycarbonyl)-2,2-dimethyloxazolidine-4-carbonyl)-2-(4-hydroxybenzylidene)hydrazin-1-ide (5c). Synthesized by general procedure 3. White solid, 69% yield. Compound is reported as 3:2 E:Z mixture of isomers. Major isomer pears denoted with *.

¹H NMR (400 MHz, (CD₃)₂CO) δ 8.02 (brs, 1H)*, 7.82 (brs 1H), 7.64–7.47 (m, 2H), 7.52–7.46 (m, 2H), 6.84–6.77 (m, 2H)*, 5.30–5.24 (m, 1H), 4.44–4.30 (m, 1H)*, 4.26–4.19 (m, 1H)*, 4.05–3.94 (m, 1H)*, 1.67–1.64 (m, 3H)*, 1.57–1.52 (m, 3H)*, 1.50 (brs, 3H)*, 1.39 (brs, 6H)*. ¹³C NMR (126 MHz, (CD₃)₂CO) δ 173.3*, 173.0, 172.8, 169.4*, 161.4, 161.0, 160.9*, 153.9, 153.3*, 153.1, 150.3, 146.4*, 130.6*, 129.9, 126.8, 126.5, 116.6*, 96.2, 96.1*, 95.9, 82.3, 82.1, 81.8, 81.3*, 67.9, 67.8*, 67.7, 61.5, 60.6*, 59.8, 59.4, 28.6*, 26.4, 26.1, 25.8, 25.5, 25.2*, 25.1, 24.8, 24.8*. HRMS: (ESI) [M + Na]⁺ calc. for C₁₈H₂₆N₃O₅⁺ 364.1867, observed 364.1844.

Tert-butyl (R,E)-4-(2-(2,3-dihydroxybenzylidene)hydrazine-1-carbonyl)-2,2-dimethyloxazolidine-3-carboxylate (5d). Synthesized by general procedure 3. White solid,88% yield. Compound is reported as a 5:1 E:Z mixture of isomers. Major isomer peaks denoted with *.

¹H NMR (400 MHz, (CD₃)₂CO) δ 8.28 (s, 1H)*, 8.20 (s, 1H), 7.00–6.95 (m, 1H), 6.90–6.82 (m, 2H)*, 6.78–6.72 (m, 1H)*, 5.20–5.14 (m, 1H), 4.46–4.39 (m, 1H), 4.39–4.31 (m, 1H)*, 4.29–4.21 (m, 1H)*, 4.06–3.95 (m, 1H)*, 1.66 (s, 3H)*, 1.54 (s, 3H)*, 1.50 (s, 3H)*, 1.39* (s, 6H)*. ¹³C NMR (126 MHz, MeOD) δ 171.6, 171.4*, 170.8, 167.7*, 167.4, 152.5, 151.8, 151.6*, 150.3, 150.2*, 146.0*, 145.4*, 145.3, 121.1, 121.0, 119.4, 119.2, 119.1, 117.9, 117.4, 117.4, 116.8, 94.8*, 94.8, 94.5, 94.5, 81.0, 80.5*, 80.1, 66.5*, 66.1, 66.1, 60.1, 59.1*, 58.3, 57.8, 29.3, 27.2*, 25.0, 24.7, 24.3, 24.0, 23.8, 23.7*, 23.4*, 23.3. HRMS: (ESI) [M + H]⁺ calc. for C₁₈H₂₆N₃O₆⁺ 380.1816, observed 380.1824.

(R,E)-N'-(2,4-Dihydroxybenzylidene)-2,2-dimethyloxazolidine-4-carbohydrazide (5e). Synthesized by general procedure 3. Off-white solid, 57% yield. Compound is reported as 5:1 E:Z mixture of isomers. Major isomer peaks denoted with *.

¹H NMR (500 MHz, (CD₃)₂CO) δ 8.19 (s, 1H)*, 8.08 (s, 1H), 7.28–7.23 (m, 1H), 7.20–7.13 (m, 1H)*, 6.39–6.29 (m, 2H)*, 5.13–5.08 (m, 1H), 4.43–4.37 (m, 1H), 4.35–4.29 (m, 1H)*, 4.23 (t, J = 8.8 Hz, 1H)*, 4.05–3.95 (m, 1H)*, 1.66 (s, 3H)*, 1.54 (s, 3H)*, 1.50 (s, 3H)*, 1.39 (s, 6H)*. ¹³C NMR (126 MHz, MeOD) δ 168.7*, 168.5, 162.7*, 162.6, 162.5, 161.4*, 160.4, 153.9, 153.0*, 152.1, 152.0*, 147.7, 133.4, 133.4*, 131.9, 112.2, 111.6*, 109.1, 108.9, 108.9*, 103.8*, 103.6, 96.2, 96.2*, 95.9, 82.4, 81.9*, 81.5, 67.9*, 67.5, 61.5, 60.5*, 59.6, 59.2, 28.7, 28.6, 28.6*, 26.4, 26.1, 25.7, 25.4, 25.2*, 25.1, 24.8*, 24.7, 24.6. HRMS: (ESI) [M + H–C₅H₉O₂]⁺

calc. for $C_{13}H_{18}N_3O_4^{\ +}$: 280.1292, observed 280.1294. HRMS represents de-BOC structure.

Tert-butyl (R,E)-4-(2-(2,5-dihydroxybenzylidene)hydrazine-1-carbonyl)-2,2-dimethyloxazolidine-3-carboxylate (5f). Synthesized by general procedure 3. Off-white solid, 75% yield. Compound is reported as 3:1 E:Z mixture of isomers. Major isomer peaks denoted with *.

¹H NMR (500 MHz, (CD₃)₂CO) δ 8.25 (s, 1H)*, 8.17 (s, 1H), 7.00–6.96 (m, 1H), 6.88–6.81 (m, 1H)*, 6.79–6.71 (m, 2H)*, 5.22–5.16 (m, 1H), 4.45–4.39 (m, 1H), 4.38–4.32 (m, 1H)*, 4.25 (t, J = 8.5 Hz, 1H)*, 4.06–3.96 (m, 1H)*, 1.66 (s, 3H)*, 1.54 (s, 3H)*, 1.50 (s, 3H)*, 1.40 (s, 6H)*. ¹³C NMR (126 MHz, MeOD) δ 171.6, 170.9, 167.8*, 167.5, 152.5, 151.9, 151.6, 151.0*, 150.2, 150.0, 149.8*, 149.4, 149.2*, 144.0, 143.8*, 119.6, 119.2*, 119.1, 118.8, 118.7, 118.1, 116.9*, 116.6, 114.8, 114.6*, 112.6, 112.4*, 94.8*, 94.8, 94.5, 94.5, 81.0, 80.8, 80.5*, 80.1, 66.5*, 66.2, 66.1, 66.0, 59.1*, 58.3, 57.9, 29.3, 27.3, 27.2, 27.2*, 24.9, 24.7, 24.3, 24.1, 23.8, 23.7*, 23.4*, 23.3. HRMS: (ESI) [M + H–C₅H₉O₂][†] calc. for C₁₃H₁₈N₃O₄[†]: 280.1292, observed 280.1294. HRMS represents de-BOC structure.

