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The parmodulin NRD-21 is an allosteric inhibitor of PAR1 Gq signaling with improved anti-inflammatory activity and stability

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Abstract

Novel analogs of the allosteric, biased PAR1 ligand ML161 (parmodulin 2, PM2) were prepared in order to identify potential anti-thrombotic and anti-inflammatory compounds of the parmodulin class with improved properties. Investigations of structure-activity relationships of the western portion of the 1,3-diaminobenzene scaffold were performed using an intracellular calcium mobilization assay with endothelial cells, and several heterocycles were identified that inhibited PAR1 at sub-micromolar concentrations. The oxazole NRD-21 was profiled in additional detail, and it was confirmed to act as a selective, reversible, negative allosteric modulator of PAR1. In addition to inhibiting human platelet aggregation, it showed superior anti-inflammatory activity to ML161 in a qPCR assay measuring the expression of tissue factor in response to the cytokine TNF-alpha in endothelial cells. Additionally, NRD-21 is much more plasma stable than ML161, and is a promising lead compound for the parmodulin class for anti-thrombotic and anti-inflammatory indications.

Graphical abstract

Abbreviations

aPC: activated Protein C; Boc: *tert*-butoxycarbonyl; DCE: 1,2-dichloroethane; DCM: dichloromethane; DIC: disseminated intravascular coagulation; DIPEA: *N,N*-diisopropylethylamine; DMAP: 4-dimethylaminopyridine; DMF: *N,N*-dimethylformamide; DMSO: dimethylsulfoxide; EDC: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; FDA: U.S. Food and Drug Administration; GPCR: G protein coupled receptor; HATU: hexafluorophosphate azabenzotriazole tetramethyl uranium; HOBT: 1-hydroxybenzotriazole; HTS: high-throughput screening; HUVEC: human umbilical vein endothelial cells; IC_{50} : half-maximal inhibitory concentration; iCa^{2+} : intracellular calcium mobilization; LC-MS: liquid chromatography-mass spectrometry; MI: myocardial infarction; NMR: nuclear magnetic resonance; PAR: protease-activated receptor; qPCR: quantitative polymerase chain reaction; SAR: structure-activity relationship; SEM: standard error of the mean; TF: tissue factor; TLC: thin layer chromatography; TNF- α : Tumor Necrosis Factor-alpha

Keywords

Parmodulin, NRD-21, ML161, PAR1, Allosteric inhibitor, Oxazole, Anti-platelet, Antithrombotic, Anti-inflammatory, Biased ligand

1. Introduction

The use of biased ligands for G protein coupled receptors (GPCRs) has emerged as a promising strategy for maximizing therapeutic signals mediated by GPCRs, while potentially mitigating undesired side effects linked to alternative signaling pathways initiated by the same receptors. Protease-activated receptors (PARs) are GPCRs that are activated by a variety of vascular proteases that cleave the *N*-termini of PARs, revealing a tethered peptide that activates the receptor¹ and initiates a plethora of signals.² PARs are present in a variety of tissues and are implicated in a variety of pathologies including thrombosis, inflammation, and cancer cell metastasis.^{3, 4} The varied phenotypic effects of PAR activation have recently been connected to the activation of specific G-proteins and arrestins,⁵ and biased signals have been observed with proteases such as activated protein C (aPC) that cleave PAR *N*-termini at alternative sites.^{6, 7, 8, 9, 10} Synthetic peptides^{11, 12} and peptidomimetics^{13, 14} based on PAR tethered ligands have also shown biased signaling by blocking or activating only a subset of signals. Pepducins, a novel class of fatty acid-tethered peptides modeled after intracellular GPCR loops developed by Kuliopulos and coworkers,^{15, 16, 17} have been reported to act as biased antagonists at PAR2.^{4, 18} Previously, we reported that small molecules identified via high-throughput screening (HTS) are capable of inhibiting platelet granule secretion, while permitting the shape change of platelets normally observed upon platelet activation via PAR1 agonism, thus acting as “biased antagonists” of PAR1.^{19, 20} Our collaborators (Flaumenhaft and coworkers) have accrued evidence that these small molecules, termed parmodulins, act at the intracellular side of PAR1 to block signaling mediated by Gq, but not G12/13.^{21, 22} The parmodulin ML161 (1, also referred to as parmodulin 2 or PM2, Figure 1) was found to promote cytoprotective and anti-inflammatory effects in endothelium in a manner similar to aPC,²² and as with aPC it was highly effective at minimizing necrosis of coronary tissue in a mouse model of myocardial infarction (MI).²³ We also recently confirmed that ML161 and its aniline analog RR-90 are selective, reversible, and allosteric inhibitors (negative allosteric

modulators) of PAR1 signaling via the G protein Gq.²⁴ The presence of PAR1 is required for the cytoprotective effects of aPC²⁵ and ML161^{21, 22} in endothelium, and targeting PAR1 with parmodulins to inhibit pro-inflammatory or pro-thrombotic signals while activating beneficial anti-inflammatory and/or cytoprotective signals could be an effective therapeutic strategy for sepsis, stroke, and thrombosis. This manuscript describes our efforts to more deeply explore structure–activity relationships (SARs) at the western side of parmodulins possessing the 1,3-diaminobenzene scaffold, exemplified by ML161.

Figure 1. Previous parmodulins and their performance in a platelet P-selectin assay.²⁰

ML161 was previously assigned as a Molecular Libraries probe,^{20, 26} and was our most potent analog to date in the P-selectin assay, a flow cytometry assay which measures levels of P-selectin on the surface of activated platelets. Our interest in measuring the effects of parmodulins in endothelial cells led us to develop a protocol for an intracellular calcium mobilization (*i*Ca²⁺) assay using adherent EA.hy926 cells in 96 well plates.²⁴ Both responses are driven by PAR1 Gq, but the endothelial calcium assay also offers higher throughput and lower variability than the platelet P-selectin assay, so we have utilized it as our primary assay for ongoing studies. Compounds were screened in 96 well plates at a concentration of 10 μM, using the PAR1 tethered ligand peptide TFLLRN-NH₂ (5 μM) as agonist. 7-point concentration–response curves were obtained for compounds demonstrating > 70% inhibition in this screen, which was an arbitrary cutoff.

A significant liability of ML161 is its low stability in mouse plasma. The branched amide **2** was equipotent to ML161 in the platelet P-selectin assay, but showed greatly improved stability in mouse plasma after 5 h (65% remaining vs 2%), presumably due to increased resistance to protease-catalyzed hydrolysis.²⁰ Unfortunately, **2** possesses decreased solubility in water with 1% DMSO (9 μM vs 58 μM for **1**),²⁰ and mediocre inhibition in the endothelial *i*Ca²⁺ assay (Table 1).²⁴ Therefore, we focused our efforts on finding alternative analogs that could offer equal or better potency than ML161 in the *i*Ca²⁺ assay but with improved plasma stability, which is particularly important for longer duration *in vivo* experiments.

Table 1. SAR of simple alkyl analogs.

Cmpd; ID#	Structure	<i>i</i> Ca ²⁺ assay %Inhib; pIC ₅₀ ^a	Cmpd; ID#	Structure	<i>i</i> Ca ²⁺ assay %Inhib; pIC ₅₀ ^a
1		82 ± 2%	8		54 ± 3%
ML161		6.1 ± 0.1			5.5 ± 0.3
2		82 ± 2%	9		88 ± 1%
CJD-125		undef. ^b	NRD-25		6.4 ± 0.2

3		65 ± 2%	10		23 ± 5% ^d
6		21% ^c	11		79%
					pIC ₅₀ < 5
7		27 ± 6%	12		63 ± 4%
					6.7 ± 0.2

- Assays were performed with adherent EA.hy926 endothelial cells according to the protocol reported in the Supporting Info. % Inhibition was measured at 10 μM with 5 μM TFLLRN-NH₂ and n = 4 wells, unless otherwise noted, with standard error of the mean (SEM) provided. pIC₅₀s (−logIC₅₀s) were estimated from curves fitted to measurements on 3 separate wells for each concentration, using 4-variable non-linear regression in GraphPad Prism v. 6. The detailed assay protocol was previously described.²⁴
- IC₅₀ is undefined— a double sigmoidal concentration–response curve was not obtained.
- In platelet P-selectin assay.²⁰
- n = 3.

2. Results

This manuscript describes our SAR studies with modifications to the “western” end of the scaffold. Many of these analogs, including the most promising analogs identified herein, could be prepared via simple acylation reactions of aniline precursors ([Scheme 1](#)). The eastern 2-bromobenzamide of ML161 that was optimized previously was fixed at this stage, though other eastern benzene substitutions are also tolerated.

Scheme 1. General conditions for the synthesis of western amide analogs.

Following up on our previous modifications at the western side exemplified by **1** and **2**, we explored the role of branching and chain length ([Table 1](#)). The cyclopentyl analog **3** showed moderate inhibition, but increasing further the level of substitution at the alpha position (**6**) greatly increased plasma stability but decreased inhibition greatly in the platelet P-selectin assay.²⁰ The acyclic analog **7** also showed weak efficacy in the *i*Ca²⁺ assay. Compound **8**, with one carbon less than ML161, also showed decreased activity, but interestingly its more lipophilic trifluoromethyl analog **9** showed increased potency (IC₅₀ = 0.38 μM) compared to **8** and similar activity to ML161. Alternatively, extension of the carbon chain of ML161 by one led to decreased efficacy and/or potency (compounds **10** and **11**), which is consistent with what we observed previously with platelets.²⁰ A close analog of the previously-reported reverse amide **4** ([Figure 1](#)) was also prepared (**12**), and showed very good potency (IC₅₀ = 0.22 μM), but only moderate efficacy (~50% maximal inhibition). Such reverse amides could address potential liabilities associated with toxic anilide or aniline hydrolysis products.

Introduction of polar functional groups was performed in order to improve the potency and drug-like properties of **1** and **2** ([Table 2](#)). Addition of hydroxy or methoxy groups (compounds **13–18**) led to significant losses of efficacy. Malonate derivatives (**19–21**) also showed weak efficacy. Two carbamates were synthesized (**22** and **23**), with **23** showing moderate activity (60% inhibition at 10 μM), as did ether **24**.

Table 2. SAR of neutral or acidic analogs.

Cmpd; ID#	Structure	<i>i</i> Ca ²⁺ assay %Inhib; pIC ₅₀ ^a	Cmpd; ID#	Structure	<i>i</i> Ca ²⁺ assay %Inhib; pIC ₅₀ ^a
13		39 ± 5%	19		11 ± 12%
14		21 ± 2%	20		37 ± 11%
15		25 ± 5%	21		36 ± 13%
16		48 ± 13%	22		42 ± 12%
17		38 ± 5%	23		60 ± 4%
18		58 ± 7%	24		57 ± 4%

- a. Assays were performed with adherent EA.hy926 endothelial cells according to the protocol reported in the Supporting Info. % Inhibition was measured at 10 μ M with 5 μ M TFLLRN-NH₂ and n = 4 wells, unless otherwise noted, with standard error of the mean (SEM) provided. pIC₅₀s (−logIC₅₀s) were estimated from curves fitted to measurements on 3 separate wells for each concentration, using 4-variable non-linear regression in GraphPad Prism v. 6. The detailed assay protocol was previously described.²⁴

Continuing with more basic amine functionality (Table 3), we found that incorporation of a dimethylamine unit at the western end (**25**) led to a complete loss of activity. However, as previously reported,^{20, 24} the aniline RR-90 (**5**) showed very good activity and similar potency to ML161. The tertiary aniline **26** showed weak activity, as did the aniline **27** with a terminal ether. Preparation of the more basic analog **28** also led to a steep drop in efficacy. Interestingly, the anilines **29** and **30** both showed evidence of activation, rather than inhibition, of PAR1. Aniline **29** was synthesized via a reductive amination reaction with the aniline precursor **S3** (Scheme 1) and cyclopentenone, and **30** was prepared by cyclization of the same precursor with 1,4-dibromobutane (see Supplementary data for details). Aniline **30** (KMK-17) is presently under investigation as a potential positive allosteric modulator of PAR1.

Table 3. SAR of amine analogs.

Cmpd; ID#	Structure	<i>i</i> Ca ²⁺ assay %Inhib; pIC ₅₀ ^a	Cmpd; ID#	Structure	<i>i</i> Ca ²⁺ assay %Inhib; pIC ₅₀ ^a
25		3 ± 3%	28		17 ± 6%
VAK-11					
5		85 ± 2%	29		−12 ± 6%
RR-90		6.0 ± 0.2			
26		13 ± 2%	30		−60 ± 8%
			KMK-17		

27		43 ± 6% ^b			
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- a. Assays were performed with adherent EA.hy926 endothelial cells according to the protocol reported in the Supporting Info. % Inhibition was measured at 10 μM with 5 μM TFLLRN-NH₂ and n = 4 wells, unless otherwise noted, with standard error of the mean (SEM) provided. pIC₅₀s (−logIC₅₀s) were estimated from curves fitted to measurements on 3 separate wells for each concentration, using 4-variable non-linear regression in GraphPad Prism v. 6. The detailed assay protocol was previously described.²⁴ ^bn = 3.

Next, we explored heterocyclic amide analogs (Table 4). The exploration of western pyrrolidine derivatives (**31–34**) initially yielded differences in activity between the *R*- and *S*-enantiomers in both the Boc-protected pyrrolidines (**31, 33**) and the deprotected derivatives (**32, 34**). However, re-synthesis and retesting of **31–34** to obtain concentration–response curves did not show significant inhibition of calcium mobilization by the PAR1 agonist TFLLRN-NH₂.

Table 4. SAR of heterocyclic amide analogs.

Cmpd; ID#	Structure	iCa ²⁺ assay %Inhib; pIC ₅₀ ^a	Cmpd; ID#	Structure	iCa ²⁺ assay %Inhib; pIC ₅₀ ^a
31		65 ± 4% ^b	36		42 ± 3%
32		63 ± 4% ^b	37		44 ± 2%
33		−8 ± 6% ^b	38		90 ± 1%
			NRD-21		6.3 ± 0.1
34		4 ± 6% ^b	39		82 ± 1%
			NRD-23		6.4 ± 0.1
35		92 ± 1%	40		58 ± 4%

- a. Assays were performed with adherent EA.hy926 endothelial cells according to the protocol reported in the Supporting Info. % Inhibition was measured at 10 μM with 5 μM TFLLRN-NH₂ and n = 4 wells, unless otherwise noted, with standard error of the mean (SEM) provided. pIC₅₀s (−logIC₅₀s) were estimated from curves fitted to measurements on 3 separate wells for each concentration, using 4-variable non-linear regression in GraphPad Prism v. 6. The detailed assay protocol was previously described.²⁴ ^bThese compounds were resynthesized and retested and were found to be inactive when 7-point concentration–response curves were obtained.

The furan **35** showed a high level of inhibition (92%, IC₅₀ = 0.32 μM), which prompted us to explore other oxygen-containing heterocycles, particularly since monosubstituted furans such **35** may suffer from undesirable oxidative metabolism. The tetrahydrofurans **36** and **37** showed only modest inhibition, but the oxazoles **38** and **39** showed very good inhibition. The phenyl analog **40** can be considered as a control compound, and it displayed moderate inhibition (58%).

The potent oxazoles **38** (NRD-21) and **39** (NRD-23) were profiled further, along with the trifluoromethyl analog **9** (NRD-25) and the furan **35** (VAK-7) (Figure 2). Concentration-response curves were obtained in our intracellular calcium mobilization assay. All four analogs showed similar potencies (IC₅₀ = 0.32 to 0.42 μM), though

interestingly the oxazole **38** showed much better efficacy than **39**, which only inhibited calcium activity at ~50% at the highest concentration (31.6 μ M).

Figure 2. Concentration-response curves for PAR1 antagonists in the TFLRN-NH₂-mediated (5 μ M) *i*Ca²⁺ mobilization assay with EA.hy926 cells: A) **35** (VAK-7), B) **39** (NRD-23), C) **9** (NRD-25), D) **38** (NRD-21).

Our previous studies showed the lead compound ML161 (**1**) and aniline **5** to have selective and reversible activity towards PAR1. We endeavored to determine if our novel analogs possess the same properties. Selectivity for PAR1 was conducted with our calcium mobilization assay and PAR1 or PAR2 agonists (Figure 3). EA.hy926 cells were dosed with either vorapaxar at 0.316 μ M or the selected parmodulin at 10 μ M. The cells were then stimulated using either the PAR1 agonist TFLRN-NH₂ or the PAR2 agonist SLIGKV-NH₂ at 3.16 μ M. All parmodulins and vorapaxar showed inhibition of the PAR1 receptor (Figure 3A). Consistent with Table 4, oxazole **39** (NRD-23) was the only parmodulin that showed <75% inhibition of PAR1, while all other parmodulins and vorapaxar exhibited > 75% inhibition (<25% stimulation) (Figure 3A). All antagonists tested with the PAR2 agonist SLIGKV-NH₂ showed no inhibition of PAR2 (Figure 3B), therefore we conclude that all analogs are highly selective for PAR1 over PAR2.

