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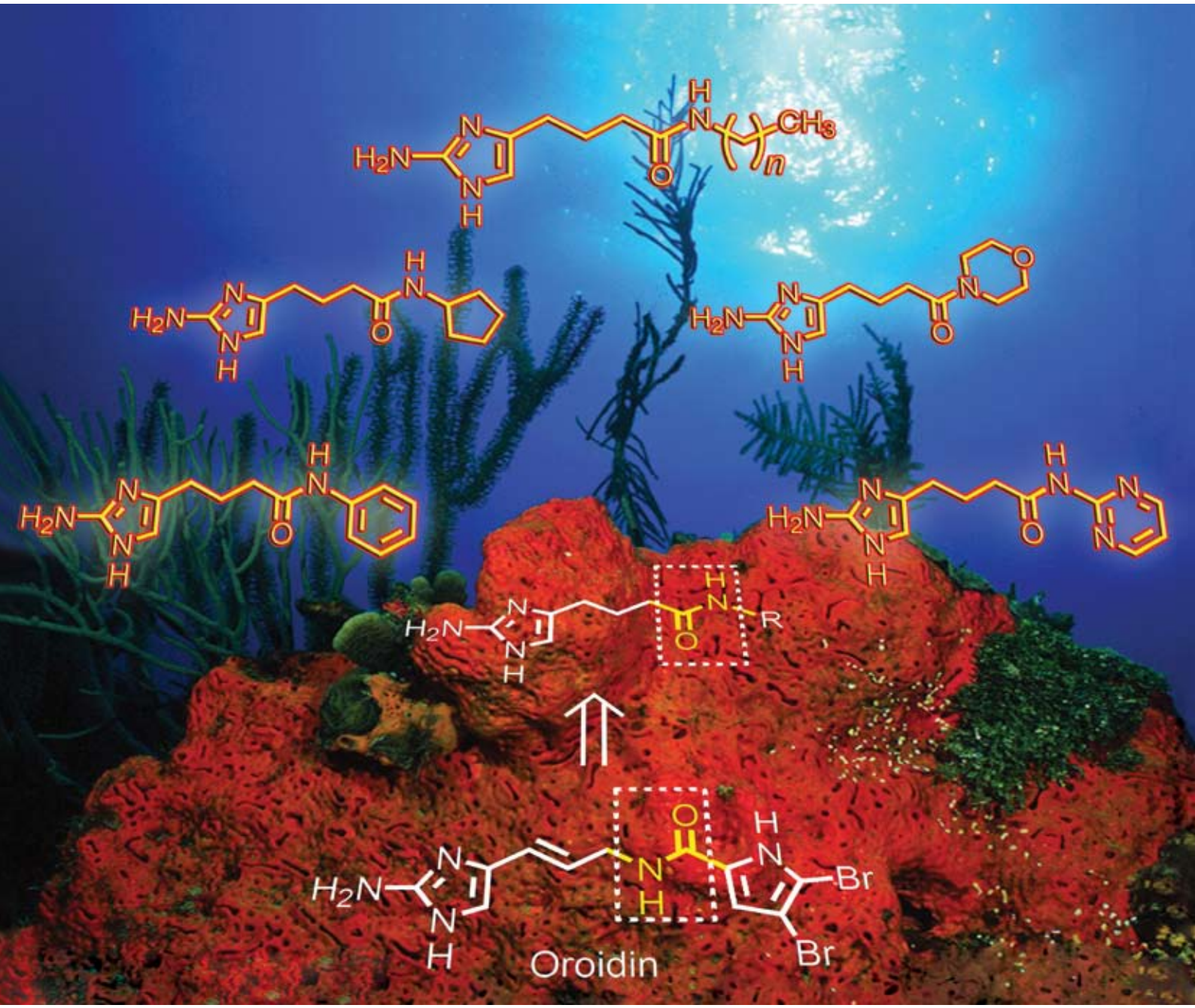

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Inhibition and dispersion of *Pseudomonas aeruginosa* biofilms with reverse amide 2-aminoimidazole oroidin analogues†

Justin J. Richards,‡ T. Eric Ballard‡ and Christian Melander*

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The marine alkaloid oroidin along with a small library of reverse amide (RA) 2-aminoimidazoles were synthesized and assayed for anti-biofilm activity against PAO1 and PA14, two strains of the medically relevant γ-proteobacterium *Pseudomonas aeruginosa.* Analogues that contained a long, linear alkyl chain were more potent inhibitors than the natural product at preventing the formation of PAO1 and PA14 biofilms. The most active compound in the series was also shown to disperse established PAO1 and PA14 biofilms at low micromolar concentrations.