Tert-butyl(R,E)-4-(2-(2,6-dihydroxybenzylidene)hydrazine-1-carbonyl)-2,2-dimethyloxazolidine-3-carboxylate (5g). Synthesized by general procedure 3. Off-white solid, 83% yield. Compound is reported as 5:1 E:Z mixture of isomers. Major isomer peaks denoted with *.

¹H NMR (500 MHz, (CD₃)₂CO) δ 8.73 (s, 1H)*, 8.57 (s, 1H), 7.08 (t, J = 9.4 Hz, 1H)*, 6.36 (d, J = 8.1 Hz, 2H)*, 5.06–5.02 (m, 1H), 4.44–4.39 (m, 1H), 4.36–4.31 (m, 1H)*, 4.24 (t, J = 8.9 Hz, 1H)*, 4.05–3.95 (m, 1H)*, 1.66 (s, 3H)*, 1.54 (s, 3H)*, 1.49 (brs, 3H)*, 1.40 (s, 6H)*. ¹³C NMR (126 MHz, MeOD) δ 167.4*, 167.2, 158.7*, 158.3, 152.5, 151.6*, 147.3*, 145.0, 132.6*, 132.4, 106.2*, 105.9, 94.8*, 94.5, 81.0, 80.5*, 66.5*, 66.1, 60.1, 59.1*, 58.2, 57.7, 29.3, 27.2*, 24.7, 24.3, 23.7*, 23.4*. HRMS: (ESI) [M + H]⁺ calc. for $C_{18}H_{26}N_3O_6^+$ 380.1816, observed 380.1818.

Tert-butyl(R,E)-4-(2-(2-hydroxy-6-methoxybenzylidene) hydrazine-1-carbonyl)-2,2-dimethyloxazolidine-3-carboxylate (5h). Synthesized by general procedure 3. Off-white solid, 89% yield. Compound is reported as 4:1 E:Z mixture of isomers. Major isomer peaks denoted with *.

¹H NMR (500 MHz, (CD₃)₂CO) δ 8.70 (s, 1H)*, 8.54 (s, 1H), 7.26–7.19 (m, 1H)*, 6.55–6.34 (m, 2H)*, 5.06–5.01 (m, 1H), 4.44–4.39 (m, 1H), 4.36–4.30 (m, 1H)*, 4.24 (t, J=8.4 Hz, 1H)*, 4.05–3.94 (m, 1H)*, 3.84 (s, 3H)*, 1.66 (s, 3H)*, 1.54 (s, 3H)*, 1.49 (s, 3H)*, 1.39 (s, 6H)*. ¹³C NMR (126 MHz, MeOD) δ 168.8*, 168.6, 160.9, 160.5*, 153.8, 152.9*, 148.1*, 145.8, 134.0*, 133.9, 110.6*, 110.3, 107.9*, 102.7, 102.3*, 96.2*, 95.9, 82.4, 81.9*, 67.8*, 67.5, 60.5*, 56.4*, 28.6*, 26.1, 25.7, 25.1*, 24.8*. HRMS: (ESI) [M + H–C₅H₉O₂]⁺ calc. for C₁₄H₂₀N₃O₄⁺: 294.1448, observed 294.1438. HRMS represents de-BOC structure.

Tert-butyl(R,E)-4-(2-(3,4-dihydroxybenzylidene)hydrazine-1-carbonyl)-2,2-dimethyloxazolidine-3-carboxylate (5i). Synthesized by general procedure 3. Off-white solid, 43% yield. Compound is reported as 3:2 E:Z mixture of isomers. Major isomer peaks denoted with *.

¹H NMR (500 MHz, (CD₃)₂CO) δ 7.96 (s, 1H)*, 7.79 (s, 1H), 7.34–7.30 (m, 1H)*, 7.20–7.18 (m, 1H), 7.04–7.00 (m, 1H)*, 6.96–6.93 (m, 1H), 6.83–6.77 (m, 1H)*, 5.31–5.25 (m, 1H), 4.44–4.33

 $\begin{array}{l} (m,1H)^*,\,4.29-4.23\;(m,1H)^*,\,4.09-3.98\;(m,1H)^*,\,1.69\;(s,3H)^*,\\ 1.68\;(s,3H),\,1.58\;(s,3H),\,1.57\;(s,3H)^*,\,1.53\;(s,3H),\,1.52\;(s,3H)^*,\\ 1.42\;(s,\,6H)^*. \ ^{13}{\rm C}\;\;{\rm NMR}\;\;(126\;\;{\rm MHz},\;\;{\rm MeOD})\;\;\delta\;\;173.3^*,\;\;172.7,\\ 169.4^*,\,169.2,\,153.9,\;153.4^*,\;153.1,\;150.4^*,\;149.7,\;149.3,\;149.2,\\ 146.9^*,\,146.6,\,146.6,\,127.4^*,\,127.1,\,122.7^*,\,121.9,\;116.2^*,\,114.0^*,\\ 113.5,\,96.2,\,96.1^*,\,95.9,\,82.3,\,82.1,\,81.8,\,81.4^*,\,67.9,\,67.9^*,\,67.7,\\ 67.5,\,60.5^*,\,59.8,\,59.4,\,28.6^*,\,26.3,\,26.1,\,25.8,\,25.5,\,25.2^*,\,25.1,\\ 24.8,\,24.8^*.\;{\rm HRMS}:\;({\rm ESI})\left[{\rm M}+{\rm H-C}_5{\rm H}_9{\rm O}_2\right]^+ {\rm calc.}\;{\rm for}\;{\rm C}_{13}{\rm H}_{18}{\rm N}_3{\rm O}_4^{+}{\rm :}\\ 280.1292,\;\;{\rm observed}\;\;280.1279.\;\;{\rm HRMS}\;\;{\rm represents}\;\;{\rm de\text{-BOC}}\;\;{\rm structure}. \end{array}$

Tert-butyl(R,E)-2,2-dimethyl-4-(2-(2,3,4-trihydroxybenzylidene)hydrazine-1-carbonyl)oxazolidine-3-carboxylate (5j). Synthesized by general procedure 3. White solid, 66% yield. Compound is reported as 9:1 E:Z mixture of isomers. Major isomer peaks denoted with *.