Figure 3. Selectivity data of antagonists in A) PAR1 (TFLRN-NH₂)- and B) PAR2 (SLIGKV-NH₂)-driven *i*Ca²⁺ mobilization. Parmodulins were used at 10 μ M; vorapaxar was used at 0.316 μ M. PAR1 agonist TFLRN-NH₂ and PAR2 agonist SLIGKV-NH₂ were used at 3.16 μ M; Vehicle (V) = 10% DMSO/water.

We also conducted “wash” studies where the endothelial cells were treated with select inhibitors, then washed twice with buffer prior to addition of the agonist and measurement of intracellular calcium levels (Figure 4).

Parmodulins were compared to vorapaxar, which is a known poorly reversible orthosteric inhibitor of PAR1. As expected, all parmoulins tested had a significant loss of inhibition after washing, while vorapaxar remained unaffected, confirming that they inhibit PAR1 in a reversible manner.

Figure 4. Reversibility studies of the PAR1 antagonist vorapaxar and selected parmoulins. Parmoulins were used at 10 μM ; vorapaxar was used at 0.316 μM . PAR1 agonist TFLLRN-NH₂ and PAR2 agonist SLIGKV-NH₂ were used at 3.16 μM ; Vehicle (V) = 10% DMSO/water. Cells containing antagonist were washed with buffer prior to the treatment with PAR1 agonist TFLLRN-NH₂ (5 μM).

Out of the four parmoulins profiled, compound **38** (NRD-21) was selected for further studies; we reasoned that the trifluoromethyl analog **9**, though potent, likely possesses the same plasma stability and solubility liabilities as ML161. ML161 was characterized as a negative allosteric modulator in both platelets and endothelial cells in our previous studies, and we reasoned that the structurally similar NRD-21 would act in a similar manner. Indeed, increasing concentrations of antagonist **38** led to a reduction in efficacy of the PAR1 agonist TFLLRN-NH₂ ([Figure 5](#)). The reduction of maximum efficacy (“ceiling effect”) of the PAR1 agonist in the presence of NRD-21, rather than the simple rightward shift in concentration–response curves, is consistent with the action of NRD-21 as a negative allosteric modulator of TFLLRN-NH₂ at PAR1, rather than a simple competitive inhibition. We previously reported this phenomenon with ML161.^{20, 24} Interestingly the drop in maximal efficacy is not as pronounced for NRD-21 as for the aniline **5** (RR-90), which we previously described.²⁴

Figure 5. $i\text{Ca}^{2+}$ concentration–response of the PAR1 agonist TFLLRN-NH₂ in the presence of increasing concentrations of NRD-21.

Next, an assay was performed to measure the ability of NRD-21 to inhibit platelet aggregation. PAR1 is highly expressed in platelets, and its activation leads to aggregation and coagulation. The platelet aggregation assay was performed with ML161 and NRD-21 ([Figure 6](#)). In both cases, human washed platelets were incubated with

parmodulins at 10 μ M, then the PAR1/2 agonist SFLLRN-NH₂ (1.5 μ M) was added. Complete inhibition of platelet aggregation by both ML161 and NRD-21 was observed.

Figure 6. Human platelet aggregation assay of A) ML161 (10 μ M, red trace) and B) NRD-21 (10 μ M, red trace) in the presence of the PAR1/2 agonist SFLLRN-NH₂ (1.5 μ M). Blue traces = DMSO.

The anti-inflammatory and cytoprotective effects reported for ML161^{21, 22, 23} begged the question if other structurally related parmodulins also share these effects. To this end, we performed a qPCR assay measuring the expression of tissue factor (TF) in endothelial cells (HUVEC) in response to the inflammatory cytokine Tumor Necrosis Factor- α (TNF- α). TF has been long established to mediate the pro-inflammatory and pro-coagulant effects²⁷ of TNF- α ^{28, 29} and endotoxins,³⁰ and unnatural TF expression is therefore the driver of disseminated intravascular coagulation (DIC) observed under conditions of cancer or sepsis.³¹ We measured the level of TF mRNA 4 h after treatment with ML161 or NRD-21 followed by the addition of TNF- α (Figure 7). Pretreatment with ML161 (column 4) blocked the $\sim$ 3-fold increase in TF expression caused by TNF- α alone (column 1), and NRD-21 was even more efficacious, dropping TF RNA levels to below baseline levels. The mechanism of this decrease in TF expression has yet to be delineated, but is presently under investigation.

Figure 7. qPCR assay (n = 3) measuring the inhibition of TNF- α (25 ng/mL) induced TF expression in HUVEC after treatment with ML161 and NRD-21 (10 μ M). Inhibitors were added at t = 0, TNF- α was added at t = 1 h, and mRNA was measured at t = 4 h. Data was analyzed with one-way ANOVA followed by Bonferroni's multiple comparisons test. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

With these promising results in hand with NRD-21, we measured its stability and a number of parameters relevant to its use as an in vivo probe (Table 5). Importantly, NRD-21 is much more plasma stable than ML161. After 4 h in mouse plasma, 32% of NRD-21 remained, while ML161 was <1%. Improved stability in human plasma was also observed for NRD-21 (97% vs 79% after 4 h), as shown in Figure 8. As with ML161, NRD-21 also shows excellent stability in the presence of human liver microsomes, with no apparent degradation after 1 h. It

also shows no measurable toxicity in a human cell line (hepG2). An area for improvement remains the low solubility of the current lead compounds of this class, with a solubility of 17 μM for NRD-21 in a kinetic aqueous solubility assay with 2.5% DMSO. Both compounds were also profiled for off-target receptor binding by the Psychoactive Drug Screening Program (PDSP).³² Both modified radioligand binding to 3 or 4 different targets, including inhibition of binding to the peripheral benzodiazepine receptor (PBR) and activation of the serotonin transporter (SERT).

Table 5. Comparison of ML161 and NRD-21.

	ML161	NRD-21
PAR1 $i\text{Ca}^{2+}$ assay^a	0.57 $\pm$ 0.08 μM (n = 10)	0.37 $\pm$ 0.13 μM (n = 6)
Average $\text{IC}_{50} \pm \text{SEM}$		
Plasma stability	<1% (4 h, mouse) 79% (4 h, human)	32% (4 h, mouse) 97% (4 h, human)
Microsomal stability	99% (1 h, human)	99% (1 h, human)
Kinetic aqueous solubility (2.5% DMSO)	24 μM	17 μM
PAR2 activity?	None observed	None observed
Off-target effects^b	4: Beta1 = 49%; Beta3 = 32%; PBR = 84%; SERT = -44%	3: 5HT-5A = -31%; NET = 31%; PBR = 67%; SERT = -44%
Cytotoxicity (CC_{50}) (human hepG2 cells)	>150 μM	>150 μM
Reversible?	Yes	Yes

- a. Average of independent assays, each with IC_{50}s determined from curve fits with n = 3. See e.g. [Table 1](#) for more details. ^bFraction of off-targets for which there is >20% inhibition (at 10 μM concentration) of 41 targets from the Psychoactive Drug Screening Program. ^bNumber of off-targets (out of 44) for which there is >30% inhibition or activation of standard radioligand binding (at 10 μM concentration) of 44 targets from the Abbreviations of target names with mean % inhibition of binding are given. 5HT = 5-hydroxytryptamine receptor; Beta = β -adrenoceptor; NET = norepinephrine transporter; PBR = peripheral benzodiazepine receptor (rat); SERT = serotonin transporter. Off-target assays were performed by the National Institute of Health Psychoactive Drug Screening Program (PDSP).

Figure 8. Human plasma stability of ML161 (left) and NRD-21 (right). Points indicate the natural logarithm of the average of 3 replicates at each time point.

3. Discussion

Our SAR studies at the western end of the 1,3-diaminobenzene scaffold have determined that lipophilic groups of limited size are best able to inhibit PAR1-driven Gq signaling. However, some heteroatom functionality is tolerated, with western heterocycles giving the most promising profiles. The oxazole NRD-21 was identified as a

compound with slightly improved potency over our previous lead compound ML161, but with much improved plasma stability, making it more suitable for *in vivo* studies.

Most notably, NRD-21 is highly efficacious in the inhibition of TNF- α -mediated TF expression in endothelium, making it a promising lead within this new class of parmodulin anti-inflammatory agents. The signaling pathway(s) leading to the anti-inflammatory effects of the parmodulins is not fully understood, but Flaumenhaft has published evidence consistent with a PAR1-mediated (via G $\beta\gamma$) signaling pathway that ultimately drives transcriptional responses.²² Conversely, the FDA-approved orthosteric PAR1 antagonist vorapaxar has shown deleterious effects in cultured endothelium, including increased levels of apoptosis and decreased barrier integrity.²¹ We have also demonstrated, here and previously,^{21, 24} that unlike vorapaxar, parmodulins are readily reversible inhibitors of PAR1, which is an important safety consideration for anti-thrombotic agents. NRD-21 also inhibited human platelet aggregation similarly to ML161. We conclude that the parmodulin class of intracellular allosteric ligands of PAR1, exemplified by NRD-21 with its 1,3-diaminobenzene scaffold, is promising for both anti-thrombotic and anti-inflammatory-related indications. Efforts are ongoing to identify additional potent parmodulins, characterize their signaling pathway(s), and further investigate their utility in thrombosis and inflammation-related and proliferative disorders.

4. Experimental section

4.1. General synthetic information

All reagents and solvents, including anhydrous solvents, were purchased from commercial vendors and used as received. Deionized water was purified by charcoal filtration to a minimum resistance of 15 M Ω and used for reaction workups and in reactions with water. NMR spectra were recorded on Varian 300 MHz or 400 MHz spectrometers as indicated. Proton and carbon chemical shifts are reported in parts per million (ppm; δ) relative to tetramethylsilane (^1H δ 0), or CDCl_3 (^{13}C δ 77.16), $(\text{CD}_3)_2\text{CO}$ (^1H δ 2.05, ^{13}C δ 29.84), d_6 -DMSO (^1H δ 2.50, ^{13}C δ 39.5), or CD_3OD (^1H δ 3.31, ^{13}C δ 49.00). NMR data are reported as follows: chemical shifts, multiplicity (obs = obscured, app = apparent, br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, comp = complex overlapping signals); coupling constant(s) in Hz; integration. Unless otherwise indicated, NMR data were collected at 25 $^\circ\text{C}$. Filtration was performed by vacuum using VWR Grade 413 filter paper, unless otherwise noted. Flash chromatography was performed using Biotage SNAP cartridges filled with 40–60 μm silica gel on Biotage Isolera automated chromatography systems with photodiode array UV detectors. Analytical thin layer chromatography (TLC) was performed on Agela Technologies 0.25 mm glass plates with 0.25 mm silica gel. Visualization was accomplished with UV light (254 nm) and KMnO_4 stain, unless otherwise noted. Chemical names were generated and select chemical properties were calculated using either ChemAxon Marvin suite or ChemDraw Professional 15.1. NMR data were processed using either MestreNova or ACD/NMR Processor Academic Edition using the JOC report format. High-resolution mass spectra (HRMS) were obtained from the University of Cincinnati Environmental Analysis Service Center using an Agilent 6540 Accurate-Mass LC-MS with Q-TOF.

4.2. LC-MS characterization methods

Tandem liquid chromatography/mass spectrometry (LC-MS) was performed on a Shimadzu LCMS-2020 with autosampler, photodiode array detector, and single-quadrupole MS with ESI and APCI dual ionization using a Peak Scientific nitrogen generator.

4.2.1. Method A

Column: Phenomenex Gemini C₁₈ (100 $\times$ 4.6 mm, 3 μm particle size, 110 $\text{\AA}$ pore size)

Column temperature: 40 $^\circ\text{C}$

Sample Injection: 1–5 μL of sample in MeCN or MeOH

Chromatographic monitoring: UV absorbance at 210 or 254 nm

Mobile Phase: Solvent A: H₂O w/ 0.1% formic acid; Solvent B: MeOH w/ 0.1% formic acid

Flow Rate: 1.0 mL/min

Gradient: 0 to 0.1 min: 25% MeOH (Isocratic)

0.1 min to 5 min: 25% to 95% MeOH (Gradient)

5 min to 7 min: 95% MeOH (Isocratic)

4.2.2. Method B

Column: Phenomenex Gemini C₁₈ (100 × 4.6 mm, 3 μm particle size, 110 Å pore size)

Column temperature: 40 °C

Sample Injection: 1–5 μL of sample in MeCN or MeOH

Chromatographic monitoring: UV absorbance at 210 or 254 nm

Mobile Phase: Solvent A: H₂O w/ 0.1% formic acid; Solvent B: MeCN w/ 0.1% formic acid

Flow Rate: 1.0 mL/min

Gradient: 0 to 0.1 min: 50% MeCN (Isocratic)

0.1 min to 5 min: 50% to 95% MeCN (Gradient)

5 min to 7 min: 95% MeCN (Isocratic)

4.2.3. Method C

Column: Phenomenex Gemini C₁₈ (100 × 4.6 mm, 3 μm particle size, 110 Å pore size)

Column temperature: 40 °C

Sample Injection: 1–5 μL of sample in MeCN or MeOH

Chromatographic monitoring: UV absorbance at 210 or 254 nm

Mobile Phase: Solvent A: H₂O w/ 0.1% formic acid; Solvent B: MeCN w/ 0.1% formic acid

Flow Rate: 1.0 mL/min

Gradient: 0 to 0.5 min: 50% MeCN (Isocratic)

0.5 min to 4 min: 50% to 95% MeCN (Gradient)

4 min to 6.3 min: 95% MeCN (Isocratic)

4.3. General procedures for synthesis of parmodulins via amide coupling.

4.3.1. Method A: Amide coupling using EDC

To a round bottom flask with stir bar under nitrogen were added the appropriate carboxylic acid and anhydrous DCM/DMF (85:15; 0.2–0.6 M). The amine HCl salt to be coupled (1.2 eq.), HOBt (1.2 eq.), EDC-HCl (1.2 eq.), and

DIPEA (2.1 eq.) were added and the reaction was stirred under nitrogen until complete, as measured by TLC and/or LC-MS. The reaction was diluted with DCM, washed with saturated aq. NaHCO₃, 1 M aq. HCl, and brine, then dried over Na₂SO₄, filtered, and concentrated under reduced pressure prior to purification by flash chromatography (SiO₂). The following parmodulins were synthesized utilizing this method: **3**, **6**, **8**, **9**, **13**, **17**, **25**, and **35**.

4.3.2. Method B: Conversion to the acid chloride and subsequent acylation

To an oven-dried round bottom flask with stir bar under nitrogen were added the carboxylic acid, dry DCM, and 3 Å molecular sieves. Oxalyl chloride (1.2 eq.) and a catalytic amount of DMF (1–2 mol %) were added and the reaction was stirred while attached to a bubbler (to monitor production of CO₂) at 20 °C for 2–3 h. The amine HCl salt (1 eq.) in DCM and DIPEA (2 eq.) were added and the reaction was stirred under nitrogen for 3–6 h. The reaction was diluted with EtOAc and washed with half-saturated aq. NaHCO₃, 1 M HCl (30 mL), and brine, then dried over Na₂SO₄, filtered, and concentrated under reduced pressure prior to purification by flash chromatography (SiO₂). The following parmodulins were synthesized utilizing this method: **7**, **11**, **18**, and **36–40**.

4.3.3. Method C: Acylation

To an oven-dried round bottom flask with stir bar and under nitrogen were added the acid chloride, dry DCM, and 3 Å molecular sieves. The amine HCl salt (1 eq.) in DCM and DIPEA (2 eq.) were added and the reaction was stirred under nitrogen for 3–6 h. The reaction was diluted with EtOAc and washed with half-saturated aq. NaHCO₃, 1 M HCl (30 mL), brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give crude product. The following parmodulins were synthesized utilizing this method: **10**, **12**, **19**, and **24**.