Introduction

Recent developments in the fields of bacteriology and infectious disease have revealed that bacteria often exist as intertwined communities rather than as independent microorganisms.[1] These surface associated microcolonies have come to be known as biofilms and are ubiquitous in nature.[2] Biofilms underpin a significant list of problems encountered in the agricultural, engineering, and medical sectors of the global economy. The NIH estimates that approximately 3 out of 4 microbial infections that occur in the body are biofilm mediated.[3] Biofilms also underlie high morbidity rates in patients who suffer from cystic fibrosis. Bacteria that reside within the biofilm state display different phenotypes than their planktonic brethren and become more resistant to many antibiotics and biocides that would often lead to their eradication.[4,5]

Despite the prevalence of biofilms in our society, examples of molecular scaffolds that inhibit biofilm formation are scarce. These limited examples include the homoserine lactones (**1**) which are naturally occurring signaling molecules that elicit their activity through disruption of bacterial communication,[6] brominated furanones (**2**) which were originally isolated from the macroalga *Delisea pulchra*,[7,8] and ursene triterpenes (**3**) from the plant *Diospyros dendo* (Fig. 1).[9]

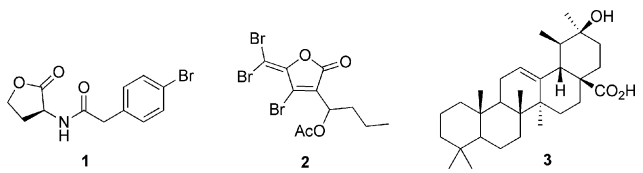


Fig. 1 Molecules known to inhibit biofilm formation.

North Carolina State University, Department of Chemistry, Raleigh, North Carolina, 27695-8204, USA. E-mail: christian_melander@ncsu.edu

† Electronic supplementary information (ESI) available: ^{1}H NMR spectra for representative compounds in the RA library, representative dose–response curves and planktonic growth curves for PAO1 and PA14 in the presence and absence of **12** and oroidin **4** are provided. See DOI: 10.1039/b719082d

‡ Both authors contributed equally to this work

Our research group has become interested in exploiting the oroidin class of marine alkaloids as molecular inspiration for developing novel compounds that possess anti-biofilm properties.[10] The activity of oroidin has been documented in a limited number of studies involving bacterial attachment and colonization.[11,12] Oroidin has also been shown to inhibit biofouling driven by the marine α-proteobacterium *R. salexigens.*[13] Due to this activity and chemical simplicity, oroidin was selected as a lead compound for structure activity relationship (SAR) studies in hopes of discovering a diverse range of compounds that possess anti-biofilm properties. One intriguing approach involved reversal of the amide bond highlighted in Fig. 2 which connects the bromopyrrole tail of oroidin **4** to the 2-aminoimidazole (2-AI) head.

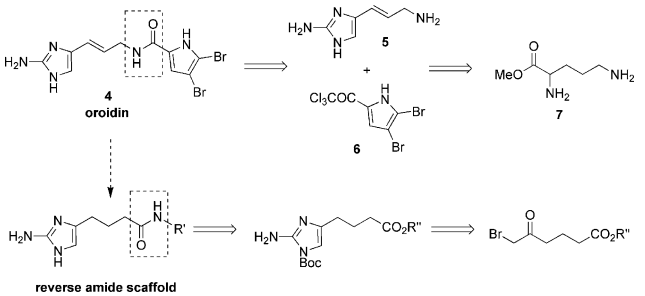


Fig. 2 Retrosynthetic analysis of oroidin and the RA scaffold.

One of the best methods for large scale preparation of the 2-AI scaffold *en route* to prepare oroidin and other family members involves Akabori reduction (Na–Hg) of ornithine methyl ester **7** followed by condensation with cyanamide under pH controlled conditions.[14–16] Derivatization can then be achieved *via* acylation of the alkyl amine off the carbon tail with variously substituted trichloroacetyl pyrroles. However, this chemistry is plagued by severe limitations, most notably the overall lack of compatibility of this system with other trichloroacetyl esters. In addition, solubility issues of the parent 2-AI leaves much to be desired. Many attempts by our group in developing other acylation conditions that would allow for the generation of greater diversity have proven unfruitful. From a practical standpoint, purifications of intermediates bearing an unprotected 2-AI often require large amounts of methanol saturated with ammonia (MeOH–NH_3),

which is cumbersome to prepare and can be difficult to remove from the pure sample after column chromatography.