¹H NMR (500 MHz, (CD₃)₂CO) δ 8.15 (s, 1H)*, 8.04 (s, 1H), 6.74–6.67 (m, 1H)*, 6.43–6.38 (m, 1H)*, 5.13–5.08 (m, 1H), 4.44–4.30 (m, 1H)*, 4.24 (t, J = 8.7 Hz, 1H)*, 4.05–3.95 (m, 1H)*, 1.66 (s, 3H)*, 1.54 (s, 3H)*, 1.50 (s, 3H)*, 1.39 (s, 6H)*. ¹³C NMR (126 MHz, MeOD) δ 173.0*, 172.3, 168.7*, 153.8, 153.0, 152.7*, 150.2*, 149.0, 148.5*, 147.8, 133.9*, 123.3*, 122.4, 112.5, 112.0*, 108.9, 108.7*, 96.2*, 95.9, 82.4, 81.9*, 67.9*, 67.5, 67.4, 67.2, 61.5, 60.5*, 59.6, 59.1, 30.7, 28.6*, 26.1, 25.7, 25.1*, 24.8*. HRMS: (ESI) [M + H]⁺ calc. for C₁₈H₂₆N₃O₇⁺: 396.1765, observed 396.1763.

Tert-butyl(R,E)-2,2-dimethyl-4-(2-(2,3,6-trihydroxybenzylidene)hydrazine-1-carbonyl)oxazolidine-3-carboxylate (5k). Synthesized by general procedure 3. Off-white solid, 3% yield. Compound is reported as 5:1 E:Z mixture of isomers. Major isomer peaks denoted with *.

¹H NMR (500 MHz, (CD₃)₂CO) δ 8.70 (s, 1H)*, 8.55 (s, 1H), 6.73 (d, J = 8.7 Hz, 1H)*, 6.20 (d, J = 8.6 Hz, 1H)*, 4.34 (t, J = 6.5 Hz, 1H), 4.24 (t, J = 9.4 Hz, 1H)*, 4.06–3.95 (m, 2H)*, 1.55 (brs, 3H)*, 1.50 (brs, 4H)*, 1.45 (brs, 2H), 1.40 (brs, 6H)*. ¹³C NMR (126 MHz, MeOD) δ 168.8*, 168.6, 153.0, 152.3*, 149.0*, 147.7, 138.7*, 120.1*, 120.0, 107.7, 106.0*, 96.2*, 95.9, 82.4, 81.9*, 67.9*, 67.5, 60.5*, 28.6*, 26.1, 25.7, 25.1*, 24.8*. HRMS: (ESI) [M - H]⁻ calc. for $C_{18}H_{24}N_3O_7^{+}$: 394.1620, observed 394.1618.

Tert-butyl(R,E)-2,2-dimethyl-4-(2-(2,3,4-trimethoxybenzylidene)hydrazine-1-carbonyl)oxazolidine-3-carboxylate (5l). Synthesized by general procedure 3. Off-white solid, 72% yield. Compound is reported as 3:2 E:Z mixture of isomers. Major isomer peaks denoted with *.

¹H NMR (500 MHz, (CD₃)₂CO) δ 8.39 (s, 1H)*, 8.19 (s, 1H), 7.82 (m, 1H)*, 7.59 (1H), 6.87 (m, 1H)*, 5.32–5.27 (m, 1H), 4.43–4.33 (m, 1H)*, 4.28–4.24 (m, 1H)*, 4.08–3.83 (m, 11H)*, 1.69 (s, 3H)*, 1.68 (s, 3H), 1.58 (s, 3H), 1.57 (s, 3H)*, 1.53 (s, 3H), 1.52 (s, 3H)*, 1.42 (s, 6H)*. ¹³C NMR (126 MHz, MeOD) δ 173.4*, 172.8, 169.5*, 169.2, 157.5*, 157.4, 157.1, 157.0, 154.6*, 154.4, 153.9, 153.3, 153.0, 145.7*, 145.6, 143.2, 143.1*, 142.0*, 122.9*, 122.1, 122.0, 121.6, 121.2, 109.5, 109.4*, 96.2, 96.1*, 95.9, 82.3, 82.1, 81.8*, 81.3, 67.9*, 67.8, 67.7, 67.5, 62.4, 62.4, 61.3*, 60.6, 59.8, 59.3, 56.6*, 28.7, 28.6*, 26.3, 26.1, 25.8, 25.5, 25.2, 25.1*, 24.8*. HRMS: (ESI) [M + H]⁺ calc. for C₂₁H₃₂N₃O₇⁺: 438.2235, observed 438.2230.

Tert-butyl(*R*)-4-(2-(2-hydroxybenzyl)hydrazine-1-carbonyl)-2,2-dimethyloxazolidine-3-carboxylate (6a). Synthesized by general procedure 4. Off-white solid, 96% yield.

¹H NMR (500 MHz, (CD₃)₂CO) δ 7.16–7.07 (m, 2H), 6.81–6.73 (m, 2H), 4.31–4.17 (m, 1H), 4.12–4.05 (m, 1H), 4.05–3.94 (m, 1H), 1.60 (brs, 3H), 1.48 (brs, 6H), 1.40 (brs, 3H). ¹³C NMR (126 MHz, MeOD) δ 171.4, 157.6, 153.2, 131.5, 130.0, 124.2, 120.4, 116.4, 96.1, 81.8, 67.9, 60.2, 52.8, 28.6, 25.2, 24.5. HRMS: (ESI) [M + H]⁺ calc. for C₁₈H₂₆N₃O₅⁻: 364.1878, observed 364.1780.

Tert-butyl(*R*)-4-(2-(3-hydroxybenzyl)hydrazine-1-carbonyl)-2,2-dimethyloxazolidine-3-carboxylate (6b). Synthesized by general procedure 4. Off-white solid, 87% yield. Compound is described with rotamers. Major peaks identified with *.

¹H NMR (500 MHz, (CD₃OD)) δ 6.75–6.68 (m, 1H), 6.66–6.57 (m, 2H), 4.31–3.75 (m, 5H), 1.61 (brs, 3H), 1.43 (brs, 6H), 1.40 (brs, 6H). ¹³C NMR (126 MHz, MeOD) δ 171.8*, 158.7*, 154.1, 154.0, 153.5, 153.2*, 140.2*, 130.5*, 121.2, 121.0*, 117.0, 116.7*, 115.5*, 96.1*, 95.7, 94.9, 94.7, 82.3, 81.9, 81.7*, 81.2, 67.9*, 67.3, 66.1*, 65.8, 62.3*, 62.0, 60.2*, 59.8, 56.4*, 56.2, 28.7, 28.6*, 27.8, 27.0, 25.2*, 24.5*, 23.2. HRMS: (ESI) [M + H]⁺ calc. for C₁₈H₂₆N₃O₅⁻: 364.1878, observed 364.1781.

Tert-butyl(R)-4-(2-(4-hydroxybenzyl)hydrazine-1-carbonyl)-2,2-dimethyloxazolidine-3-carboxylate (6c). Synthesized by general procedure 4. Off-white solid, 92% yield. Compound is reported at 1:1 with rotamers.