4.3.4. Method D: Amide coupling using HATU

To a round bottom flask with stir bar under nitrogen was added the carboxylic acid and anhydrous DCM. The amine (1.2 eq.), HATU (1.2 eq.), and DIPEA (1.2 eq.) were added and the reaction was stirred under nitrogen. The reaction was diluted with DCM (75 mL) and washed with saturated NaHCO₃, 1 M HCl (30 mL), brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give crude material. The following parmodulins were synthesized utilizing this method: **14–16**, **31**, and **33**.

4.4. Preparation of *N*-[3-(2-bromobenzamido)phenyl]-1,3-oxazole-5-carboxamide (**38**, NRD-21, Scheme S1)

To a vial with a magnetic stir bar was added aniline **53** (50.2 mg, 0.173 mmol), oxazole-5-carboxylic acid (27 µL, 0.347 mmol), EDC-HCl (33.9 mg, 0.177 mmol), and HOBt (26.7 mg, 0.174). The vial was sealed and flushed with nitrogen for 5 min., then DCE (1.5 mL) and DMF (0.5 mL) were added. To the resulting solution was added a 10% solution of pyridine in DCE (0.14 mL, 0.173 mmol) by syringe, and the reaction was stirred for 24 h. A sample aliquot was taken from the reaction, concentrated under reduced pressure, dissolved in a minimal amount of HPLC grade MeCN, and analyzed by LC-MS to confirm reaction completion. The reaction was then diluted with EtOAc (30 mL) and washed with half-saturated aq. NaHCO₃ (3 × 15 mL), brine (2 × 10 mL), dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude product was dissolved in a minimal amount of DCM, loaded onto a 10 g silica gel column, and purified by flash chromatography (MeOH:DCM, 0–8%) to give **38** as a yellow oil (40 mg, 60%). TLC: mobile phase: MeOH:DCM (6:94), R_f = 0.30; LC-MS t_R = 4.29 min. (Characterization Method A); *m/z* = 387.29 (M + H); ¹H NMR (300 MHz, CD₃OD) δ = 8.36 (s, 1H), 8.15 (t, *J* = 2.0 Hz, 1H), 7.85 (s, 1H), 7.64 (dd, *J* = 1.0, 7.9 Hz, 1H), 7.53–7.41 (m, 5H), 7.41–7.28 (m, 3H); ¹³C NMR (100 MHz, CD₃OD) δ = 169.1, 157.2, 154.7, 147.0, 140.2, 140.1, 139.3, 134.2, 132.3, 131.2, 130.2, 129.8, 128.7, 120.5, 118.5, 118.1, 114.4. HRMS (ESI⁺) calculated for C₁₇H₁₂BrN₃O₃ (M + H) 386.0135, found 386.0147.

Author contributions

D.M.G. and R.R. contributed equally to this manuscript. Conceived project: C.D.; Designed compounds: C.D., D.M.G., R.R.; Synthesized and characterized analogs: D.M.G., R.R., N.D.-R.D., E.G., A.S., K.M.K., T.H.Y.Y., K.E.K.; Performed pharmacology and analyzed data: D.M.G., R.R. C.D.; Performed physicochemical/physiochemical profiling: E.D., L.A.A.; Performed RNA assay and analyzed related data: S.S., H.W; Wrote manuscript: C.D., R.R.; Prepared Supporting Information and edited the manuscript: R.R., D.M.G., C.D., L.A.A., S.S., E.G.

Funding sources

C.D. thanks the National Heart, Lung, and Blood Institute (NHLBI) (R15HL127636) for support, and Marquette University for startup funding. H.W. and S.S. thank NHLBI for funding (R01HL133348). K.M.K. (Honors Program), D.M.G., and R.R. thank Marquette University for summer support. N.D.-R.D thanks the National Science Foundation (via Milwaukee Area Technical College BEST program) for a fellowship.

Notes

A patent application describing compounds reported in this manuscript has been submitted. C.D. is an inventor on a patent (WO 2012/040636) containing previously reported compounds included in this paper. An earlier version of this manuscript was posted to the preprint server ChemRxiv.³³

Acknowledgments

We thank Irene Hernandez, and Trudy Holyst (Blood Research Institute) for assistance with cell culture and assay troubleshooting, Dr. Peter Newman and Dr. Huiying Zhi (Blood Research Institute) for assistance with the platelet aggregation assay, and Dr. Sheng Cai and Dennis Wiedenhoef (Marquette University) for assistance with LC-MS and NMR instruments. We thank ACD/Labs (NMR processing) and ChemAxon (NMR prediction and compound naming and property prediction) for providing access to software. Receptor binding profiles were provided by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract # HHSN-271-2018-00023-C (NIMH PDSP). The NIMH PDSP is Directed by Bryan L. Roth MD, PhD at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscoll at NIMH, Bethesda MD, USA.

Appendix A. Supplementary data

The Parmodulin NRD-21 is an Allosteric Inhibitor of PAR1 Gq Signaling with Improved Anti-Inflammatory Activity and Stability

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1. Abbreviations

AcOH	acetic acid
aPC	activated protein C
conc.	concentrated
Boc	<i>tert</i> -butoxycarbonyl (protecting group)
DCE	1,2-dichloroethane (solvent)
DCM	dichloromethane (solvent)
DIC	disseminated intravascular coagulation
DMAP	4-dimethylaminopyridine (nucleophilic catalyst)
DMF	<i>N,N</i> -dimethylformamide (solvent)
DMSO	dimethylsulfoxide (solvent)
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (amide coupling reagent)
DIPEA	<i>N,N</i> -diisopropylethylamine (base)
EtOAc	ethyl acetate (solvent)
FBS	fetal bovine serum

GFP	green fluorescent protein
GPCR	G-protein coupled receptor
HATU	1-[bis(dimethylamine)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (amide coupling reagent)
HBSS	Hank's balanced salt solution
HEPES	4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (buffering agent)
HOBt	1-hydroxybenzotriazole (additive for amide coupling)
HPLC	high performance liquid chromatography
HTS	high-throughput screening
HUVEC	human umbilical vein cells
iCa²⁺	intracellular calcium mobilization
IC₅₀	half-maximal inhibitory concentration
IPA	isopropyl alcohol
LCMS	liquid chromatography-mass spectrometry
NMR	nuclear magnetic resonance
MI	myocardial infarction
MeCN	acetonitrile (solvent)
PAR	protease-activated receptor
Pd/C	palladium on carbon (charcoal)
PGE1	prostaglandin E1
Phe	phenylalanine
PRP	platelet-rich plasma
SAR	structure-activity relationship
SEM	standard error of the mean
TEA	triethylamine (base)
TFA	trifluoroacetic acid
THF	tetrahydrofuran (solvent)
TNF-α	Tumor Necrosis Factor-alpha

2. Assay protocols

a) Intracellular calcium mobilization assay

All assays, including washing studies, were run according to our previously published protocol.¹

b) Platelet aggregation assay

Tyrode's buffer preparation:

137 mM NaCl	4 g
20 mM HEPES	2.6 g
13.8 mM NaHCO ₃	0.58 g
2.5 mM KCl	0.093 g
0.36 mM NaH ₂ PO ₄	0.0216 g
	500 mL pH 7.4

Just before use, 0.25% BSA and 0.1% glucose were added.

Human platelet-rich plasma (PRP) preparation:

Whole blood (10 mL) was collected from a volunteer. The blood was diluted with an equal volume of Tyrode's buffer (10 mL) and then transferred into two 17x100 mm tubes and 50 ng/mL of PGE1 (final concentration) was added. The samples were centrifuged in a Beckman GS-6 at 200 g (1700 rpm) for 7 minutes at room temperature. After completion, the PRP was removed and transferred to a new set of 17x100 tubes, then 2 mM

EDTA (final concentration) and 40 μ L per 10 mL of 0.5 M EDTA were added. The cells were again centrifuged at 700 g (1700 rpm) for 10 minutes at room temperature. The platelet cells were resuspended in Tyrode's buffer containing 50 ng/mL PGE1 and 2 mM EDTA. A small aliquot of platelet cells was taken for cell counting with a Scil Vet ABC Hematology Analyzer. After counting, the suspension was centrifuged at 700 g (1700 rpm) for 10 min. at room temperature, and then resuspended in Tyrode's buffer at 1×10^8 cells per mL.

Platelet aggregation:

Platelet aggregation was measured using a whole-blood lumi-aggregometer (Chrono-Log). Platelet cells in Tyrode's buffer (300 μ L) were transferred to a siliconized glass cuvette at 37 °C with constant stirring at 1000 rpm. The cells were dosed with a parmodulin (10 μ M final concentration) and incubated for 2 min. The agonist SFLLRN-NH2 (1.5 μ M final concentration) was then added to the cuvette and response was monitored for five minutes after the addition of the agonist.

c) Tissue Factor gene expression assay

EA.hy926 cells were seeded on a 12 well plate and treated with the parmodulins ML161 (10 μ M) and NRD-21 (10 μ M) for 4 hours. Some wells received TNF- α (25 ng/mL) 1 h after the addition of parmodulins. At the end of the 4 h experiment, cells were washed with PBS, and total RNA was extracted using TRIzol (Life Technologies, Carlsbad, CA). RNA was reverse transcribed into cDNA using the QuantiTect[®] Reverse transcription kit (Qiagen), and the levels of tissue factor (TF) gene expression was quantified by Quantitative PCR reactions using TaqMan, consisting of 5 μ L TaqMan Fast Advanced Master Mix (Applied Biosystems), 0.5 μ L each of TF and GAPDH TaqMan primers/probes (final concentrations of 900 nM/250 nM, respectively), and 20–30 ng of genomic DNA (1 μ L) in a 20 μ L reaction. Thermal cycling conditions were as follows: 1 cycle of 95 °C for 10 min., followed by 50 cycles of 95 °C for 10 s and 60 °C for 20 s. Real-time PCR primer/probes were purchased from Integrated DNA Technologies (Coralville, IA). TF Primers: forward 5'-ACCCGTCAATCAAGTCTACAC-3'; Reverse 5'-GTCTGCTTCACATCCTTCACA-3'. GAPDH Primers: forward 5'-GGATTGGTCTGATTGGG-3'; Reverse 5'-GGAAGATGGTGATGGGATT-3'

Data was analyzed with one-way ANOVA followed by Bonferroni's multiple comparisons test. *P<0.05 **P<0.01, ***P<0.001, ****P<0.0001.

d) Plasma stability protocol

Solid NRD-21 and ML-161 were dissolved in dimethyl sulfoxide (DMSO) to give 1 mM solutions. Human plasma was incubated for 30 min at 37 °C prior to the addition of each of the compounds. NRD-21 and ML-161 solutions were added to 490 μ L and 495 μ L aliquots of plasma to final concentrations of 20 μ M and 10 μ M respectively. Three replicates for each compound were incubated for 24 h at 37°C. 50 μ L aliquots of each sample were collected and quenched in 100 μ L of methanol stored on ice at seven time points of 0 min, 30 min, 1 h, 2 h, 4 h, 8 h, and 24 h. Samples were vortexed and centrifuged at 12,000 rpm. The supernatant was collected and loaded on a 0.2 μ m nylon filter plate for vacuum filtration. 50 μ L of the filtrate was diluted in 100 μ L of methanol containing 300 nM 4,5-diphenylimidazole (4,5-DPI) as an internal standard.

3. Synthetic procedures

a) General information

All reagents and solvents, including anhydrous solvents, were purchased from commercial vendors and used as received. Deionized water was purified by charcoal filtration to a minimum resistance of 15 MΩ and used for reaction workups and in reactions with water. NMR spectra were recorded on Varian 300 MHz or 400 MHz spectrometers as indicated. Proton and carbon chemical shifts are reported in parts per million (ppm; δ) relative to tetramethylsilane (^1H δ 0), or CDCl_3 (^{13}C δ 77.16), $(\text{CD}_3)_2\text{CO}$ (^1H δ 2.05, ^{13}C δ 29.84), $\text{d}_6\text{-DMSO}$ (^1H δ 2.50, ^{13}C δ 39.5), or CD_3OD (^1H δ 3.31, ^{13}C δ 49.00). NMR data are reported as follows: chemical shifts, multiplicity (obs = obscured, app = apparent, br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, comp = complex overlapping signals); coupling constant(s) in Hz; integration. Unless otherwise indicated, NMR data were collected at 25 °C. Filtration was performed by vacuum using VWR Grade 413 filter paper, unless otherwise noted. Flash chromatography was performed using Biotage SNAP cartridges filled with 40-60 μm silica gel on Biotage Isolera automated chromatography systems with photodiode array UV detectors. Analytical thin layer chromatography (TLC) was performed on Agela Technologies 0.25 mm glass plates with 0.25 mm silica gel. Visualization was accomplished with UV light (254 nm) and KMnO_4 stain, unless otherwise noted. Chemical names were generated and select chemical properties were calculated using either ChemAxon Marvin suite or ChemDraw Professional 15.1. NMR data were processed using either MestreNova or ACD/NMR Processor Academic Edition using the JOC report format. High-resolution mass spectra (HRMS) were obtained from the University of Cincinnati Environmental Analysis Service Center using an Agilent 6540 Accurate-Mass LC-MS with Q-TOF.

b) LC-MS characterization methods

Tandem liquid chromatography/mass spectrometry (LC-MS) was performed on a Shimadzu LCMS-2020 with autosampler, photodiode array detector, and single-quadrupole MS with ESI and APCI dual ionization using a Peak Scientific nitrogen generator.

Method A

Column:	Phenomenex Gemini C18 (100 x 4.6 mm, 3 μm particle size, 110 Å pore size)
Column temperature:	40 °C
Sample Injection:	1–5 μL of sample in MeCN or MeOH
Chromatographic monitoring:	UV absorbance at 210 or 254 nm
Mobile Phase:	Solvent A: H_2O w/ 0.1% formic acid; Solvent B: MeOH w/ 0.1% formic acid
Flow Rate:	1.0 mL/min
Gradient:	0 to 0.1 min: 25% MeOH (Isocratic) 0.1 min to 5 min: 25% to 95% MeOH (Gradient) 5 min to 7 min: 95% MeOH (Isocratic)

Method B

Column: Phenomenex Gemini C₁₈ (100 x 4.6 mm, 3 µm particle size, 110 Å pore size)
Column temperature: 40 °C
Sample Injection: 1–5 µL of sample in MeCN or MeOH
Chromatographic monitoring: UV absorbance at 210 or 254 nm
Mobile Phase: Solvent A: H₂O w/ 0.1% formic acid; Solvent B: MeCN w/ 0.1% formic acid
Flow Rate: 1.0 mL/min
Gradient: 0 to 0.1 min: 50% MeCN (Isocratic)
0.1 min to 5 min: 50% to 95% MeCN (Gradient)
5 min to 7 min: 95% MeCN (Isocratic)

Method C

Column: Phenomenex Gemini C₁₈ (100 x 4.6 mm, 3 µm particle size, 110 Å pore size)
Column temperature: 40 °C
Sample Injection: 1–5 µL of sample in MeCN or MeOH
Chromatographic monitoring: UV absorbance at 210 or 254 nm
Mobile Phase: Solvent A: H₂O w/ 0.1% formic acid; Solvent B: MeCN w/ 0.1% formic acid
Flow Rate: 1.0 mL/min
Gradient: 0 to 0.5 min: 50% MeCN (Isocratic)
0.5 min to 4 min: 50% to 95% MeCN (Gradient)
4 min to 6.3 min: 95% MeCN (Isocratic)

c) Preparative HPLC purification methods

Preparative liquid chromatography was performed on a Shimadzu LC-20AP preparative HPLC with autosampler, dual wavelength detector, and fraction collector.