Implementation of a reverse amide approach, coupled with a practical protecting group strategy, would effectively eliminate many of the aforementioned handicaps with current methods. Installation of the reverse amide bond could be obtained by direct aminolysis of an intermediate Boc-2AI alkyl ester or through couplings of a carboxylic acid (Fig. 2). These intermediates could be accessed through α-bromoketones which are obtained by diazomethane homologation with the proper acyl chloride. Additionally, significant diversity can be achieved by incorporating any commercially available amine with a common RA intermediate. Herein we report the synthesis of a focused reverse amide (RA) library (Fig. 3) and subsequent biological evaluation of the library in comparison to the natural product oroidin in the context of anti-biofilm activity of biofilms formed by the medically relevant γ-proteobacterium *Pseudomonas aeruginosa*.

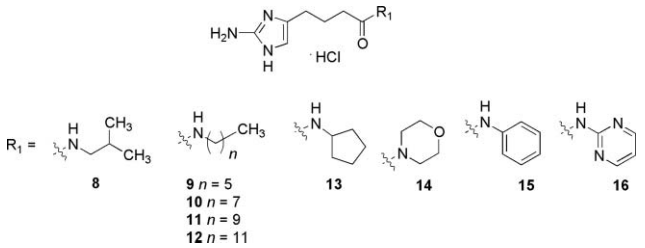
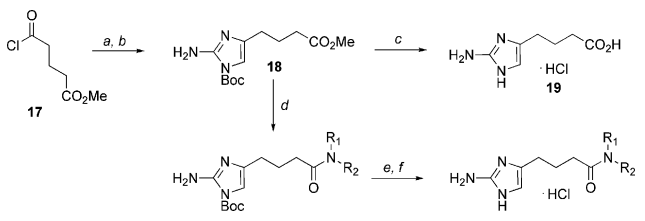


Fig. 3 Members of the RA pilot library.

Results and discussion

Synthesis of RA Library

Scaffold synthesis began with treatment of the commercially available acid chloride **17** with diazomethane (Scheme 1).[17] Quenching with concentrated HCl or HBr delivered the corresponding α-haloketones in excellent yields which were isolable by column chromatography. Installation of the protected 2-aminoimidazole moiety was achieved through a Boc-guanidine condensation in DMF at ambient temperature to yield **18**. Significantly higher yields for this step were obtained when two equivalents of sodium iodide were added to the reaction mixture and represents a significant improvement over previous reports.[18,19] It was also observed during this sequence that the α-bromoketone afforded higher yields than its α-chloro counterpart in the cyclization reaction.

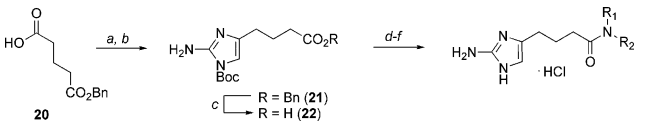


Scheme 1 1st Generation synthesis of the RA scaffold. *Reaction conditions*: (*a*) *i*. CH_2N_2, Et_2O–CH_2Cl_2, 0 °C *ii*. conc. HCl (90%) or conc. HBr (93%) (*b*) Boc-guanidine. NaI, DMF, 65% (*c*) LiOH, MeOH–THF–H_2O (3 : 1 : 1) then 1 N HCl to pH = 5, 94% (*d*) $AlMe_3$, NR_1R_2, DCE, 0 °C to 60 °C (*e*) TFA, CH_2Cl_2, (*f*) 2 M HCl in Et_2O.