¹H NMR (400 MHz, (CD₃OD)) δ 7.19 (d, J = 8.1 Hz, 2H), 6.78 (d, J = 8.7 Hz, 2H), 4.33–4.20 (m, 1H), 4.14–4.07 (m, 1H), 3.92–3.75 (m, 3H), 1.67–1.58 (m, 3H), 1.54–1.46 (m, 6H), 1.45–1.37 (m, 6H). ¹³C NMR (126 MHz, MeOD) δ 171.6*, 158.1*, 153.1*, 131.4*, 129.2*, 116.1*, 96.0*, 95.7, 82.3, 81.7*, 67.9*, 67.3, 60.2*, 56.0*, 55.7, 28.6*, 26.4, 25.3*, 24.5*. HRMS: (ESI) [M + H]⁺ calc. for C₁₈H₂₈N₃O₅⁺: 366.2023, observed 366.2022.

Tert-butyl(*R*)-4-(2-(2,3-dihydroxybenzyl)hydrazine-1-carbonyl)-2,2-dimethyloxazolidine-3-carboxylate (6d). Synthesized by general procedure 4. Off-white solid, 77% yield. Compound is reported at 1:1 with rotamers.

¹H NMR (400 MHz, (CD₃OD)) δ 6.76–6.68 (m, 1H), 6.67–6.59 (m, 2H), 4.30–4.16 (m, 1H), 4.13–3.90 (m, 3H), 3.91–3.75 (m, 1H), 1.61 (s, 3H), 1.48 (s, 6H), 1.41 (s, 6H). ¹³C NMR (126 MHz, MeOD) δ 176.3, 175.8, 171.6*, 171.5, 153.9*, 153.7, 153.3, 153.2*, 146.4*, 146.3, 145.9, 145.6*, 145.5, 144.4, 124.4*, 122.0*, 120.4*, 115.9*, 96.1*, 95.8, 95.7, 95.6, 82.3, 81.9, 81.8*, 81.2, 67.9, 67.8*, 67.5, 67.3, 60.2*, 52.9*, 28.6*, 26.4, 26.2, 25.6, 25.3, 25.1, 24.7, 24.5. HRMS: (ESI) [M + H]⁺ calc. for C₁₈H₂₈N₃O₆⁺: 382.1973, observed 382.1974.

Tert-butyl(*R*)-4-(2-(2,4-dihydroxybenzyl)hydrazine-1-carbonyl)-2,2-dimethyloxazolidine-3-carboxylate (6e). Synthesized by general procedure 4. Off-white solid, 68% yield.

¹H NMR (400 MHz, (CD₃OD)) δ 6.91 (d, J = 8.2 Hz, 1H), 6.30 (d, J = 2,4 Hz, 1H), 6.24 (dd, J = 2.3 Hz, 8.1 Hz, 1H), 4.33–4.18 (m, 1H), 4.15–4.04 (m, 1H), 3.97–3.75 (m, 3H), 1.64–1.56 (m, 3H), 1.51–1.44 (m, 6H), 1.43–1.37 (m, 6H). ¹³C NMR (126 MHz, MeOD) δ 170.0, 158.1, 157.2, 151.8, 130.9, 113.9, 106.0, 102.3, 94.7, 80.4, 66.5, 58.9, 51.1, 27.2, 23.9, 23.1. HRMS: (ESI) [M + H]⁺ calc. for C₁₈H₂₈N₃O₆⁺: 382.1973, observed 382.1965.

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Tert-butyl(R)-4-(2-(2,5-dihydroxybenzyl)hydrazine-1carbonyl)-2,2-dimethyloxazolidine-3-carboxylate (6f). Synthesized by general procedure 4. Off-white solid, 89% yield.

¹H NMR (400 MHz, (CD₃OD)) δ 6.64–6.60 (m, 2H), 6.58–6.54 (m, 1H), 4.30-4.17 (m, 1H), 4.13-4.07 (m, 1H), 3.98-3.76 (m, 3H), 1.65-1.56 (m, 3H), 1.49 (s, 6H), 1.40 (s, 6H). ¹³C NMR (126 MHz, MeOD) δ 171.5*, 153.2*, 151.0*, 150.4*, 124.9*, 117.9*, 117.1*, 116.2*, 96.1*, 95.8, 82.4, 81.8*, 67.9*, 67.4, 60.2*, 52.9*, 28.6° , 26.4, 25.3, 25.2° , 24.5° . HRMS: (ESI) [M - H]⁻ calc. for C₁₈H₂₆N₃O₆⁻: 380.1827, observed 380.1813.

Tert-butyl(R)-4-(2-(2,6-dihydroxybenzyl)hydrazine-1carbonyl)-2,2-dimethyloxazolidine-3-carboxylate (6g). Synthesized by general procedure 4. Off-white solid, 89% yield.

¹H NMR (400 MHz, (CD₃OD)) δ 6.92 (t, J = 7.9 Hz, 1H), 6.33 (d, J = 7.9 Hz, 2H), 4.32-4.20 (m, 1H), 4.15-4.05 (m, 3H), 3.94-3.80 (m, 1H), 1.63-1.59 (m, 3H), 1.52-1.47 (m, 6H), 1.44-1.41 (m, 6H). 13 C NMR (126 MHz, MeOD) δ 171.0*, 158.5*, 153.2, 129.8*, 110.9*, 107.6*, 96.0*, 95.7, 82.3, 81.8*, 67.8*, 67.3, $60.2^*, 45.9^*, 28.6^*, 26.3, 25.4, 25.2^*, 24.6^*$. HRMS: (ESI) [M + H]⁺ calc. for C₁₃H₂₀N₃O₄⁺: 282.1448, observed 282.1422.

(R)-2-Amino-3-hydroxy-N'-(2-hydroxybenzyl)propanehydrazide hydrochloride (7a). Synthesized by general procedure 5. Off-white solid, 95% yield. Compound is reported with rotamers. Major rotamer peaks designated with *.

 1 H NMR (400 MHz, (CD₃OD)) δ 7.36–7.27 (m, 2H)*, 6.96–6.87 (m, 2H)*, 4.18 (s, 2H)*, 4.08-4.01 (m, 1H), 4.01-3.94 (m, 1H)*, 3.91–3.82 (m, 1H)*, 3.79–3.72 (m, 1H)*, 3.70–3.62 (m, 1H). ¹³C NMR (126 MHz, CDCl₃) δ 167.1, 157.6, 132.4, 131.7, 131.2, 130.2, 121.2, 120.7, 116.8, 116.4, 61.5, 60.1, 55.1, 52.3. *. HRMS: (ESI) $[M + H]^+$ calc. for $C_{10}H_{16}N_3O_3^+$: 226.1186, observed 226.1181.

(R)-2-Amino-3-hydroxy-N'-(3-hydroxybenzyl)propanehydrazide hydrochloride (7b). Synthesized by general procedure 5. Off-white solid, 87% yield.