Method A

Column: Phenomenex Gemini C₁₈ Semi Preparative Column (250 x 10 mm, 5 µm particle size, 110 Å pore size)
Mobile Phase: Solvent A: H₂O w/ 0.1% formic acid; Solvent B: MeOH w/ 0.1% formic acid
Peak collection: measured by UV absorbance at 210 or 254 nm
Sample Injection: 0.1–1.9 mL (2 mL sample loop) of sample in DMSO
Flow Rate: 6.0 mL/min
Gradient: 0 to 1.5 min: 25% MeOH
1.5 min to 8 min: 25% to 95% MeOH
8 min to 10.5 min: 95% MeOH

Method B

Column: Phenomenex Gemini C₁₈ Semi Preparative Column (250 x 10 mm, 5 µm particle size, 110 Å pore size)
Mobile Phase: Solvent A: H₂O w/ 0.1% formic acid; Solvent B: MeCN w/ 0.1% formic acid
Peak collection: measured by UV absorbance at 210 or 254 nm
Sample Injection: 0.1–1.9 mL (2 mL sample loop) of sample in DMSO
Flow Rate: 6.0 mL/min
Gradient: 0 to 1.5 min: 25% MeCN
1.5 min to 8 min: 25% to 95% MeCN
8 min to 10.5 min: 95% MeCN

d) Synthetic schemes

Scheme S1. Synthesis of oxazole NRD-21

Scheme S2. Synthesis of butoxyphenylether TY-13

Scheme S3. Synthesis of methyl carbamate VAK-9

Scheme S4. Synthesis of ethyl carbamate VAK-10

Scheme S5. Synthesis of secondary amine DG-75

Scheme S6. Synthesis of pyrrolidine analog KMK-17

e) Experimental procedures for select parmodulins

2-bromo-N-(3-nitrophenyl)benzamide (S2)

S2 was synthesized according to a previously published protocol.²

N-(3-aminophenyl)-2-bromobenzamide hydrochloride (S3)

To a round bottom flask with stir bar was added a solution of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ (6.15 g, 0.03 mol) in conc. HCl (5 mL). To this solution was added a solution of 2-bromo-N-(3-nitrophenyl)benzamide (S2, 1.75 g, 0.01 mol) in ethanol (50 mL), and the resulting suspension was stirred at 55 °C for 2 h. A sample aliquot was taken from the reaction, basified with 2M aq. NaOH, filtered, and the filtrate was concentrated under reduced pressure, dissolved in a minimal amount of HPLC grade MeCN, and analyzed with LCMS to confirm reaction completion. The reaction was cooled to 20 °C, basified with 2 M aq. NaOH (until pH 9), and filtered. The filtrate was concentrated under reduced pressure to half volume and then extracted with DCM (150 mL). The organic layer was washed with brine, dried over Na_2SO_4 , filtered, and concentrated under reduced pressure to give crude product. The crude material was dissolved in DCM and added to 0.9 M HCl in ether (20 mL) and stirred for 5 min. at 20 °C to form a precipitate. The precipitate was filtered, washed with DCM, collected, and dried to give aniline HCl salt S3 (1.6 g, 81%) as a yellow solid. This compound has been previously reported and characterized (CAS# 331445-38-6).

2-bromo-N-(3-butanamidophenyl)benzamide (1)

1 was synthesized according to a previously published protocol.²

2-bromo-N-[3-(3-methylbutanamido)phenyl]benzamide (**2**)

2 was synthesized according to a previously published protocol.²

2-bromo-N-(3-cyclopentaneamidophenyl)benzamide (**3**)

3 was synthesized according to a previously published protocol.²

3-(2-chlorobenzamido)-N-propylbenzamide (**4**)

4 was synthesized according to a previously published protocol.²

2-bromo-N-[3-(butylamino)phenyl]benzamide (**5**)

5 was synthesized according to a previously published protocol.¹

2-bromo-N-[3-(2-methylbutanamido)phenyl]benzamide (**7**)

Analogue (**7**) was synthesized by coupling **S3** with 2-methylbutanoyl chloride (59 mg, 0.489 mmol) according to general method B. The crude material was dissolved in a minimal amount of DCM, loaded onto a 10 g silica column, and purified by flash chromatography (EtOAc:hexanes, 0–47%) to give **7** as a white solid (80 mg) in 44% yield. LC/MS t_R = 5.57 min (Characterization Method A); m/z = 375.00 (M + H); ¹H NMR (400 MHz, (CD₃)₂CO) δ = 9.54 (br. s., 1 H), 9.22 (br. s., 1 H), 8.16 (br. s., 1 H), 7.72 - 7.20 (m, 7 H), 2.51 - 2.38 (m, 1 H), 1.78 - 1.64 (m, 1 H), 1.51 - 1.37 (m, 1 H), 1.19 - 1.11 (m, 3 H), 0.97 - 0.86 (m, 3 H).

2-bromo-N-[3-(3,3,3-trifluoropropanamido)phenyl]benzamide (9)

Analogue (9) was synthesized by coupling S3 with 3,3,3-trifluoropropanoic acid (15 μ L, 0.170 mmol) according to general method A. The crude material was dissolved in a minimal amount of DCM, loaded onto a 5 g silica column, and purified by flash chromatography (EtOAc:hexanes, 0–80%) to give 9 as a yellow oil (19 mg) in 28% yield. LC/MS tR = 4.93 min (Characterization Method A); m/z = 402.70 (M + H); ^1H NMR (300 MHz, CD_3OD) δ = 8.00 (t, J = 1.9 Hz, 1 H), 7.65 (dd, J = 0.7, 7.9 Hz, 1 H), 7.52 - 7.24 (m, 6 H), 3.33 (q, J = 1.0 Hz, 2 H).

3-nitro-N-propylbenzamide (S4)

To an oven-dried round bottom flask with stir bar under nitrogen were added 3-nitrobenzoic acid (1 g, 5.98 mmol), dry DCM, and 3 $\text{\AA}$ molecular sieves. Oxalyl chloride (1.2 eq.) and a catalytic amount of DMF (1 mol %) were added and the reaction was stirred while attached to a bubbler (to monitor production of CO_2) at 20 $^\circ\text{C}$ for 2 h. propylamine (1 eq.) in DCM and DIPEA (2 eq.) were added and the reaction was stirred under nitrogen for 23 h. The reaction was diluted with EtOAc and washed with half-saturated aqueous NaHCO_3 , 1M HCl (30 mL), brine, dried over Na_2SO_4 , filtered, and concentrated under reduced pressure to give S4 (1 g) as a beige solid in 83% yield. The material was pushed forward without purification. LC/MS tR = 6.88 min (Characterization Method A); m/z = 209.70 (M + H); ^1H NMR (400MHz, CDCl_3) δ = 8.55 (t, J = 1.9 Hz, 1 H), 8.21 - 8.15 (m, 1 H), 8.12 (td, J = 1.2, 7.8 Hz, 1 H), 7.77 (t, J = 5.4 Hz, 1 H), 7.49 (t, J = 8.0 Hz, 1 H), 3.39 - 3.28 (m, 2 H), 1.57 (sxt, J = 7.3 Hz, 2 H), 0.86 (t, J = 7.4 Hz, 3 H).

3-amino-N-propylbenzamide (S5)

A solution of S4 (940 mg, 4.51 mmol) in EtOH (20 mL) was flushed with nitrogen for 5 min. To a 250 mL round bottom hydrogenator flask under nitrogen was suspended 10% Pd/C (240 mg, 0.226 mmol) in EtOH/ H_2O (4:1) and the resultant suspension was added to the solution of S4. The flask was attached to the hydrogenator and the reaction was purged with H_2 gas for 2 min. The reaction was left to stir at room temperature under 25 bar of H_2 for 1 h, after which an aliquot was taken from the reaction, filtered through celite and cotton, and concentrated under reduced pressure. The aliquot was used for LCMS monitoring which showed reaction completion. The reaction was filtered through a funnel packed with Celite. The filter cake was washed with MeOH and the filtrate was concentrated under reduced pressure. The free-amine was dissolved in DCM and added to 0.6 M HCl in ether (20 mL) to give S5 (900 mg) as a clear yellow oil in 93% yield. The material was pushed forward without purification. LC/MS tR = 3.84 min (Characterization Method A); m/z = 178.85 (M + H); ^{13}C NMR (75 MHz, CD_3OD) δ = 166.8, 136.6, 131.3, 130.1, 127.0, 125.6, 122.0, 41.5, 22.3, 10.4.

3-(2-bromobenzamido)-N-propylbenzamide (12)

Analogue (9) was synthesized by acylating S5 with 2-bromobenzoyl chloride (53 μ L, 0.408 mmol) according to general method C. The crude material was dissolved in a minimal amount of DCM, loaded onto a 25 g silica column, and purified by flash chromatography (EtOAc:hexanes, 0–50%) to give 12 as a white solid (26 mg) in 23% yield. LC/MS tR = 5.41 min (Characterization Method A); m/z = 384.80 (M + Na); ¹H NMR (400 MHz, CD₃OD) δ = 8.11 (s, 1 H), 7.83 (d, J = 8.0 Hz, 1 H), 7.69 (d, J = 8.0 Hz, 1 H), 7.61 - 7.50 (m, 2 H), 7.50 - 7.36 (m, 3 H), 3.34 (t, J = 7.3 Hz, 2 H), 1.64 (sxt, J = 7.2 Hz, 2 H), 0.98 (t, J = 7.3 Hz, 3 H).

2-bromo-N-[3-(3-methoxypropanamido)phenyl]benzamide (13)

Analogue (13) was synthesized by coupling S3 with 3-methoxypropanoic acid (16 μ L, 0.174 mmol) according to general method A. The crude material was dissolved in a minimal amount of DCM, loaded onto a 5 g silica column, and purified by flash chromatography (EtOAc:hexanes, 0–50%) to give 13 as a white solid (26 mg) in 40% yield. LC/MS tR = 4.36 min (Characterization Method A); m/z = 478.70 (M + H); ¹H NMR (300 MHz, CD₃OD) δ = 7.99 (t, J = 1.9 Hz, 1 H), 7.67 (dd, J = 0.9, 8.0 Hz, 1 H), 7.54 - 7.42 (m, 2 H), 7.42 - 7.34 (m, 3 H), 7.29 (q, J = 1.0 Hz, 1 H), 3.71 (t, J = 6.1 Hz, 2 H), 3.35 (s, 3 H), 2.61 (t, J = 6.1 Hz, 2 H).

2-bromo-N-[3-(2-hydroxybutanamido)phenyl]benzamide (14)

To an oven-dried round bottom flask with stir bar under nitrogen were added [(2S)-2-hydroxybutanoyl]oxysodium (50 mg, 0.40 mmol), PyBOP (210 mg, 0.40 mmol), and anhydrous DCE (4.0 mL). The resulting solution was stirred for 10 min at room temperature before aniline S3 (100 mg, 0.34 mmol) and DIPEA (60 μ L, 0.35 mmol) were added. The reaction was heated at 45 $^{\circ}$ C for 48 h. After 48 h, a small aliquot was taken from the reaction, concentrated under reduced pressure, dissolved in minimal amount of HPLC grade MeCN, and analyzed with LCMS to confirm reaction completion. The reaction was diluted with DCM (10 mL), washed with saturated aqueous NaHCO₃, brine, dried over Na₂SO₄, filtered, and concentrated under vacuum to give crude material which dissolved in minimal DCM, loaded on to a 10 g silica column, and purified by flash chromatography (EtOAc:hexanes 0–100%) to give 25 mg of 14 in 19% yield. LC/MS tR = 5.54 min (Characterization Method A); m/z = 400.75 (M + Na); ¹H NMR (300 MHz, CD₃OD) δ = 7.95 (t, J = 2.1 Hz, 1H), 7.66 (dd, J = 8.0, 1.1 Hz, 1H), 7.53-7.42 (m, 4H), 7.39-7.28 (m, 2H), 4.09 (dd, J = 7.3, 4.2 Hz, 1H), 1.92-1.84 (m, 1H), 1.79-1.65 (m, 1H), 1.01 (t, J = 7.4 Hz, 3H).

2-bromo-N-{3-[(2R)-2-hydroxy-3-methylbutanamido]phenyl}benzamide

To an oven-dried round bottom flask with stir bar under nitrogen were added [(2R)-2-hydroxy-3-methylbutanoic acid (39 mg, 0.33 mmol), PyBOP (160 mg, 0.31 mmol), and anhydrous DCE (3.0 mL). The resulting solution was stirred for 10 min at room temperature before aniline S3 (75.0 mg, 0.34 mmol) and DIPEA (50 μ L, 0.29 mmol) were added. The reaction was heated at 45 $^{\circ}$ C for 48 h. After 48 h, a small aliquot was taken from the reaction, concentrated under reduced pressure, dissolved in minimal amount of HPLC grade MeCN, and analyzed with LCMS to confirm reaction completion. The reaction was diluted with DCM (10 mL), washed with saturated aqueous NaHCO₃, brine, dried over Na₂SO₄, filtered, and concentrated under vacuum to afford crude material which was purified using preparative HPLC (MeOH:H₂O with 0.1% formic acid, 50-95%) to give 8 mg of 15 in 8% yield. LC/MS t_R = 5.43 min (Characterization Method A); m/z = 414.75 (M + Na); ¹H NMR (300 MHz, CD₃OD) δ = 8.99 (br. s., 1H), 7.92–7.88 (m, 1H), 7.69–7.30 (m, 8H), 4.05–4.02 (m, 1H), 3.47–3.38 (m, 1H), 2.27–2.19 (m, 1H), 1.09–1.05 (m, 3H), 0.93–0.89 (m, 3H).

2-bromo-N-{3-[(2S)-2-hydroxy-3-methylbutanamido]phenyl}benzamide

To an oven-dried round bottom flask with stir bar under nitrogen were added [(2S)-2-hydroxy-3-methylbutanoic acid (39 mg, 0.33 mmol), PyBOP (160 mg, 0.31 mmol), and anhydrous DCE (3.0 mL). The resulting solution was stirred for 10 min at room temperature before aniline S3 (75.0 mg, 0.34 mmol) and DIPEA (50 μ L, 0.29 mmol) were added. The reaction was heated at 45 $^{\circ}$ C for 48 h. After 48 h, a small aliquot was taken from the reaction, concentrated under reduced pressure, dissolved in minimal amount of HPLC grade MeCN, and analyzed with LCMS to confirm reaction completion. The reaction was diluted with DCM (10 mL), washed with saturated aqueous NaHCO₃, brine, dried over Na₂SO₄, filtered, and concentrated under vacuum to afford crude material which was purified using preparative HPLC (MeOH:H₂O with 0.1% formic acid, 50-95%) to give 9 mg of 16 in 9% yield. LC/MS t_R = 5.44 min (Characterization Method A); m/z = 414.75 (M + Na); ¹H NMR (300 MHz, CD₃OD) δ = 7.97 (t, J = 2.0 Hz, 1H), 7.67 (dd, J = 8.0, 1.1 Hz, 1H), 7.54–7.29 (m, 6H), 3.97 (d, J = 3.8 Hz, 1H), 2.22–2.12 (m, 1H), 1.05 (d, J = 6.9 Hz, 3H), 0.92 (d, J = 6.8 Hz, 3H).

2-bromo-N-[3-(2-methoxyacetamido)phenyl]benzamide (17)

Analogue (17) was synthesized via a peptide coupling between S3 (50.0 mg, 0.172 mmol) and methoxyacetic acid (23.2 mg, 0.258 mmol) according to general method A. The crude material was dissolved in a minimal amount of DCM, loaded onto a 10 g silica column, and purified by flash chromatography (MeOH:DCM, 0-10%) to give 17 as a yellow oil (46 mg) in 73% yield. LC/MS t_R = 4.39 min (Characterization Method A); m/z = 362.70 (M + H); ¹H NMR (300 MHz, CDCl₃) δ 8.00 (t, J = 1.9 Hz, 1H), 7.68 (dd, J = 7.9, 0.9 Hz, 1H), 7.54–7.29 (m, 7 H), 4.04 (s, 2H), 3.48 (s, 3H).

2-bromo-N-[3-(2-methoxypropanamido)phenyl]benzamide (18)

Analogue (18) was synthesized by acylating S3 with 2-methoxypropanoyl chloride (35.0 mg, 0.286 mmol) according to general method C. The crude material was dissolved in a minimal amount of DCM, loaded onto a 10 g silica column, and purified by flash chromatography (EtOAc:hexanes, 0–60%) to give 18 as a white solid (36 mg) in 33% yield. LC/MS tR = 5.20 min (Characterization Method A); m/z = 400.75 (M + Na); ¹H NMR (400 MHz, CDCl₃) δ = 8.43 (br. s, 1 H), 8.04 (br. s, 1 H), 7.99 (t, J = 2.0 Hz, 1 H), 7.61 (dd, J = 1.1, 7.9 Hz, 1 H), 7.57 (dd, J = 1.8, 7.6 Hz, 1 H), 7.47 - 7.40 (m, 2 H), 7.38 (dt, J = 1.2, 7.5 Hz, 1 H), 7.34 - 7.27 (m, 2 H), 3.79 (q, J = 6.8 Hz, 1 H), 3.46 (s, 3 H), 1.42 (d, J = 6.8 Hz, 3 H).

methyl 2-[[3-(2-bromobenzamido)phenyl]carbamoyl]acetate (19)

Analogue (19) was synthesized by acylating S3 with methyl 3-chloro-3-oxo-propanoate (120 μL, 1.12 mmol) according to general method C. The crude material was dissolved in a minimal amount of DCM, loaded onto a 10 g silica column, and purified by flash chromatography (EtOAc:hexanes, 0–80%) to give 19 as a pale yellow solid (100 mg) in 26% yield. ¹H NMR (300 MHz, CDCl₃) δ = 9.30 (s, 1 H), 8.38 (s, 1 H), 7.87 (s, 1 H), 7.59 - 7.43 (m, 3 H), 7.35 - 7.26 (m, 2 H), 7.24 - 7.19 (m, 1 H), 3.71 (s, 3 H), 3.36 (s, 2 H).

2-[[3-(2-bromobenzamido)phenyl]carbamoyl]acetic acid (20)

To a 25 mL round bottom flask with a stir bar was added 19 (23.0 mg, 0.0588 mmol) and THF (4 mL) and H₂O (2 mL). LiOH (3 mg, 0.134 mmol) was added and the reaction was stirred for 1 h at room temperature. The reaction was diluted with EtOAc (15 mL) and washed with 1 M HCl (15 mL). The layers were separated, and a second extraction was performed on the aqueous layer with EtOAc (15 mL). The organic layers were combined and washed with brine (2X 10 mL), dried over with NaSO₄, filtered, and concentrated under reduced pressure to give the desired product 20 as a light brown solid (20 mg) in 90% yield. ¹H NMR (300 MHz, CD₃OD) δ = 8.01 (d, J = 1.3 Hz, 1 H), 7.68 (d, J = 8.0 Hz, 1 H), 7.56 - 7.44 (m, 2 H), 7.44 - 7.37 (m, 3 H), 7.36 - 7.25 (m, 1 H), 3.46 (s, 2 H).

N'-[3-(2-bromobenzamido)phenyl]propanediamide (21)

To a 20 mL scintillation vial with a stir bar was added 19 (25.0 mg, 0.0639 mmol) and via syringe was added THF (0.5 mL) and 7 N NH₄OH (1 mL), respectively. The reaction solvent was removed under reduced pressure to give the crude product. The product was dissolved in a minimal amount of DMSO, and loaded onto a 12 g C18 column, and purified via reverse phase chromatography (MeOH:water, 0-100%) to give product 21 as a white

crystalline solid (10 mg) in 42% yield. ¹H NMR (300 MHz, CD₃OD) δ = 8.02 - 7.96 (m, 1 H), 7.67 (dd, J = 1.1, 8.0 Hz, 1 H), 7.54 - 7.45 (m, 2 H), 7.44 - 7.36 (m, 3 H), 7.35 - 7.25 (m, 1 H), 3.37 (s, 2 H).

methyl N-[3-(2-bromobenzamido)phenyl]carbamate (22)

To an oven-dried round bottom flask with stir bar under nitrogen were added aniline S3 (50 mg, 0.172 mmol), anhydrous DCE, and TEA (23.9 μ L, 0.172 mmol). The vessel was flushed with nitrogen gas for an additional 5 min and then methyl chloroformate (13 μ L, 0.172 mmol) was added and the reaction was allowed to stir at room temperature for 48 h. A sample aliquot was taken from the reaction, concentrated under reduced pressure, dissolved in a minimal amount of HPLC grade MeCN, and analyzed with LCMS to confirm reaction completion. The reaction was diluted with EtOAc and washed with half-saturated aqueous NaHCO₃, 1M HCl (30 mL), brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give crude material which was dissolved in a minimal amount of DCM, loaded onto a 10 g silica column, and purified by flash chromatography (MeOH:DCM, 0–4%) to give 22 as a colorless oil (15 mg) in 25% yield. LC/MS tR = 4.70 min (Characterization Method A); m/z = 350.65 (M + H). ¹H NMR (400 MHz, CD₃OD) δ 7.87–7.85 (m, 1H), 7.68 (ddd, J = 8.0, 1.2, 0.4 Hz, 1H), 7.52 (ddd, J = 7.6, 1.9, 0.4 Hz, 1H), 7.47 (td, J = 7.5, 1.2 Hz, 1H), 7.38 (ddd, J = 8.0, 7.3, 1.9 Hz, 1H), 7.33–7.25 (m, 3H), 3.74 (s, 3H).

ethyl N-[3-(2-bromobenzamido)phenyl]carbamate (23)

To an oven-dried round bottom flask with stir bar under nitrogen were added aniline S3 (50 mg, 0.172 mmol), anhydrous DCE, and TEA (23.9 μ L, 0.172 mmol). The vessel was flushed with nitrogen gas for an additional 5 min and then ethyl chloroformate (16 μ L, 0.172 mmol) was

1-butoxy-3-nitrobenzene (S6)

To a round bottom flask with stir bar under nitrogen were added 3-nitrophenol (500 mg, 3.59 mmol), 60% NaH in mineral oil (0.154 mL, 5.39 mmol), and DMF (6 mL). The flask was flushed with nitrogen for an additional 5 min and 1-bromobutane (0.466 mL, 4.31 mmol) was syringed into the vessel. The reaction was stirred under nitrogen overnight at 125 °C. A small aliquot of the reaction was taken and monitored via TLC which showed consumption of starting material and formation of desired product. The reaction was diluted with EtOAc (30 mL) and transferred to a 500 mL separatory funnel. The organic layer was washed with water (3x 20mL) and then collected in an Erlenmeyer flask and dried with sodium sulfate. The dry organic solvent was transferred to a pre-weighed round bottom flask, concentrated down on the rotovap and placed on the hi-vac overnight to give S6 (692 mg) as a clear oil in 99% yield. The material was pushed forward without purification. This compound has been previously reported and characterized (CAS#122329-01-5). TLC: mobile phase: EtOAc:hexanes (25:75), Rf:

0.9; ¹H NMR (400 MHz, CDCl₃) δ = 7.80 (td, J = 0.9, 8.2 Hz, 1 H), 7.71 (t, J = 1.0 Hz, 1 H), 7.41 (t, J = 8.2 Hz, 1 H), 7.21 (td, J = 1.0, 8.2 Hz, 1 H), 4.04 (t, J = 1.0 Hz, 2 H), 1.85 - 1.76 (m, 2 H), 1.57 - 1.46 (m, 2 H), 0.99 (t, J = 7.4 Hz, 3 H).

S7

3-butoxyaniline (S7)

A solution of S6 (692 mg, 3.54 mmol) in MeOH (5 mL) was flushed with nitrogen for 5 min. To a 50 mL round bottom flask under nitrogen was suspended 10% Pd/C (197 mg, 3.54 mmol) in MeOH/H₂O (4:1) and the resultant suspension was added to the solution of S6. A balloon was filled with H₂ gas and inserted into the flask and the reaction was purged with H₂ gas for 2 min. The reaction was left to stir under a full balloon of H₂ for 1 h, after which an aliquot was taken from the reaction, filtered through celite and cotton, and concentrated under reduced pressure. The aliquot was used for TLC monitoring which showed reaction completion. The reaction was filtered through a funnel packed with Celite. The filter cake was washed with MeOH and the filtrate was concentrated under reduced pressure, to give S7 (553 mg) as a dark yellow oil in 94% yield. The material was pushed forward without purification. This compound has been previously reported and characterized (CAS#23079-68-7). TLC: mobile phase: EtOAc:hexanes (25:75), R_f: 0.55; ¹H NMR (300 MHz, CDCl₃) δ = 7.04 (t, J = 8.0 Hz, 1 H), 6.38 - 6.21 (m, 3 H), 3.91 (t, J = 6.5 Hz, 2 H), 1.80 - 1.67 (m, 2 H), 1.47 (m, 2 H), 0.96 (t, J = 7.4 Hz, 3 H). filled with H₂ gas and inserted into the flask and the reaction was purged with H₂ gas for 2 min. The reaction was left to stir under a full balloon of H₂ for 1 h, after which an aliquot was taken from the reaction, filtered through celite and cotton, and concentrated under reduced pressure. The aliquot was used for TLC monitoring which showed reaction completion. The reaction was filtered through a funnel packed with Celite. The filter cake was washed with MeOH and the filtrate was concentrated under reduced pressure, to give S7 (553 mg) as a dark yellow oil in 94% yield. The material was pushed forward without purification. This compound has been previously reported and characterized (CAS#23079-68-7). TLC: mobile phase: EtOAc:hexanes (25:75), R_f: 0.55; ¹H NMR (300 MHz, CDCl₃) δ = 7.04 (t, J = 8.0 Hz, 1 H), 6.38 - 6.21 (m, 3 H), 3.91 (t, J = 6.5 Hz, 2 H), 1.80 - 1.67 (m, 2 H), 1.47 (m, 2 H), 0.96 (t, J = 7.4 Hz, 3 H).

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2-bromo-N-(3-butoxyphenyl)benzamide (24)

Analogue (24) was synthesized by coupling S7 with 2-bromobenzoyl chloride (956 mg, 4.35 mmol) according to general method C. The crude material was dissolved in a minimal amount of DCM, loaded onto a 50 g silica column, and purified by flash chromatography (EtOAc:hexanes, 0–30%) to give 24 as a white solid (138 mg) in 12% yield. ¹H NMR (400 MHz, CDCl₃) δ = 7.69 - 7.63 (m, 2 H), 7.62 (br. s., 1 H), 7.46 - 7.38 (m, 2 H), 7.37 - 7.30 (m, 1 H), 7.26 - 7.22 (m, 1 H), 7.10 - 7.04 (m, 1 H), 6.75 - 6.70 (m, 1 H), 4.00 (t, J = 6.5 Hz, 2 H), 1.83 - 1.73 (m, 2 H), 1.54 - 1.45 (m, 2 H), 0.98 (t, J = 7.4 Hz, 3 H).

2-bromo-N-{3-[2-(dimethylamino)acetamido]phenyl}benzamide (25)

Analogue (25) was synthesized via a peptide coupling between S3 (50.0 mg, 0.172 mmol), and 2-(dimethylamino)acetic acid (26.6 mg, 0.252 mmol) according to general method A. The crude material was dissolved in minimal DCM, loaded on to a 25 g silica column, and purified by flash chromatography (EtOAc:hexanes 0–100%) to give 25 as an off white solid in 44% yield. LC/MS tR = 1.68 min (Characterization Method A); m/z = 377.75 (M + H); ¹H NMR (300 MHz, CDCl₃) δ 7.99 (t, J = 1.9 Hz, 1H), 7.68 (dd, J = 7.9, 0.9 Hz, 1H), 7.54–7.28 (m, 7 H), 3.14 (s, 2H), 2.38 (s, 6H).

2-bromo-N-{3-(dipropylamino)phenyl}benzamide (26)

To a 50 mL round bottom flask was added a magnetic stir bar and 40 mg of 3 Å molecular sieves. Propanal (352 µL, 4.91 mmol) was added to the round bottom flask alongside methanol (4 mL) and Acetic acid (14.1 µL, 0.246 mmol). The vessel was sealed and flushed with nitrogen gas for 2 min and to this S3 was added into the flask. The mixture was cooled to 0°C and allowed to stir for 30 min before NaBH₃CN (61.7 mg, 0.982 mmol) was added. The reaction was allowed to stir at 0°C and gradually allowed to warm up to room temperature for an additional 1 h. A sample aliquot was taken from the reaction, diluted with DCM, and washed with half saturated Na₂CO₃. The organic layer was separated and analyzed with LCMS to confirm reaction completion. The reaction was diluted with DCM (10 mL), washed with half-saturated aq. NaHCO₃ (2 x 15 mL), dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give crude product. The crude product was dissolved in a minimal amount of DCM, loaded onto a 10 g silica column, and purified with flash chromatography (MeOH:DCM, 2–10%) to give amine 26 (96 mg) as a yellow oil in 59% yield. LC/MS tR = 5.24 min (Characterization Method A); m/z = 375.90 (M + H); ¹H NMR (400 MHz, CDCl₃) δ = 7.64 - 7.55 (m, 1 H), 7.42 - 7.23 (m, 1 H), 7.19 - 7.11 (m, 1 H), 6.74 (d, J = 7.8 Hz, 0 H), 6.44 (dd, J = 2.2, 8.4 Hz, 0 H), 3.25 (t, J = 1.0 Hz, 2 H), 1.68 - 1.57 (m, 2 H), 0.93 (t, J = 7.4 Hz, 3 H); ¹³C NMR (101 MHz, CDCl₃) δ = 165.5, 148.9, 138.7, 138.2, 133.4, 131.4, 129.7, 129.6, 127.7, 119.3, 108.3, 106.7, 103.3, 52.9, 20.4, 11.5.

2-bromo-N-{3-[(2-methoxyethyl)amino]phenyl}benzamide (27)

To a 50 mL round bottom flask was added aniline S3 (50 mg, 0.172 mmol), K₂CO₃ (48 mg, 0.343 mmol), and a magnetic stir bar. The flask was sealed and flushed with nitrogen gas for 5 min. The contents were dissolved in anhydrous DMF (5 mL) and then 1-bromo-2-methoxyethane (24 µL, 0.258 mmol) was added via syringe to the flask and stirred at 80 °C for 24 h. After 24 h, the reaction was diluted with EtOAc (30 mL), washed with half-saturated aq. NaHCO₃ (2 x 15 mL), dried over with Na₂SO₄, filtered, and concentrated under reduced pressure to give crude product. The crude product was dissolved in a minimal amount of DCM, loaded onto a 50 g silica column, and purified with flash chromatography (EtOAc:hexane, 0–65%) to give 27 (8 mg) as a yellow oil in 14% yield. ¹H NMR (400MHz, CDCl₃) δ = 7.67 - 7.62 (m, 2 H), 7.54 (br. s., 1 H), 7.42 (dt, J = 1.2, 7.6 Hz, 1 H), 7.36 -

7.28 (m, 1 H), 7.22 (t, J = 2.2 Hz, 1 H), 7.16 (t, J = 8.0 Hz, 1 H), 6.76 (dd, J = 1.2, 7.8 Hz, 1 H), 6.45 (dd, J = 1.8, 7.6 Hz, 1 H), 3.62 (t, J = 5.1 Hz, 2 H), 3.40 (s, 3 H), 3.32 (t, J = 5.3 Hz, 2 H).

3-[[[tert-butyldimethylsilyl]oxy]methyl]aniline (S8)

3-aminobenzyl alcohol (1.20 g, 9.74 mmol) was added to a 100 mL round bottom flask with a magnetic stir bar, and then dissolved in anhydrous DMF (25 mL). To the solution was added TBDMSCl (1.73 g, 11.5 mmol), DMAP (0.03 g, 0.025 mmol), and TEA (1.63 mL, 11.7 mmol) and the reaction stirred at room temperature for 24 h. A small aliquot of the reaction was taken and used for TLC monitoring, which showed consumption of starting material. The reaction was transferred to a separatory funnel and diluted with DI water and extracted with EtOAc (2x 125 mL). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give crude product. The crude material was dissolved in a minimal amount of DCM, loaded onto a 100 g silica column, and purified by flash chromatography (EtOAc:hexanes, 0–50%) to give S8 as a clear liquid (2.1 g) in 91% yield. This compound has been previously reported and characterized (CAS#159217-95-5). TLC: mobile phase: EtOAc:hexanes (30:70), R_f: 0.64; ¹H NMR (400 MHz, CDCl₃) δ = 7.12 (t, J = 7.7 Hz, 1 H), 6.74 - 6.68 (m, 2 H), 6.58 (dd, J = 2.1, 7.7 Hz, 1 H), 4.68 (s, 2 H), 3.66 (br. s., 2 H), 0.96 (s, 9 H), 0.12 (s, 6 H).

2-bromo-N-(3-[[[tert-butyldimethylsilyl]oxy]methyl]phenyl)benzamide (S9)

Aniline S8 (250 mg, 1.05 mmol) was added to a 100 mL round bottom flask with a magnetic stir bar, and then dissolved in anhydrous DCM (6 mL). The vessel was flushed with nitrogen for 5 min and to the suspension was added 2-bromobenzoic acid (240 mg, 1.19 mmol), TEA (0.31 mL, 2.21 mmol), and BOP-Cl (293 mg, 1.09 mmol). The reaction mixture stirred for 19 h at room temperature and then monitored by TLC which showed consumption of starting material. The reaction was diluted with EtOAc (30 mL) and washed with half-saturated aq. NaHCO₃, 1M HCl (30 mL), brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give crude product. The crude material was passed through a silica plug using 5% MeOH:DCM to yield S9 as a clear yellow liquid (380mg) in 86% yield. TLC: mobile phase: EtOAc: hexanes (30:70), R_f: 0.66; ¹H NMR (400 MHz, CDCl₃) δ = 7.59 (br. s., 1 H), 7.35 - 7.21 (m, 4 H), 7.11 - 6.93 (m, 3 H), 6.84 (d, J = 7.6 Hz, 1 H), 4.45 (s, 2 H), 0.65 (s, 9 H), -0.19 (s, 6 H).