¹H NMR (400 MHz, (CD₃OD)) δ 7.30 (t, J = 9.0 Hz, 1H), 7.02– 6.98 (m, 2H), 6.93-6.89 (m, 1H), 4.40 (dd, I = 12.3, 17.1 Hz, 2H), 4.15-4.12 (m, 1H), 3.96-3.91 (m, 1H), 3.89-3.84 (m, 1H). ¹³C NMR (126 MHz, MeOD) δ 165.8, 157.9, 130.4, 130.0, 121.3, 117.2, 116.6, 59.8, 54.3, 53.8. HRMS: (ESI) [M + H]⁺ calc. for $C_{10}H_{16}N_3O_3^+$: 226.1186, observed 226.1107.

(R)-2-Amino-3-hydroxy-N'-(4-hydroxybenzyl)propanehydrazide hydrochloride (7c). Synthesized by general procedure 5. Off-white solid, 74% yield.

¹H NMR (400 MHz, (CD₃OD)) δ 7.36–7.30 (m, 2H), 6.86–6.81 (m, 2H), 4.32-4.24 (m, 2H), 4.09-4.05 (m, 1H), 3.89 (d, J =4.25 Hz, 1H), 3.85 (d, J = 5.8 Hz, 1H). ¹³C NMR (126 MHz, MeOD) δ 167.1, 160.1, 133.3, 121.9, 116.8, 66.9, 61.3, 55.4, 55.1. HRMS: (ESI) $[M + H]^+$ calc. for $C_{10}H_{16}N_3O_3^+$: 226.1186, observed

(R)-2-Amino-N'-(2,3-dihydroxybenzyl)-3-

hydroxypropanehydrazide hydrochloride (7d). Synthesized by general procedure 5. Off-white solid,76% yield.

¹H NMR (400 MHz, (CD₃OD)) δ 6.87–6.83 (m, 1H), 6.79–6.75 (m, 1H), 6.74-6.68 (m, 1H), 4.39-4.29 (m, 2H), 4.03-3.98 (m, 1H), 3.87 (d, J = 4.4 Hz, 1H), 3.81 (d, J = 6.2 Hz, 1H). ¹³C NMR (126 MHz, MeOD) δ 167.0, 146.5, 146.3, 123.2, 120.8, 118.9,

117.5, 61.3, 55.2, 51.8. HRMS: (ESI) [M + H]⁺ calc. for C₁₀H₁₄N₃O₄⁺: 242.1135, observed 242.1135.

(R)-2-Amino-N'-(2,4-dihydroxybenzyl)-3-

hydroxypropanehydrazide hydrochloride (7e). Synthesized by general procedure 5. Off-white solid, 78% yield. Compound is reported as 1:1 with rotamers. Major peak denoted with *.

¹H NMR (400 MHz, (CD₃OD)) δ 7.01–6.73 (m, 1H), 6.45–6.17 (m, 2H), 4.20-4.06 (m, 1H), 5.02-3.85 (m, 2H), 3.85-3.56 (m, 2H). 13 C NMR (126 MHz, MeOD) δ 167.7, 166.9, 161.3, 159.0, 134.0, 120.5, 109.2, 108.1, 103.4, 68.0, 66.9, 61.3, 55.1, 51.7. ¹³C NMR (126 MHz, MeOD) δ 167.7*, 166.9*, 161.3*, 159.0*, 134.0*, 120.5, 109.2, 108.1*, 103.4*, 68.0*, 66.9, 61.3*, 55.1*, 51.7. HRMS: (ESI) $[M + H]^+$ calc. for $C_{10}H_{16}N_3O_4^+$: 242.1135, observed 242.1138.

(R)-2-Amino-N'-(2,5-dihydroxybenzyl)-3-

hydroxypropanehydrazide hydrochloride (7f). Synthesized by general procedure 5. White solid, 95% yield. Compound is reported 1:1 with rotamers. Major peak denoted with *.

¹H NMR (400 MHz, (CD₃OD)) δ 6.80–6.74 (m, 3H)*, 4.36 (s, 2H)*, 4.18-4.14 (m, 1H)*, 4.09-4.06 (m, 1H)*, 4.01-3.96 (m, 1H), 3.96-3.89 (m, 1H)*, 3.86-3.81 (m, 1H). ¹³C NMR (126 MHz, MeOD) δ 167.7, 167.0, 151.5, 150.7, 119.3, 119.0, 117.1, 61.2, 55.2, 52.0. HRMS: (ESI) $[M + H]^+$ calc. for $C_{10}H_{16}N_3O_4^+$: 242.1135, observed 242.1128.

(R)-2-Amino-N'-(2,6-dihydroxybenzyl)-3-

hydroxypropanehydrazide hydrochloride (7g). Synthesized by general procedure 5. Off-white solid, 87% yield. Compound is reported with minor diethyl ether impurity. Compound is reported with major rotamers. Major rotamer peaks designated with *.

¹H NMR (400 MHz, (CD₃OD)) δ 7.08 (t, J = 8.9 Hz, 1H), 6.47– 6.34 (m, 2H), 4.59-4.47 (m, 1H), 4.31-4.05 (m, 2H), 3.96-3.82 (m, 2H). 13 C NMR (126 MHz, MeOD) δ 167.4, 166.8, 159.0, 156.4, 132.1, 107.8, 107.5, 61.4, 55.1, 54.8. HRMS: (ESI) [M + H]⁺ calc. for C₁₀H₁₆N₃O₄⁺: 242.1135, observed 242.1138.

(R,E)-2-Amino-3-hydroxy-N'-(2-hydroxybenzylidene)propanehydrazide hydrochloride (8a). Synthesized by general procedure 5. Off-white solid, 78% yield. Compound is reported as 7:3E:Zmixture of isomers. Major isomer peaks designated with *.

¹H NMR (400 MHz, (CD₃OD)) δ 8.45 (s, 1H)*, 8.36 (1H), 7.69-7.65 (m, 1H), 7.46-7.42 (m, 1H)*, 7.33-7.23 (m, 1H)*, 6.93-6.85 $(m, 2H)^*, 4.74 (q, J = 3.7 Hz, 1H), 4.18-4.09 (m, 1H)^*, 4.04-3.92$ (m, 2H)*. 13 C NMR (126 MHz, MeOD) δ 168.8*, 167.7, 164.7*, 159.4*, 158.4, 152.1*, 146.0, 133.2*, 133.0, 131.6*, 128.4, 120.8, 120.7*, 119.2, 117.6*, 117.1, 61.6*, 61.2, 60.5, 55.6*, 55.4, 55.1. HRMS: (ESI) $[M + H]^+$ calc. for $C_{10}H_{14}N_3O_3^+$: 224.1030, observed 224.1023.

(R,E)-2-Amino-3-hydroxy-N'-(3-hydroxybenzylidene)propanehydrazide hydrochloride (8b). Synthesized by general procedure 5. Off-white solid, 88% yield. Compound is reported as 3:7 E:Zmixture of isomers. E isomer denoted with *.