2-bromo-N-[3-(hydroxymethyl)phenyl]benzamide (S10)

To a 100 mL round bottom flask, S9 (300 mg, 0.714 mmol) was added alongside a magnetic stir bar and diluted with anhydrous THF (6 mL). To the stirring reaction, 1 M TBAF in THF (0.80 mL, 0.800 mmol) was added via syringe. The reaction was stirred at room temperature for 2 h before a sample aliquot was taken from the reaction, diluted with DCM, and washed with half saturated Na₂CO₃. The organic layer was separated and analyzed with TLC to confirm reaction completion. The reaction was diluted with sat. ammonium chloride (25

mL) and transferred to a separatory funnel and extracted with EtOAc (2 x 25mL). The organic layer was washed with brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give crude product. The crude material was dissolved in a minimal amount of DCM, loaded onto a 10 g silica column, and purified by flash chromatography (EtOAc: hexanes, 20–100%) to give S10 as a white solid (164 mg) in 90% yield. TLC: mobile phase: EtOAc: hexanes (30:70), R_f: 0.23; ¹H NMR (400 MHz, (CD₃)₂CO) δ = 9.49 (br. s., 1 H), 7.84 (s, 1 H), 7.72 - 7.65 (m, 2 H), 7.57 (dd, J = 1.6, 7.6 Hz, 1 H), 7.48 (t, J = 7.5 Hz, 1 H), 7.43 - 7.36 (m, 1 H), 7.32 (t, J = 7.8 Hz, 1 H), 7.14 (d, J = 7.6 Hz, 1 H), 4.64 (s, 2 H), 4.31 (br. s., 1 H).

2-bromo-N-(3-formylphenyl)benzamide (S11)

To a 100 mL round bottom flask, alcohol S10 (105mg, 0.343 mmol) was added alongside a magnetic stir bar. The starting material was dissolved in DCM (6 mL) and DMP (382 mg, 0.901 mmol) was added with along with DI water (0.02 mL). The reaction was heated to 30 °C for 2.5 h and was then monitored by TLC which showed consumption of starting material. The reaction was filtered through a funnel packed with Celite. The filter cake was washed with DCM and the filtrate was concentrated under reduced pressure, to give crude material. The crude material was dissolved in a minimal amount of DCM and loaded onto a 10 g silica column, and purified by flash chromatography (EtOAc:hexanes, 20–100%) to give S11 as a white solid (80 mg) in 77% yield. This compound has been previously reported and characterized (CAS#1968124-06-2). TLC: mobile phase: MeOH:DCM (4:96), R_f: 0.80; ¹H NMR (400 MHz, CD₃OD) δ = 9.99 (s, 1 H), 8.28 (t, J = 1.8 Hz, 1 H), 7.95 (ddd, J = 1.1, 2.2, 8.1 Hz, 1 H), 7.74 - 7.66 (m, 2 H), 7.61 - 7.52 (m, 2 H), 7.48 (dt, J = 1.2, 7.5 Hz, 1 H), 7.43 - 7.35 (m, 1 H).

2-bromo-N-{3-[(propylamino)methyl]phenyl}benzamide (28)

To a 100 mL round bottom flask was added a magnetic stir bar and 40 mg of 3 Å molecular sieves. Aldehyde S11 (38.5 mg, 0.127 mmol) was added to the round bottom flask alongside DCE (4 mL) and acetic acid (170 μ L, 0.297 mmol). The vessel was sealed and flushed with nitrogen gas for 5 min and to this propylamine (250 μ L, 0.304 mmol) was syringed into the flask. The mixture stirred at room temperature for 1 h before STAB (59.1 mg, 0.279 mmol) was added, and the reaction was allowed to stir at room temperature for an additional 21 h. A sample aliquot was taken from the reaction, diluted with DCM, and washed with half saturated Na₂CO₃. The organic layer was separated and analyzed with TLC to confirm reaction completion. The reaction was diluted with DCM (10 mL), washed with half-saturated aq. NaHCO₃ (2 x 15 mL), dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give crude product. The crude product was dissolved in a minimal amount of DCM, loaded onto a 10 g silica column, and purified with flash chromatography (MeOH:DCM, 2–16% to give amine 28 (32 mg) as a clear oil in 73% yield. The product was converted to the HCl salt by adding 0.9 M HCl in ether (0.14 mL) and lyophilizing overnight to give the HCl salt as an off-white solid. TLC: mobile phase: MeOH:DCM (10:90), R_f: 0.46; ¹H NMR (300 MHz, D₂O) δ = 7.58 - 7.33 (m, 2 H), 7.33 - 7.01 (m, 7 H), 4.61 (s, 2 H), 2.85 - 2.69 (m, 2 H), 1.52 - 1.35 (m, 2 H), 0.74 - 0.61 (m, 3 H).

2-bromo-N-[3-(cyclopentylamino)phenyl]benzamide (29)

To a 50 mL round bottom flask was added a magnetic stir bar and 40 mg of 3 Å molecular sieves. cyclopentanone (988 µL, 11.2 mmol) was added to the round bottom flask alongside methanol (8 mL) and Acetic acid (32.0 µL, 0.558 mmol). The vessel was sealed and flushed with nitrogen gas for 2 min and to this S3 was added into the flask. The mixture was cooled to 0°C and allowed to stir for 30 min before NaBH₃CN (140 mg, 2.23 mmol) was added. The reaction was allowed to stir at 0°C and gradually allowed to warm up to room temperature for an additional 1 h. A sample aliquot was taken from the reaction, diluted with DCM, and washed with half saturated Na₂CO₃. The organic layer was separated and analyzed with LCMS to confirm reaction completion. The reaction was diluted with DCM (10 mL), washed with half-saturated aq. NaHCO₃ (2 x 15 mL), dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give crude product. The crude product was dissolved in a minimal amount of DCM, loaded onto a 10 g silica column, and purified with flash chromatography (EtOAc:hexanes, 10–60%) to give amine 29 (385 mg) as a yellow solid in 96% yield. LC/MS t_R = 5.24 min (Characterization Method A); m/z = 360.85 (M + H); ¹H NMR (300 MHz, CDCl₃) δ 7.75 (br. s., 1H), 7.57 (ddd, J = 9.7, 7.7, 1.6 Hz, 2H), 7.35 (td, J = 7.5, 1.3 Hz, 1H), 7.31–7.23 (m, 1H), 7.17 (t, J = 2.0 Hz, 1H), 7.11 (t, J = 8.0 Hz, 1H), 6.74–6.70 (m, 1H), 6.39 (ddd, J = 8.2, 2.3, 0.9 Hz, 1H), 3.83–3.73 (m, 1H), 2.16–1.92 (m, 4H), 1.74–1.45 (m, 4H). ¹³C NMR (75 MHz, CDCl₃) δ = 165.8, 149.1, 138.8, 138.3, 133.6, 131.7, 129.9, 127.9, 119.5, 109.7, 108.5, 105.0, 54.8, 38.6, 33.8, 24.3, 23.5.

2-bromo-N-[3-(pyrrolidin-1-yl)phenyl]benzamide KMK-17 (30)

To a round bottom flask was added aniline S3 (264 mg, 0.908 mmol), K₂CO₃ (157 mg, 1.13 mmol), a cat. amount of KI (0.753 mg, 0.00454 mmol) and a magnetic stir bar. The flask was sealed and flushed with nitrogen gas for 5 min. The contents were dissolved in anhydrous acetonitrile (10 mL) and then 1,4-dibromobutane (54 µL, 0.454 mmol) was added via syringe to the flask and stirred at 50 °C for 30 min. After 30 min, a sample aliquot was taken from the reaction, concentrated under reduced pressure, dissolved in a minimal amount of HPLC grade MeCN, and analyzed with LCMS which showed no reaction. Toluene (10 mL) was introduced into the reaction and the reaction was heated to 90 °C for 24 h. A sample aliquot was taken from the reaction and analyzed with LCMS to show reaction completion. The reaction was diluted with EtOAc (30 mL), washed with half-saturated aq. NaHCO₃ (2 x 15 mL), dried over with Na₂SO₄, filtered, and concentrated under reduced pressure to give crude product. The crude product was dissolved in a minimal amount of DCM, loaded onto a 10 g silica column, and purified with flash chromatography (EtOAc:hexane, 10–70% to give 30 (135 mg) as a yellow solid in 86% yield. LC/MS t_R = 6.05 min (Characterization Method A); m/z = 346.85 (M + H); ¹H NMR (300 MHz, CDCl₃) δ = 7.63 (td, J = 1.8, 7.7 Hz, 2 H), 7.59 (br. s., 1 H), 7.39 (dt, J = 1.2, 7.5 Hz, 1 H), 7.36–7.27 (m, 1 H), 7.19 (t, J = 8.1 Hz, 1 H), 7.03 (t, J = 2.0 Hz, 1 H), 6.79 (dd, J = 1.2, 7.9 Hz, 1 H), 6.38 (dd, J = 2.0, 8.1 Hz, 1 H), 3.37–3.26 (m, 4 H), 2.06–1.94 (m, 4 H).

tert-butyl (2R)-2-[[3-(2-bromobenzamido)phenyl]carbamoyl]pyrrolidine-1-carboxylate (31)

Analogue (31) was synthesized by coupling S3 with Boc-D-proline (133 mg, 0.619 mmol) according to general method D. The crude material was dissolved in a minimal amount of DCM, loaded onto a 25 g silica column, and purified by flash chromatography (EtOAc:hexanes, 0–50%) to give 31 as a white solid (150 mg) in 74% yield.

LC/MS tR = 5.70 min (Characterization Method A); m/z = 488.00 (M + H); ¹H NMR (400 MHz, CD₃OD) δ = 8.05 - 7.95 (m, 1 H), 7.66 (d, J = 8.0 Hz, 1 H), 7.54 - 7.22 (m, 7 H), 4.36 - 4.22 (m, 1 H), 3.59 - 3.40 (m, 2 H), 2.35 - 2.20 (m, 1 H), 2.03 (br s, 3 H), 1.88 (d, J = 6.8 Hz, 1 H), 1.45 (br s, 3 H), 1.37 (s, 7 H).

(2R)-N-[3-(2-bromobenzamido)phenyl]pyrrolidine-2-carboxamide (32)

To a 25 mL round bottom flask with a stir bar was added 31 (76 mg, 0.156 mmol) and anhydrous DCM (5 mL), which was subsequently flushed with nitrogen gas for 5 minutes. To the sealed flask, via syringe, was added 4 M HCl in dioxane (1.20 mL). The reaction was allowed to stir at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure (passed through a base trap) to give crude material that was suspended in EtOAc and washed with half-saturated aq. NaHCO₃, and brine. The reaction was dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give crude material. The crude material was dissolved in a minimal amount of DCM, loaded onto a 10 g silica column, and purified by flash chromatography (MeOH:DCM, 0-6%) to give 32 as a white solid (42 mg) in 69% yield. Note: after the purification the free-amine was converted to the HCl salt by introducing excess 4 M HCl in dioxane and subsequently removing the acid under reduced pressure (through a base trap). LC/MS tR = 2.59 min (Characterization Method A); m/z = 389.00 (M + H); ¹H NMR (400 MHz, CD₃OD) δ = 8.00 (t, J = 2.0 Hz, 1 H), 7.69 - 7.63 (m, 1 H), 7.55 - 7.33 (m, 5 H), 7.33 - 7.23 (m, 1 H), 3.83 (dd, J = 6.0, 8.7 Hz, 1 H), 3.14 - 2.94 (m, 2 H), 2.30 - 2.17 (m, 1 H), 1.95 - 1.76 (m, 3 H).

tert-butyl (2S)-2-[[3-(2-bromobenzamido)phenyl]carbamoyl]pyrrolidine-1-carboxylate (33)

Analogue (33) was synthesized by coupling S3 with Boc-L-proline (150 mg, 0.697 mmol) according to general method D. The crude material was dissolved in a minimal amount of DCM, loaded onto a 25 g silica column, and purified by flash chromatography (EtOAc:hexanes, 0–50%) to give 33 as a white solid (158 mg) in 70% yield.

LC/MS tR = 5.69 min (Characterization Method A); m/z = 488.95 (M + H); ¹H NMR (400 MHz, CD₃OD) δ = 8.05 - 7.95 (m, 1 H), 7.66 (d, J = 8.0 Hz, 1 H), 7.54 - 7.23 (m, 7 H), 4.37 - 4.20 (m, 1 H), 3.59 - 3.37 (m, 2 H), 2.36 - 2.19 (m, 1 H), 2.05 - 1.93 (m, 3 H), 1.93 - 1.80 (m, 1 H), 1.45 (s, 3 H), 1.39 (s, 7 H).

(2S)-N-[3-(2-bromobenzamido)phenyl]pyrrolidine-2-carboxamide (34)

To a 25 mL round bottom flask with a stir bar was added 33 (36 mg, 0.074 mmol) and anhydrous DCM (5 mL), which was subsequently flushed with nitrogen gas for 5 minutes. To the sealed flask, via syringe, was added 4 M HCl in dioxane (1 mL). The reaction was allowed to stir at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure (passed through a base trap) to give crude material that was suspended in EtOAc and washed with half-saturated aq. NaHCO₃, and brine. The reaction was dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give crude material. The crude material was dissolved in a minimal amount of DCM, loaded onto a 10 g silica column, and purified by flash chromatography (MeOH:DCM, 0-6%) to give 34 as a white solid (22 mg) in 78% yield. Note: after the purification the free-amine was converted to the HCl salt by introducing excess 4 M HCl in dioxane and subsequently removing the acid under reduced pressure (through a base trap). LC/MS t_R = 2.55 min (Characterization Method A); m/z = 389.85 (M + H); ¹H NMR (300 MHz, CD₃OD) δ = 7.98 - 7.78 (m, 1 H), 7.65 - 7.44 (m, 5 H), 7.44 - 7.25 (m, 6 H), 6.91 (d, J = 7.6 Hz, 1 H), 4.03 (dd, J = 4.6, 9.5 Hz, 1 H), 3.20 - 2.92 (m, 1 H), 2.69 - 2.56 (m, 1 H), 2.29 - 2.16 (m, 1 H), 2.08 - 1.72 (m, 3 H).

N-[3-(2-bromobenzamido)phenyl]furan-2-carboxamide (35)

Analogue (25) was synthesized via a peptide coupling between S3 (50.0 mg, 0.172 mmol), and furan-2-carboxylic acid (19.6 mg, 0.172 mmol) according to general method A. The crude material was dissolved in minimal DCM, loaded on to a 10 g silica column, and purified by flash chromatography (MeOH:DCM 0-10%) to give 35 as an off white solid in 44% yield. TLC: mobile phase: MeOH:DCM (10:90), R_f: 0.85; LC/MS t_R = 4.82 min (Characterization Method A); m/z = 386.75 (M + H); ¹H NMR (300 MHz, CDCl₃) δ 8.17 (br. s., 1H), 8.09 (s, 1H), 7.86 (br. s., 1H), 7.64-7.60 (m, 2H), 7.55-7.50 (m, 2H), 7.46-7.29 (m, 5H), 7.22 (dd, J = 3.5, 0.8 Hz, 1H), 6.56 (dd, J = 3.5, 1.8 Hz, 1H).

N-[3-(2-bromobenzamido)phenyl]oxolane-2-carboxamide (36)

Analogue (36) was synthesized by acylating S3 with tetrahydrofuran-2-carboxylic acid (41 μL, 0.431 mmol) according to general method B. The crude material was dissolved in a minimal amount of DCM, loaded onto a 10 g silica column, and purified by flash chromatography (EtOAc:hexanes, 0-100%) to give 36 as a white solid (55 mg) in 33% yield. LC/MS t_R = 5.22 min (Characterization Method A); m/z = 413.05 (M + Na); ¹H NMR (300 MHz, CDCl₃) δ = 8.54 (s, 1 H), 7.98 (t, J = 1.9 Hz, 1 H), 7.89 (br. s., 1 H), 7.67 - 7.55 (m, 2 H), 7.48 - 7.27 (m, 5 H), 4.41 (dd, J = 5.9, 8.3 Hz, 1 H), 4.11 - 3.87 (m, 2 H), 2.42 - 2.25 (m, 1 H), 2.21 - 2.06 (m, 1 H), 2.02 - 1.85 (m, 2 H).

N-[3-(2-bromobenzamido)phenyl]oxolane-3-carboxamide (37)

Analogue (37) was synthesized by acylating S3 with tetrahydrofuran-3-carboxylic acid (41 μ L, 0.431 mmol) according to general method B. The crude material was dissolved in a minimal amount of DCM, loaded onto a 10 g silica column, and purified by flash chromatography (EtOAc:hexanes, 0–100%) to give 37 as a white solid (32 mg) in 19% yield. LC/MS tR = 5.22 min (Characterization Method B); m/z = 387.05 (M + H); ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{SO}$) δ = 10.09 (br. s., 1 H), 8.08 (s, 1 H), 7.70 (d, J = 7.7 Hz, 1 H), 7.55 – 7.45 (m, 2 H), 7.44 – 7.35 (m, 2 H), 7.33 – 7.18 (m, 2 H), 3.92 (t, J = 8.2 Hz, 1 H), 3.81 – 3.63 (m, 3 H), 3.21 – 3.07 (m, 1 H), 2.13 – 1.97 (m, 2 H).

N-[3-(2-bromobenzamido)phenyl]-1,3-oxazole-4-carboxamide (39)

Analogue (39) was synthesized by acylating S3 with oxazole-4-carboxylic acid (19.7 mg, 0.174 mmol) according to general method A. The crude material was dissolved in a minimal amount of DCM, loaded onto a 5 g silica column, and purified by flash chromatography (MeOH:DCM, 0–8%) to give 39 as a yellow oil (19 mg) in 29% yield. LC/MS tR = 4.56 min (Characterization Method A); m/z = 387.05 (M + H); ^1H NMR (300 MHz, $(\text{CD}_3)_2\text{CO}$) δ = 9.50 (d, J = 1.0 Hz, 1 H), 8.58 (d, J = 1.0 Hz, 1 H), 8.40 – 8.30 (m, 2 H), 7.73 – 7.55 (m, 5 H), 7.55 – 7.30 (m, 4 H).

N-(3-benzamidophenyl)-2-bromobenzamide (40)

Analogue (40) was synthesized by acylating S3 with benzoyl chloride (29.1 mg, 0.207 mmol) according to general method A. The crude material was dissolved in a minimal amount of DCM, loaded onto a 5 g silica column, and purified by flash chromatography (MeOH:DCM, 0–8%) to give 40 as a yellow oil (32 mg) in 46% yield. LC/MS tR = 5.23 min (Characterization Method A); m/z = 396.75 (M + H); ^1H NMR (300 MHz, CD_3OD) δ = 8.14 (t, J = 1.9 Hz, 1 H), 7.96 – 7.89 (m, 2 H), 7.68 (dd, J = 0.8, 7.9 Hz, 1 H), 7.62 – 7.42 (m, 7 H), 7.41 – 7.30 (m, 2 H).

4. Compound characterization data

Shimadzu Open Solution

Project:	Dockendorff Lab
Experiment:	egreve_20170906_04
Experiment Description:	Wizard-generated sample plate
Sample:	emg-par-022-3
Sample Description:	emg-par-022-3
Data File Name:	C:\Data\Envi\emg-par-022-3.lcd
Sample Location:	Plate Number: 1 - Position: 27
Run By:	egreve
Run Started:	Wednesday, September 06, 2017 11:09:21 AM
Run Finished:	Wednesday, September 06, 2017 11:32:54 AM
Method:	051817 Std Gemini 25 MeCN

MS Chromatogram

Group#1 Scan(+) EI: TIC

MS Spectrum

Group#1 - PDA Peak: 14, RT: 5.17 to 5.49 min

PDA Chromatogram

1: Wavelength 254 nm, Band Width 4 nm

LC/MS (+ mode) for compound 3

LC/MS (+ mode) for compound 3

RR-90
5

Project: Dockendorff Lab
 Experiment: ricardo_20160416_12
 Experiment Description: Wizard-generated sample plate
 Sample: RR-PAR-090-2 Fractions 20-32
 Sample Description: RR-PAR-090-2 Fractions 20-32
 Data File Name: C:\Data\docken\RICARDO\RR-PAR-090-2 Fractions 20-34.lcd
 Sample Location: Plate Number: 1 - Position: 45
 Run By: ricardo
 Run Started: Saturday, April 16, 2016 2:20:18 PM
 Run Finished: Saturday, April 16, 2016 2:30:21 PM
 Method: Standard Gemini

MS Chromatogram
 Group#1 Scan(+) EI : TIC

MS Spectrum
 Group#1 - PDA Peak: 11, RT: 6.88 to 8.98 min

PDA Chromatogram
 1: Wavelength 254 nm, Band Width 4 nm

LC/MS (+ mode) for compound 5

S-30LC/MS (+ mode) for compound 5

Project:	Dockendorff Lab
Experiment:	dibyn_20180528_08
Experiment Description:	Wizard-generated sample plate
Sample:	NDRD-PAR-028-1-3-1
Sample Description:	NDRD-PAR-028-1-3-1
Data File Name:	C:\Data\docken\Roland\NDRD-PAR-028-1-3-1.jod
Sample Location:	Plate Number: 1 - Position: 62
Run By:	dibyn
Run Started:	Monday, May 28, 2018 2:18:43 PM
Run Finished:	Monday, May 28, 2018 2:28:46 PM
Method:	051817 Std Gemini 25 MeCN

MS Chromatogram

Group#1 Scan(+) EI : TIC
Int

MS Spectrum

Group#1 - PDA Peak: 13, RT: 5.34 to 5.61 min
Int

PDA Chromatogram

1: Wavelength 254 nm, Band Width 4 nm
mAU

LC/MS (+ mode) for compound **6**

LC/MS (+ mode) for compound **6**

Shimadzu Open Solution

Project: Dockendorff Lab
Experiment: 6485Stephaa_20140220_03
Experiment Description: Wizard-generated sample plate
Sample: AS-PAR-001-2-
Sample Description: AS-PAR-001-2-
Data File Name: C:\LabSolutions\Data\disha\AS-PAR-001-2-Jcd
Sample Location: Plate Number: 1 - Position: 42
Run By: 6485Stephaa
Run Started: Thursday, February 20, 2014 4:52:12 PM
Run Finished: Thursday, February 20, 2014 5:03:12 PM
Method: Standard Gemini

MS Chromatogram

MS Spectrum

PDA Chromatogram

LC/MS (- mode) for compound 7

LC/MS (- mode) for compound 7

¹H NMR (400 MHz, (CD₃)₂CO) of compound 7

¹H NMR (400 MHz, (CD₃)₂CO) of compound 7

EMG-21
8

Project:	Dockendorf Lab
Experiment:	egreve_20170908_03
Experiment Description:	Wizard-generated sample plate
Sample:	emg-par-021-3-r2
Sample Description:	emg-par-021-3-r2
Data File Name:	C:\Data\Eric\emg-par-021-3-r2.lcd
Sample Location:	Plate Number: 1 - Position: 40
Run By:	egreve
Run Started:	Friday, September 08, 2017 8:14:37 AM
Run Finished:	Friday, September 08, 2017 8:24:37 AM
Method:	051817_Std_Gemini_25_MeCN

MS Chromatogram

Group#1 Scan(+) EI : TIC
Int

MS Spectrum

Group#1 - PDA Peak: 9, RT: 4.36 to 8.97 min
Int

PDA Chromatogram

1: Wavelength 254 nm, Band Width 4 nm
mAU

LC/MS (+ mode) for compound 8

LC/MS (+ mode) for compound 8

Project:	Dockendorff Lab
Experiment:	dibyn_20180312_02
Experiment Description:	Wizard-generated sample plate
Sample:	NRD-PAR-025-3-1
Sample Description:	NRD-PAR-025-3-1
Data File Name:	C:\Data\docken\Roland\NRD-PAR-025-3-1.lcd
Sample Location:	Plate Number: 1 - Position: 62
Run By:	dibyn
Run Started:	Monday, March 12, 2018 4:31:41 PM
Run Finished:	Monday, March 12, 2018 4:41:43 PM
Method:	051817 Std. Gemini 25 MeCN

MS Chromatogram

Group#1 Scan(+) EI : TIC
Int

MS Spectrum

Group#1 - PDA Peak: 8, RT: 4.85 to 8.97 min
Int

PDA Chromatogram

1: Wavelength 254 nm, Band Width 4 nm
mAU

LC/MS (+ mode) for compound **9**

LC/MS (+ mode) for compound **9**

¹H NMR (400 MHz, CD₃OD) of compound **9**

¹H NMR (400 MHz, CD₃OD) of compound **9**

Project: Dockendorff Lab
Experiment: egreve_20170908_02
Experiment Description: Wizard-generated sample plate
Sample: emg-par-023-2
Sample Description: emg-par-023-2
Data File Name: C:\Data\Eric\emg-par-023-2.lcd
Sample Location: Plate Number: 1 - Position: 41
Run By: egreve
Run Started: Friday, September 08, 2017 7:55:10 AM
Run Finished: Friday, September 08, 2017 8:14:33 AM
Method: 051817 Std Gemini 25 MeCN

MS Chromatogram

Group#1 Scan(+) EI : TIC

MS Spectrum

Group#1 - PDA Peak: 10, RT: 5.03 to 8.99 min

PDA Chromatogram

1: Wavelength 254 nm, Band Width 4 nm

LC/MS (+ mode) for compound 10

LC/MS (+ mode) for compound 10

Project: Dockendorff Lab
Experiment: ricardo_20171117_01
Experiment Description: Wizard-generated sample plate
Sample: RR-PAR-010-4
Sample Description: RR-PAR-010-4
Data File Name: C:\Data\docken\RICARDO\RR-PAR-010-4.lcd
Sample Location: Plate Number: 1 - Position: 56
Run By: ricardo
Run Started: Friday, November 17, 2017 12:42:05 PM
Run Finished: Friday, November 17, 2017 1:01:42 PM
Method: 051817_Std_Gemini_25_MeCN

MS Chromatogram

Group#1 Scan(+) EI : TIC

MS Spectrum

Group#1 - PDA Peak: 9, RT: 6.33 to 8.98 min

PDA Chromatogram

1: Wavelength 254 nm, Band Width 4 nm

LC/MS (+ mode) for compound **11**

LC/MS (+ mode) for compound **11**

Project: Dockendorff Lab
 Experiment: ricardo_20160216_01
 Experiment Description: Wizard-generated sample plate
 Sample: RR-PAR-071-2
 Sample Description: RR-PAR-071-2
 Data File Name: C:\Data\docken\RICARDO\RR-PAR-071-3.lcd
 Sample Location: Plate Number: 1 - Position: 95
 Run By: ricardo
 Run Started: Tuesday, February 16, 2016 10:33:58 AM
 Run Finished: Tuesday, February 16, 2016 10:54:57 AM
 Method: Standard Gemini

LC/MS (+ mode) for compound 12

^1H NMR (400 MHz, CD_3OD) of compound 12

Project: Dockendorff Lab
 Experiment: dibyn_20180316_01
 Experiment Description: Wizard-generated sample plate
 Sample: NDRD-PAR-026-3-1
 Sample Description: NDRD-PAR-026-3-1
 Data File Name: C:\Data\docken\Roland\NDRD-PAR-026-3-1.lcd
 Sample Location: Plate Number: 1 - Position: 63
 Run By: dibyn
 Run Started: Friday, March 16, 2018 4:24:50 PM
 Run Finished: Friday, March 16, 2018 4:34:40 PM
 Method: 051817 Std Gemini 25 MeCN

MS Chromatogram

Group#1 Scan(+): EI : TIC
Int

MS Spectrum

Group#1 - PDA Peak: 11, RT: 4.26 to 4.69 min
Int

PDA Chromatogram

1: Wavelength 254 nm, Band Width 4 nm
mAU

LC/MS (+ mode) for compound **13**

LC/MS (+ mode) for compound **13**

¹H NMR (300 MHz, CD₃OD) of compound **13**

¹H NMR (300 MHz, CD₃OD) of compound **13**

Project: Dockendorff Lab
 Experiment: 1895GandhiD_20160305_02
 Experiment Description: Wizard-generated sample plate
 Sample: DG-PAR-119-4-FR13
 Sample Description: DG-PAR-119-4-FR13
 Data File Name: C:\LabSolutions\Data\disha\DG-PAR-119-4-FR13.lcd
 Sample Location: Plate Number: 1 - Position: 73
 Run By: 1895GandhiD
 Run Started: Saturday, March 05, 2016 3:49:48 PM
 Run Finished: Saturday, March 05, 2016 4:01:02 PM
 Method: Standard Gemini

MS Chromatogram

Group#1 Scan(+) EI : TIC

MS Spectrum

Group#1 - PDA Peak: 15, RT: 5.34 to 5.79 min

PDA Chromatogram

1: Wavelength 254 nm, Band Width 4 nm

LC/MS (+ mode) for compound **14**

LC/MS (+ mode) for compound **14**

¹H NMR (300 MHz, CD₃OD) of compound **14**

¹H NMR (300 MHz, CD₃OD) of compound **14**

Project:	Dockendorff Lab
Experiment:	1895GandhiD_20160223_05
Experiment Description:	Wizard-generated sample plate
Sample:	DG-PAR-120-2
Sample Description:	DG-PAR-120-2
Data File Name:	C:\LabSolutions\Data\dishai\DG-PAR-120-2.lcd
Sample Location:	Plate Number: 1 - Position: 53
Run By:	1895GandhiD
Run Started:	Tuesday, February 23, 2016 5:29:49 PM
Run Finished:	Tuesday, February 23, 2016 5:39:42 PM
Method:	Standard Gemini

MS Chromatogram

Group#1 Scan#1 El: TIC

MS Spectrum

Group#1 - PDA Peak: 11, RT: 5.26 to 5.67 min

PDA Chromatogram

1: Wavelength 254 nm, Band Width 4 nm

LC/MS (+ mode) for compound **15**

LC/MS (+ mode) for compound **15**

¹H NMR (300 MHz, CDCl₃) of compound **15**

¹H NMR (300 MHz, CDCl₃) of compound **15**

Project:	Dockendorff Lab
Experiment:	1895GandhiD_20160223_06
Experiment Description:	Wizard-generated sample plate
Sample:	DG-PAR-121-2
Sample Description:	DG-PAR-121-2
Data File Name:	C:\LabSolutions\Data\disha\DG-PAR-121-2.lcd
Sample Location:	Plate Number: 1 - Position: 54
Run By:	1895GandhiD
Run Started:	Tuesday, February 23, 2016 5:39:45 PM
Run Finished:	Tuesday, February 23, 2016 5:49:47 PM
Method:	Standard Gemini

LC/MS (+ mode) for compound **16**

¹H NMR (300 MHz, CD₃OD) of compound 16

^1H NMR (300 MHz, CD_3OD) of compound **16**

Project: Dockendorff Lab
Experiment: vlada_20180223_01
Experiment Description: Wizard-generated sample plate
Sample: VAK-PAR-012-1 workup
Sample Description: VAK-PAR-012-1 workup
Data File Name: C:\Data\docken\vlada\VAK-PAR-012-1 workup.lcd
Sample Location: Plate Number: 1 - Position: 51
Run By: vlada
Run Started: Friday, February 23, 2018 11:51:01 AM
Run Finished: Friday, February 23, 2018 12:04:25 PM
Method: 051817_Std_Gemini_25_MeCN

MS Chromatogram

Group#1 Scan(+) EI: TIC

MS Spectrum

Group#1 - MS Peak: 4, RT: 4.33 to 4.75 min

PDA Chromatogram

1: Wavelength 254 nm, Band Width 4 nm

LC/MS (+ mode) for compound **17**

LC/MS (+ mode) for compound **17**

¹H NMR (300 MHz, CDCl₃) of compound **15**

¹H NMR (400 MHz, CDCl₃) of compound **18**

¹H NMR (400 MHz, CDCl₃) of compound **18**

¹H NMR (300 MHz, CDCl₃) of compound **19**

¹H NMR (300 MHz, CDCl₃) of compound **19**

¹H NMR (300 MHz, CD₃OD) of compound **20**

¹H NMR (300 MHz, CD₃OD) of compound **20**

¹H NMR (300 MHz, CD₃OD) of compound **21**

¹H NMR (300 MHz, CD₃OD) of compound **21**

MS Chromatogram
Group#1 Scan(+) EI : TIC

LC/MS (+ mode) for compound **22**

¹H NMR (400 MHz, CD₃OD) of compound 22

^1H NMR (400 MHz, CD_3OD) of compound **22**

Project:	Dockendorff Lab
Experiment:	viada_20180216_01
Experiment Description:	Wizard-generated sample plate
Sample:	VAK-PAR-010-2 Peak 1
Sample Description:	VAK-PAR-010-2 Peak 1
Data File Name:	C:\Data\docker\viada\VAK-PAR-010-2 Peak 1.lcd
Sample Location:	Plate Number: 1 - Position: 31
Run By:	viada
Run Started:	Friday, February 16, 2018 2:52:52 PM
Run Finished:	Friday, February 16, 2018 3:02:54 PM
Method:	051817 Std_Gemini_25_MeCN