¹H NMR (400 MHz, (CD₃OD)) δ 8.14 (s, 1H), 7.92 (s, 1H)*, 7.26-7.11 (m, 3H)*, 6.88-6.84 (s, 1H)*, 4.75 (q, J = 3.4 Hz1H)*, 4.15-4.09 (m, 1H), 3.97-3.89 (m, 2H)*. ¹³C NMR (126 MHz, MeOD) δ 169.1, 167.7*, 159.1*, 151.3, 147.7*, 136.4*, 136.2, 131.0*, 120.8, 120.1*, 119.2, 118.8*, 114.5, 114.2*, 61.6, 61.2*,

60.6, 55.6, 55.5*, 55.1. HRMS: (ESI) $[M + H]^+$ calc. for $C_{10}H_{14}N_3O_3^+$: 224.1030, observed 224.1033.

(R,E)-2-Amino-3-hydroxy-N'-(4-hydroxybenzylidene)propane-hydrazide hydrochloride (8c). Synthesized by general procedure 5. White solid, 99% yield. Compound is reported as $3:7 \ E:Z$ mixture of isomers. E isomer denoted with *.

¹H NMR (400 MHz, (CD₃OD)) δ 8.14 (s, 1H), 7.92 (s, 1H)*, 7.66–7.61 (m, 2H), 7.58–7.53 (m, 2H)*, 6.86–6.80 (m, 2H)*, 4.76 (q, J = 3.6 Hz, 1H)*, 4.18–4.11 (m, 1H), 4.03–3.89 (m, 2H)*. ¹³C NMR (126 MHz, MeOD) δ 168.8*, 167.7, 165.1, 161.6, 161.3*, 151.5, 147.9*, 130.8, 130.1*, 126.4*, 126.2, 116.7*, 61.7, 61.2, 60.6*, 55.6, 55.5*, 55.1. HRMS: (ESI) [M + H]⁺ calc. for $C_{10}H_{14}N_3O_3^+$: 224.1030, observed 224.1033.

(R,E)-2-Amino-N'-(2,3-dihydroxybenzylidene)-3-

hydroxypropanehydrazide hydrochloride (8d). Synthesized by general procedure 5. Off-white solid, 83% yield. Compound is reported as 7:3 E:Z mixture of isomers. Major isomer denoted with *. Compound spectra matches previously reported spectra.³²

¹H NMR (400 MHz, (CD₃OD)) δ 8.42 (s, 1H)*, 8.34 (s, 1H), 7.14–7.11 (m, 1H), 6.93–6.84 (m, 2H)*, 6.79–6.69 (m, 1H)*, 4.76–4.72 (m, 1H)*, 4.66–4.63 (m, 1H), 4.22–4.17 (m, 1H), 4.15–4.09 (m, 1H)*, 4.06–3.78 (m, 3H)*. HRMS: (ESI) [M + H]⁺ calc. for C₁₀H₁₄N₃O₃⁺: 240.0979, observed 240.0983.

(R,E)-2-Amino-N'-(2,4-dihydroxybenzylidene)-3-

hydroxypropanehydrazide hydrochloride (8e). Synthesized by general procedure 5. Off-white solid, 77% yield. Compound is reported as 4:1 E:Z mixture of isomers. Major isomer peak denoted with *.

¹H NMR (400 MHz, (CD₃OD) δ 8.40 (s, 1H)*, 8.21 (s, 1H), 7.45 (d, J = 8.3 Hz, 1H), 7.33 (d, J = 8.6 Hz, 1H)*, 6.45–6.41-m, 1H)*, 6.38–6.34 (m, 1H)*, 6.33 (d, J = 3.0 Hz, 1H), 4.69 (q, J = 3.3 Hz,1H), 4.19–4.14 (m, 1H), 4.13–4.07 (m, 1H)*, 4.04–3.89 (m, 2H)*. ¹³C NMR (126 MHz, MeOD) δ 168.3, 167.6*, 164.7*, 164.5, 162.7, 162.4*, 160.2, 160.1, 154.9*, 147.5*, 134.5*, 130.6, 112.5, 110.7, 109.6*, 109.1, 103.7*, 103.4, 61.6*, 61.2, 60.7, 60.4, 55.5*, 55.3, 55.1. HRMS: (ESI) [M + H]⁺ calc. for C₁₀H₁₄N₃O₃⁺: 240.0979, observed 240.0975.

(R,E)-2-Amino-N'-(2,5-dihydroxybenzylidene)-3-

hydroxypropanehydrazide hydrochloride (8f). Synthesized by general procedure 5. Off-white solid, 90% yield. Compound is reported as 1:1 E:Z mixture of isomers. Major isomer denoted with *. Spectra matches previously reported spectra.³²

¹H NMR (400 MHz, (CD₃OD)) δ 8.33 (s, 1H)*, 8.29 (s, 1H), 7.11–7.08 (m, 1H), 6.91–6.88 (m, 1H)*, 6.81–6.68 (m, 2H)*, 4.74–4.69 (m, 1H), 4.16–3.87 (m, 3H)*. ¹³C NMR (126 MHz, MeOD) δ 168.8*, 164.7, 152.5*, 151.7, 151.3*, 151.3, 145.6*, 121.2, 120.9*, 120.5*, 119.4, 118.3*, 117.9, 115.9*, 113.0, 61.5*, 61.2, 60.5, 55.6*, 55.4, 55.1. HRMS: (ESI) [M + H]⁺ calc. for C₁₀H₁₄N₃O₃⁺: 240.0979, observed 240.0966.

(R,E)-2-Amino-N'-(2,6-dihydroxybenzylidene)-3-

hydroxypropanehydrazide hydrochloride (8g). Synthesized by general procedure 5. Off-white solid, 56% yield. Compound is reported as 8.5:1.5 E:Z mixture of isomers. Major isomer denoted with *

¹H NMR (400 MHz, (CD₃OD)) δ 8.83 (s, 1H)*, 8.63 (s, 1H), 7.12 (t, J = 8.5 Hz, 1H)*, 6.38–6.34 (m, 2H)*, 4.64–4.59 (m, 1H),

4.20–4.15 (m, 1H), 4.14–4.09 (m, 1H)*, 4.05–3.92 (m, 2H)*. 13 C NMR (126 MHz, MeOD) δ 167.9, 167.6*, 164.4*, 162.4, 160.2*, 159.7, 149.9*, 147.7, 134.4*, 133.9, 107.0*, 61.6*, 61.2, 60.6, 60.2, 56.0, 55.6, 55.6*, 55.1. HRMS: (ESI) [M + H]* calc. for $C_{10}H_{14}N_{3}O_{3}^{+}$: 240.0979, observed 240.0965.