MS Chromatogram

Group#1 Scan(+) EI : TIC

MS Spectrum

Group#1 - PDA Peak 11, RT: 4.94 to 8.93 min

PDA Chromatogram

1: Wavelength 254 nm, Band Width 4 nm

LC/MS (+ mode) for compound **23**

LC/MS (+ mode) for compound **23**

¹H NMR (300 MHz, CDCl₃) of compound **23** ¹H NMR (400 MHz, CDCl₃) of compound **S6**

¹H NMR (400 MHz, CDCl₃) of compound **S7**

¹H NMR (400 MHz, CDCl₃) of compound **S7**

¹H NMR (400 MHz, CDCl₃) of compound **24**

¹H NMR (400 MHz, CDCl₃) of compound **24**

Project: Dockendorff Lab
Experiment: vlada_20180219_03
Experiment Description: Wizard-generated sample plate
Sample: VAK-PAR-011-2 Fraction 11
Sample Description: VAK-PAR-011-2 Fraction 11
Data File Name: C:\Data\docken\vlada\VAK-PAR-011-2 Fraction 11.lod
Sample Location: Plate Number: 1 - Position: 49
Run By: vlada
Run Started: Monday, February 19, 2018 3:55:43 PM
Run Finished: Monday, February 19, 2018 4:15:24 PM
Method: 051817.Std Gemini 25 MeCN

MS Chromatogram

Group#1 Scan(+) EI: TIC

MS Spectrum

Group#1 - PDA Peak: 11, RT: 1.43 to 1.96 min

PDA Chromatogram

1: Wavelength 254 nm, Band Width 4 nm

LC/MS (+ mode) for compound 25

LC/MS (+ mode) for compound 25

¹H NMR (300 MHz, CDCl₃) of compound 25

¹H NMR (300 MHz, CDCl₃) of compound 25

Project: Dockendorff Lab
Experiment: kientala_20150623_01
Experiment Description: Wizard-generated sample plate
Sample: KMK-PAR-022-2-greenfrac
Sample Description: KMK-PAR-022-2-greenfrac
Data File Name: C:\Data\docken\kientala\KMK-PAR-022-2-greenfrac.lcd
Sample Location: Plate Number: 1 - Position: 23
Run By: kientala
Run Started: Tuesday, June 23, 2015 9:35:59 AM
Run Finished: Tuesday, June 23, 2015 9:46:22 AM
Method: Standard Gemini

MS Chromatogram

Group#1 Scan(+) EI : TIC

MS Spectrum

Group#1 - PDA Peak: 12, RT: 5.08 to 5.51 min

PDA Chromatogram

1: Wavelength 254 nm, Band Width 4 nm

LC/MS (+ mode) for compound 26

LC/MS (+ mode) for compound 26

¹³C NMR (101 MHz, CDCl₃) of compound 26

¹³C NMR (101 MHz, CDCl₃) of compound 26

^1H NMR (400 MHz, CDCl_3) of compound **27**

^1H NMR (400 MHz, CDCl_3) of compound **27**

^1H NMR (400 MHz, CDCl_3) of compound **S8**

^1H NMR (400 MHz, CDCl_3) of compound **S8**

¹H NMR (400 MHz, CDCl₃) of compound **S9** ¹H NMR (400 MHz, (CD₃)₂CO) of compound **S10**

¹H NMR (400 MHz, CD₃OD) of compound **S11**

¹H NMR (400 MHz, CD₃OD) of compound **S11**

Project: Dockendorff Lab
 Experiment: 1895GandhiD_20140611_01
 Experiment Description: Wizard-generated sample plate
 Sample: DG-PAR-075-4
 Sample Description: DG-PAR-075-4
 Data File Name: C:\LabSolutions\Data\disha\DG-PAR-075-4.lcd
 Sample Location: Plate Number: 1 - Position: 101
 Run By: 1895GandhiD
 Run Started: Wednesday, June 11, 2014 12:37:36 PM
 Run Finished: Wednesday, June 11, 2014 12:48:53 PM
 Method: DG-PAR rXNmon

MS Chromatogram

Group#1 Scan(+): EI : TIC

MS Spectrum

Group#1 - PDA Peak: 11, RT: 3.62 to 4.42 min

PDA Chromatogram

1: Wavelength 254 nm, Band Width 4 nm

LC/MS (+ mode) for compound **28**

LC/MS (+ mode) for compound **28**

¹H NMR (300 MHz, D₂O) of compound **28**

¹H NMR (300 MHz, D₂O) of compound **28**

Project: Dockendorff Lab
 Experiment: kkentala_20150611_01
 Experiment Description: Wizard-generated sample plate
 Sample: KMK-PAR-018-1-OVERNIGHT
 Sample Description: KMK-PAR-018-1-OVERNIGHT
 Data File Name: C:\Data\docken\kkentala\KMK-PAR-018-1-OVERNIGHT.lcd
 Sample Location: Plate Number: 1 - Position: 67
 Run By: kkentala
 Run Started: Thursday, June 11, 2015 10:20:18 AM
 Run Finished: Thursday, June 11, 2015 10:30:25 AM
 Method: Standard Gemini

LC/MS (+ mode) for compound **29**

LC/MS (+ mode) for compound **29**

¹H NMR (300 MHz, CDCl₃) of compound **29**

¹H NMR (300 MHz, CDCl₃) of compound **29**

¹³C NMR (75 MHz, CDCl₃) of compound **29**

¹³C NMR (75 MHz, CDCl₃) of compound **29**

Project: Dockendorff Lab
Experiment: kkentala_20150610_01
Experiment Description: Wizard-generated sample plate
Sample: KMK-PAR-017-2
Sample Description: KMK-PAR-017-2
Data File Name: C:\Data\docken\kkentala\KMK-PAR-017-2.lcd
Sample Location: Plate Number: 1 - Position: 14
Run By: kkentala
Run Started: Wednesday, June 10, 2015 8:55:45 AM
Run Finished: Wednesday, June 10, 2015 9:05:47 AM
Method: Standard Gemini

MS Chromatogram

Group#1 Scan(+) EI : TIC

MS Spectrum

Group#1 - MS Peak: 3, RT: 6 to 6.34 min

PDA Chromatogram

1: Wavelength 254 nm, Band Width 4 nm

LC/MS (+ mode) for compound **30**

LC/MS (+ mode) for compound **30**

¹H NMR (300 MHz, CDCl₃) of compound **30**

¹H NMR (300 MHz, CDCl₃) of compound **30**

KMK-14A
31

Project: Dockendorff Lab
 Experiment: kktentala_20150609_02
 Experiment Description: Wizard-generated sample plate
 Sample: KMK-PAR-014-1
 Sample Description: KMK-PAR-014-1
 Data File Name: C:\Data\docken\kktentala\KMK-PAR-014-1.lcd
 Sample Location: Plate Number: 1 - Position: 13
 Run By: kktentala
 Run Started: Tuesday, June 09, 2015 2:00:12 PM
 Run Finished: Tuesday, June 09, 2015 2:13:57 PM
 Method: Standard Gemini

MS Chromatogram

Group#2 Scan(-) EI: TIC

MS Spectrum

Group#2 - MS Peak: 8, RT: 5.61 to 5.96 min

PDA Chromatogram

1: Wavelength 254 nm, Band Width 4 nm

LC/MS (+ mode) for compound 31

LC/MS (+ mode) for compound 31

KMK-14A
31

¹H NMR (400 MHz, CD₃OD) of compound 31

¹H NMR (400 MHz, CD₃OD) of compound 31

KMK-14B
32

Project: Dockendorff Lab
Experiment: kkentala_20150610_03
Experiment Description: Wizard-generated sample plate
Sample: KMK-PAR-014-1-DEPRO
Sample Description: KMK-PAR-014-1-DEPRO
Data File Name: C:\Data\docken\kkentala\KMK-PAR-014-1-DEPRO001.lcd
Sample Location: Plate Number: 1 - Position: 55
Run By: kkentala
Run Started: Wednesday, June 10, 2015 3:36:22 PM
Run Finished: Wednesday, June 10, 2015 3:46:39 PM
Method: Standard Gemini

MS Chromatogram

Group#1 Scan(+) EI : TIC
Int

MS Spectrum

Group#1 - PDA Peak: 11, RT: 2.41 to 3.63 min
Int

PDA Chromatogram

1: Wavelength 254 nm, Band Width 4 nm
mAU

LC/MS (+ mode) for compound 32

LC/MS (+ mode) for compound 32

KMK-14B
32

¹H NMR (400 MHz, CD₃OD) of compound 32

¹H NMR (400 MHz, CD₃OD) of compound 32

Project: Dockendorff Lab
 Experiment: kkentala_20150601_02
 Experiment Description: Wizard-generated sample plate
 Sample: KMK-PAR-015-1-FRACYELLOWBLUE
 Sample Description: KMK-PAR-015-1-FRACYELLOWBLUE
 Data File Name: C:\Data\docken\kkentala\KMK-PAR-015-1-FRACYELLOWBLUE.lcd
 Sample Location: Plate Number: 1 - Position: 48
 Run By: kkentala
 Run Started: Monday, June 01, 2015 11:35:59 AM
 Run Finished: Monday, June 01, 2015 11:46:05 AM
 Method: Standard Gemini

MS Chromatogram

Group#2 Scan(-) EI - TIC

MS Spectrum

Group#2 - MS Peak: 7, RT: 5.50 to 7.04 min

PDA Chromatogram

1: Wavelength 254 nm, Band Width 4 nm

LC/MS (+ mode) for compound 33

LC/MS (+ mode) for compound 33

¹H NMR (400 MHz, CD₃OD) of compound 33

¹H NMR (400 MHz, CD₃OD) of compound 33

KMK-15B
34

Project: Dockendorff Lab
Experiment: kkentala_20150605_05
Experiment Description: Wizard-generated sample plate
Sample: KMK-PAR-015-3-REDPROD-BOCDEPRO-GREENFRA2
Sample Description: KMK-PAR-015-3-REDPROD-BOCDEPRO-GREENFRA2
Data File Name: C:\Data\docken\kkentala\KMK-PAR-015-3-REDPROD-BOCDEPRO-GREENFRA2.lcd
Sample Location: Plate Number: 1 - Position: 18
Run By: kkentala
Run Started: Friday, June 05, 2015 2:23:29 PM
Run Finished: Friday, June 05, 2015 2:37:09 PM
Method: Standard Gemini

MS Chromatogram

Group#1 Scan(+): EI : TIC

MS Spectrum

Group#1 - MS Peak: 1, RT: 2.51 to 2.84 min

PDA Chromatogram

1: Wavelength 254 nm, Band Width 4 nm

LC/MS (+ mode) for compound 34

LC/MS (+ mode) for compound 34

KMK-15B
34

¹H NMR (300 MHz, CDCl₃) of compound 34

¹H NMR (300 MHz, CDCl₃) of compound 34

VAK-7
35

Project: Dockendorf Lab
Experiment: vlada_20180205_01
Experiment Description: Wizard-generated sample plate
Sample: VAK-PAR-007-2
Sample Description: VAK-PAR-007-2
Data File Name: C:\Data\docken\vlada\VAK-PAR-007-2.lcd
Sample Location: Plate Number: 1 - Position: 64
Run By: vlada
Run Started: Monday, February 05, 2018 2:56:27 PM
Run Finished: Monday, February 05, 2018 3:20:48 PM
Method: 051817 Std Gemini 25 MeCN

LC/MS (+ mode) for compound 35

VAK-7
35

^1H NMR (300 MHz, CDCl_3) of compound 35

Project: Dockendorff Lab
Experiment: AS-PAR-002-2
Experiment Description: Wizard-generated sample plate
Sample: AS-PAR-002-2
Sample Description: AS-PAR-002-2
Data File Name: C:\LabSolutions\Data\isha\AS-PAR-002-2.lcd
Sample Location: Plate Number: 1 - Position: 55
Run By: 6485Stephaa
Run Started: Thursday, March 06, 2014 7:26:49 PM
Run Finished: Thursday, March 06, 2014 7:40:38 PM
Method: Standard Gemini

LC/MS (+ mode) for compound **36**

¹H NMR (300 MHz, CDCl₃) of compound **36**

Project: Dockendorff Lab
 Experiment: AS-PAR-004-4 t14
 Experiment Description: Wizard-generated sample plate
 Sample: AS-PAR-004-4 t14
 Sample Description: C:\LabSolutions\Data\isha\AS-PAR-004-4 t14.lcd
 Data File Name: Plate Number: 1 - Position: 104
 Sample Location: 6485Stephaa
 Run By: Wednesday, April 30, 2014 2:56:45 PM
 Run Started: Wednesday, April 30, 2014 3:12:53 PM
 Run Finished: DG:25-100-MEOH-15MIN-GEMINI
 Method:

MS Chromatogram

Group#1 Scan(-) EI : TIC
 Int

MS Spectrum

Group#1 - PDA Peak: 10, RT: 7.16 to 8.80 min
 Int

PDA Chromatogram

1: Wavelength 254 nm, Band Width 4 nm
 mAU

LC/MS (+ mode) for compound **37**

LC/MS (+ mode) for compound **37**

¹H NMR (300 MHz, (CD₃)₂SO) of compound **37**

¹H NMR (300 MHz, (CD₃)₂SO) of compound **37**

Project:	Dockendorff Lab
Experiment:	dibyn_20180613_02
Experiment Description:	Wizard-generated sample plate
Sample:	NRD-PAR-033-1-2
Sample Description:	NRD-PAR-033-1-2
Data File Name:	C:\Data\docken\Roland\NRD-PAR-033-1-2.lcd
Sample Location:	Plate Number: 1 - Position: 62
Run By:	dibyn
Run Started:	Wednesday, June 13, 2018 11:50:29 AM
Run Finished:	Wednesday, June 13, 2018 12:04:51 PM
Method:	051817 Std. Gemini 25 MeCN

LC/MS (+ mode) for compound **38**

LC/MS (+ mode) for compound **38**

¹H NMR (300 MHz, CD₃OD) of compound **38**

^{13}C NMR (75 MHz, CD_3OD) of compound **38**

Project: Dockendorf Lab
 Experiment: dibyn_20180530_02
 Experiment Description: Wizard-generated sample plate
 Sample: NDRD-PAR-023-4-2
 Sample Description: NDRD-PAR-023-4-2
 Data File Name: C:\Data\docken\Roland\NDRD-PAR-023-4-2.lcd
 Sample Location: Plate Number: 1 - Position: 61
 Run By: dibyn
 Run Started: Wednesday, May 30, 2018 1:56:28 PM
 Run Finished: Wednesday, May 30, 2018 2:06:30 PM
 Method: 051817_Std_Gemini_25_MeCN

MS Chromatogram
 Group#1 Scan(+) EI: TIC

MS Spectrum
 Group#1 - PDA Peak: 11, RT: 4.45 to 8.87 min

PDA Chromatogram
 1: Wavelength 254 nm, Band Width 4 nm

LC/MS (+ mode) for compound **39**

LC/MS (+ mode) for compound **39**

¹H NMR (300 MHz, (CD₃)₂CO) of compound **39**

¹H NMR (300 MHz, (CD₃)₂CO) of compound **39**

Project:	Dockendorf Lab
Experiment:	dibyn_20180224_01
Experiment Description:	Wizard-generated sample plate
Sample:	NRD-PAR-022-2-1
Sample Description:	NRD-PAR-022-2-1
Data File Name:	C:\Data\docken\Roland\NRD-PAR-022-2-1.lcd
Sample Location:	Plate Number: 1 - Position: 77
Run By:	dibyn
Run Started:	Saturday, February 24, 2018 3:39:47 PM
Run Finished:	Saturday, February 24, 2018 4:04:16 PM
Method:	051817_Std_Gemini_25_MeCN

MS Chromatogram

Group#1 Scan(+) EI : TIC

MS Spectrum

Group#1 - PDA Peak: 11, RT: 5.12 to 5.44 min

PDA Chromatogram

1: Wavelength 254 nm, Band Width 4 nm

LC/MS (+ mode) for compound **40**

LC/MS (+ mode) for compound **40**

¹H NMR (300 MHz, (CD₃OD) of compound **40**

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Note

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