(R,E)-2-Amino-3-hydroxy-N'-(2-hydroxy-6-

methoxybenzylidene)propanehydrazide hydrochloride (8h). Synthesized by general procedure 5. White solid, 99% yield. Compound is reported as 5:1 E:Z mixture of isomers. Major isomer denoted with *.

 1H NMR (400 MHz, (CD₃OD)) δ 8.78 (s, 1H)*, 8.59 (s, 1H), 7.28–7.21 (m, 1H)*, 6.55–6.46 (m, 2H)*, 4.64–4.60 (m, 1H), 4.12–4.07 (m, 1H)*, 4.06–3.91 (m, 2H)*, 3.88–3.83 (m, 3H). $^{13}\mathrm{C}$ NMR (126 MHz, MeOD) δ 168.0, 167.8*, 164.4*, 161.0, 160.6*, 148.9*, 146.9, 134.2*, 110.6*, 110.3, 108.0, 107.8*, 102.9, 102.4*, 61.6*, 61.5, 60.2, 56.4*, 55.6*, 55.1. HRMS: (ESI) [M + H]^+ calc. for $\mathrm{C_{11}H_{16}N_3O_4}^+$: 254.1135, observed 254.1132.

(R,E)-2-Amino-N'-(3,4-dihydroxybenzylidene)-3-

hydroxypropanehydrazide hydrochloride (8i). Synthesized by general procedure 5. Off-white solid, 44% yield. Compound is reported as 1:1 E:Z mixture of isomers. E isomer denoted with *. Spectra matches previously reported spectra.³²

¹H NMR (400 MHz, (CD₃OD)) δ 8.10 (1H)*, 7.86 (1H), 7.28 (d, J = 1.9 Hz, 1H)*, 7.22 (d, J = 2.2 Hz, 1H), 7.04 (dd, J = 2.4, 8.6 Hz, 1H)*, 6.98 (dd, J = 2.3, 8.1 Hz, 1H), 6.80 (d, J = 3.0 Hz, 1H)*, 6.79 (d, J = 2.9 Hz, 1H), 4.78–4.74 (m, 1H), 4.17–4.10 (m, 1H)*, 4.08–3.91 (m, 3H)*. HRMS: (ESI) [M + H]⁺ calc. for C₁₀H₁₄N₃O₃⁺: 240.0979, observed 240.0979.

(R,E)-2-Amino-3-hydroxy-N'-(2,3,4-trihydroxybenzylidene) propanehydrazide hydrochloride (8j). Synthesized by general procedure 5. Off-white solid, 93% yield. Compound is reported as 4:1 E:Z mixture of isomers. Major isomer denoted with *. Spectra matches previously reported spectra.³²

¹H NMR (400 MHz, (CD₃OD)) δ 8.27 (s, 1H)*, 8.15 (s, 1H), 6.89 (d, J = 9.2 Hz, 1H), 6.73 (d, J = 8.6 Hz, 1H)*, 6.44 (d, J = 9.2 Hz, 1H)*, 4.70–4.67 (m, 1H), 4.13–4.08 (m, 1H)*, 4.06–3.91 (m, 2H)*. HRMS: (ESI) [M + H]⁺ calc. for $C_{10}H_{14}N_3O_5^{-1}$: 256.0928, observed 256.0935.

(*R,E*)-2-Amino-3-hydroxy-*N*'-(2,3,6-trihydroxybenzylidene) propanehydrazide hydrochloride (8k). Synthesized by general procedure 5. White solid, 96% yield. Compound reported as 5: 1 E: Z mixture of isomers. Major isomer peaks denoted by *.

¹H NMR (400 MHz, (CD₃OD)) δ 8.76 (s, 1H)*, 8.61 (s, 1H), 6.76–6.71 (m, 1H)*, 6.24 (d, J = 9.0 Hz, 1H), 6.21 (d, J = 8.5 Hz, 1H)*, 4.64–4.59 (m, 1H), 4.05–4.01 (m, 1H)*, 4.00–3.90 (m, 2H)*. ¹³C NMR (126 MHz, MeOD) δ 168.0, 164.3*, 152.4*, 152.2, 149.7*, 147.9, 147.8*, 138.7*, 120.4*, 107.7, 107.6*, 106.3, 106.0*, 61.9, 61.6*, 60.6, 60.2, 55.6*, 55.5, 55.2. HRMS: (ESI) [M + H]⁺ calc. for C₁₀H₁₄N₃O₅⁺: 256.0928, observed 256.0924.

(R,E)-2-Amino-3-hydroxy-N'-(2,3,4-trimethoxybenzylidene) propanehydrazide hydrochloride (8l). Synthesized by general procedure 5. White solid, 99% yield. Compound is reported as 1:2 E:Z mixture of isomers. E isomer is denoted with *.

¹H NMR (400 MHz, (CD₃OD)) δ 8.46 (s, 1H)* 8.23 (s, 1H), 7.76, (d, J = 9.62, 1H)*, 7.61 (d, J = 9.4 Hz, 1H), 6.85 (d, J = 9.6 Hz, 1H)*, 4.77–4.73 (m, 1H), 4.14–3.80 (m, 13H). ¹³C NMR (126 MHz, MeOD) δ 168.9*, 165.2*, 157.7*, 157.4, 154.8, 154.6*,

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146.9*, 143.5*, 143.2, 122.9, 122.2*, 121.2*, 121.0, 109.5*, 62.5, 62.4*, 61.6, 61.6*, 61.3*, 60.6, 56.6, 55.6*, 55.5*, 55.2. HRMS: (ESI) $[M + H]^+$ calc. for $C_{13}H_{20}N_3O_5^+$: 298.1397, observed 298.1396.

Tert-butyl(2-hydrazineyl-2-oxoethyl)carbamate (9aa). Synthesized by general procedure 2. White solid, 92% yield.

¹H NMR (400 MHz, (CD₃OD)) δ 3.68 (s, 2H), 1.44 (s, 9H). ¹³C NMR (126 MHz, MeOD) δ 170.4, 157.0, 79.3, 41.9, 27.3. HRMS: (ESI) [M + Na]⁺ calc. for C₇H₁₅N₃NaO₅⁺: 212.1011, observed 212.1009.

Tert-butyl(*R*)-(1-hydrazineyl-1-oxopropan-2-yl)carbamate (9ab). Synthesized by general procedure 2. White solid, 78% vield.

¹H NMR (400 MHz, (CD₃OD)) δ 4.06 (q, J = 7.0 Hz, 1H), 1.43 (s, 9H), 1.27 (d, J = 7.5 Hz, 3H). ¹³C NMR (126 MHz, MeOD) δ 175.0*, 157.5*, 152.9, 80.5*, 50.3*, 28.7*, 24.6, 18.5*, 15.5. HRMS: (ESI) [M + Na]⁺ calc. for C₈H₁₇N₃NaO₅⁺: 226.1168, observed 226.1161.

Tert-butyl(*S*)-(1-hydrazineyl-3-mercapto-1-oxopropan-2-yl) carbamate (9ac). Synthesized by general procedure 2. White solid, 86% yield.

¹H NMR (400 MHz, (CD₃OD)) δ 4.14 (s, 1H), 2.86–2.68 (m, 2H), 1.44 (s, 9H). ¹³C NMR (126 MHz, MeOD) δ 170.8, 156.2, 79.5, 55.8, 27.3, 25.8. HRMS: (ESI) [M – H]⁻ calc. for C₈H₁₆N₃O₃S: 234.0918, observed 234.0913.

Tert-butyl(E)-(2-(2-(2,6-dihydroxybenzylidene)hydrazineyl)-2-oxoethyl)carbamate (10a). Synthesized by general procedure 3. White solid, 69% yield. Compound reported as 5:1E:Z mixture of isomers. Major isomer denoted with *.

¹H NMR (400 MHz, (CD₃OD)) δ 8.75 (s, 1H)*, 8.58 (s, 1H), 7.11–7.06 (m, 1H)*, 6.39–6.32 (m, 2H)*, 4.17 (s, 2H), 3.84 (s, 2H)*, 1.48 (s, 9H). ¹³C NMR (126 MHz, MeOD) δ 171.6, 168.2*, 160.1*, 159.7, 158.5, 148.5*, 146.1, 133.7*, 107.6*, 80.8*, 61.5*, 43.4*, 42.6, 28.7*. HRMS: (ESI) [M + H]⁺ calc. for C₁₄H₂₀N₃O₅⁺: 310.1397, observed 310.1395.

Tert-butyl(R,E)-(1-(2-(2,6-dihydroxybenzylidene)

hydrazineyl)-1-oxopropan-2-yl)carbamate (10b). Synthesized by general procedure **3.** White solid, 54% yield. Compound reported as 9:1 E:Z mixture of isomers. Major isomer denoted with *.

¹H NMR (500 MHz, (CD₃)₂CO) δ 8.77 (s, 1H)*, 8.61 (s, 1H), 7.08 (t, J=8.3 Hz, 1H)*, 6.36 (d, J=8.9 Hz, 2H), 4.18 (q, J=6.9 Hz, 1H)*, 1.46 (s, 9H)*, 1.40 (d, J=7.6 Hz, 3H)*. ¹³C NMR (126 MHz, MeOD) δ 173.0, 171.7*, 161.1, 160.0*, 159.7, 157.7, 148.7*, 146.4, 135.3, 133.8*, 107.6*, 80.7*, 61.5*, 50.6*, 28.7*, 20.9, 18.4*, 17.2, 14.5*. HRMS: (ESI) [M + H]⁺ calc. for $C_{15}H_{22}N_3O_5^+$: 324.1554, observed 324.1553.

Tert-butyl(S,E)-(1-(2-(2,6-dihydroxybenzylidene)hydrazineyl)-3-mercapto-1-oxopropan-2-yl)carbamate (10c). Synthesized by general procedure 2. White solid, 88% yield. Compound is reported as 9:1 E:Z mixture of isomers. Major isomer denoted with *.

¹H NMR (500 MHz, (CD₃)₂CO) δ 8.77 (s, 1H)*, 8.59 (s, 1H), 7.06 (t, J = 7.6 Hz, 1H)*, 6.34 (d, J = 8.0 Hz, 2H)*, 4.26 (s, 1H)*, 2.93–2.76 (m, 2H)*, 1.44 (s, 9H)*. ¹³C NMR (126 MHz, MeOD) δ 173.0, 168.8*, 160.0*, 157.6, 149.2*, 133.9*, 107.6*, 107.3,

81.0*, 61.5*, 57.4*, 28.6*, 27.0, 20.9*, 14.4*. *. HRMS: (ESI) [M - H] $^-$ calc. for $C_{15}H_{20}N_3O_5S^-$: 354.1129, observed 354.1124.

(*E*)-2-Amino-N'-(2,6-dihydroxybenzylidene)acetohydrazide hydrochloride (11a). Synthesized by general procedure 5. White solid, 99% yield. Compound is reported as 5:1 E:Z mixture of isomers. Major isomer denoted with *.

¹H NMR (500 MHz, (CD₃)₂CO) δ 8.74 (s, 1H)*, 8.62 (s, 1H), 7.10 (t, J = 8.5 Hz, 1H)*, 6.38–6.32 (m, 2H)*, 4.16* (s, 1H)*, 3.83 (s, 2H)*. ¹³C NMR (126 MHz, MeOD) δ 167.5*, 163.4*, 160.2*, 159.8, 149.2*, 147.3, 134.3*, 134.1*, 107.6*, 40.9, 40.7*. HRMS: (ESI) [M + H]⁺ calc. for C₉H₁₂N₃O₃⁺: 210.0873, observed 210.0870.

(R,E)-2-amino-N'-(2,6-dihydroxybenzylidene)propanehydrazide hydrochloride (11b). Synthesized by general procedure 5. White solid, 81% yield. Compound is reported as 9:1 E: Z mixture of isomers. Major isomer denoted with *.

¹H NMR (500 MHz, (CD₃)₂CO) δ 8.80 (s, 1H)*, 8.64 (s, 1H), 7.13–7.06 (m, 1H)*, 6.41–6.31 (m, 2H)*, 4.11–4.01 (m, 1H)*, 1.63–1.54 (m, 3H)*. ¹³C NMR (126 MHz, MeOD) δ 170.7*, 170.0, 167.0*, 160.1*, 159.8, 149.6*, 147.6, 134.3*, 134.2, 107.6*, 107.1, 17.6, 16.1*. *. HRMS: (ESI) [M + H]⁺ calc. for C₁₀H₁₄N₃O₃⁺: 224.1030, observed 224.1030.

(S,E)-2-Amino-N'-(2,G-dihydroxybenzylidene)-3-mercaptopropanehydrazide hydrochloride (11c). Synthesized by general procedure 5. White solid, 92% yield. Compound is reported as 9:1 E:Z mixture of isomers. Major peaks denoted with *.

¹H NMR (400 MHz, (CD₃OD)) δ 9.92–9.76 (m, 1H)*, 7.15–7.02 (m, 1H)*, 6.97–6.87 (m, 1H), 6.40–6.26 (m, 2H)*, 4.74–4.68 (m, 1H), 4.55–4.50 (m, 1H), 4.12–4.06 (m, 1H)*, 3.23–3.08 (m, 1H)*, 3.08–2.95 (m, 1H)*. ¹³C NMR (126 MHz, MeOD) δ 164.4, 160.2, 149.9, 134.3, 107.7, 107.1, 55.6, 26.2 HRMS: (ESI) [M + H]⁺ calc. for C₁₀H₁₄N₃O₃S⁺: 256.0750, observed 256.0744.

Data availability

All data generated or analyzed during this study are included in this published article and its ESI.†

Conflicts of interest

There are no conflicts to declare.

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