The first approach to the RA scaffold relied heavily on the aminolysis of intermediate **18** since this would afford the Boc-protected RA precursors in a single synthetic step. After deprotection with TFA and HCl salt exchange, isolation of the targets would require only filtration with no need for further purification. Based upon the seminal paper published by Weinreb and co-workers on the transformation, trimethylaluminium was used as the Lewis acid to affect the direct aminolysis reaction.[20] Numerous reaction factors were taken into account such as choice of solvent, equivalents of aluminium–amine complex, reagent order of addition, time, and temperature. Despite all of the conditions scanned (data not shown), the highest yielding reaction occurred in only 55% yield when aniline was used as the amine partner. Triazabicyclo[4.4.0]dec-5-ene (TBD) was also examined as a potential catalyst to promote the direct aminolysis of ester **18**.[21] Heating both starting materials in the presence of 30 mol% of TBD in toluene at elevated temperatures for extended periods of time failed to produce any desired product as evident by TLC analysis (data not shown).

Due to the problems encountered utilizing aminolysis, we opted for a more conventional route to access the RA scaffold through the intermediacy of an activated carboxylic acid. Unfortunately, saponification of the methyl ester **18** proved problematic on this system as cleavage of the Boc group was observed under the basic conditions of both LiOH–MeOH–THF–H_2O or LiI–pyridine. Decomposition of the methyl ester was also observed when TMSOK in methylene chloride or $(Bu_3Sn)_2O$ in toluene at either ambient temperature or reflux were employed as the saponification agents (data not shown).

Persuaded by these results that the current route required revision, we began a second generation approach to our core scaffold (Scheme 2). This approach relied on a different protecting group strategy, substituting the methyl ester for a benzyl ester which, in the case of another failed attempt at aminolysis, would undergo hydrogenolysis under mild conditions to deliver the corresponding Boc-protected acid **22**. Synthesis began with the known monobenzyl ester acid[22] **20** which was transformed into the benzyl protected α-bromoketone by conversion to its acid chloride followed by diazomethane homologation and concomitant quench with concentrated HBr. Cyclization of this intermediate afforded the Boc-protected 2-AI **21** in 66% yield. All attempts at direct aminolysis of benzyl ester **21** resulted in sluggish reactions that were plagued by the formation of multiple side products.



Scheme 2 2nd Generation synthesis of the RA scaffold. *Reaction conditions*: (*a*) *i*. $(COCl)_2$, DMF (cat.), CH_2Cl_2 *ii*. CH_2N_2, Et_2O–CH_2Cl_2, 0 °C *iii*. conc. HBr, 88% (*b*) Boc-guanidine, DMF, 66% (*c*) H_2 (1 atm), 10% Pd/C, THF, 98% (*d*) EDC, HOBt, NR_1R_2, DMF (*e*) TFA, CH_2Cl_2 (*f*) 2 M HCl in Et_2O.

Given the failure of the direct aminolysis conversion, the two-step approach to the RA scaffold was investigated. Deprotection proceeded as planned and was accomplished by subjecting **21** to a hydrogen atmosphere at balloon pressure which cleanly afforded pure Boc-protected acid **22** in near quantitative yield (98%). With

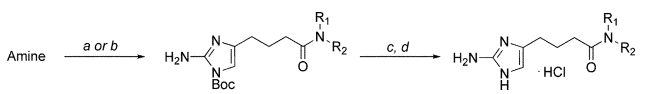
the acid now in hand and available on a multi-gram scale, attempts to install the key amide bond were assessed. A number of activating agents were scanned including DCC, EDC, HCTU, CDI, and cyanuric chloride to affect the transformation. Of those listed only EDC and HCTU were able to give consistent and tangible results. EDC was chosen over HCTU due to ease of purification in separating side products during column chromatography. It was during this optimization that the limitation of the synthetic route was identified to be the reactivity of the Boc group. A significant quantity of a Boc-protected starting amine was isolated and characterized, signifying the lability of the Boc-group due to Boc-transfer under the reaction conditions regardless of which activating agent was used.

With two routes in hand to generate the RA scaffold, we assembled the focused library outlined in Table 1. EDC–HOBt couplings of acid **22** were used to generate most of the linear alkyl chain analogues (28–34%) while aminolysis of the methyl ester intermediate **18** furnished the remaining compounds (11–55%) in the library (Table 1). The final step of the synthetic approach required removal of the Boc group, which proceeded at room temperature in TFA–DCM. The resulting trifluoroacetate salts of each target were then traded out for their HCl counterparts before characterization and assessment of their biological activity.

Biological evaluation of RA library

Nosocomial infections are driven by a persistent bacterial colonization of hospital facilities, wherein the bacteria are extremely resistant to eradication because they exist in a biofilm state. *P. aeruginosa* is an opportunistic γ-proteobacterium that is a serious threat to immunocompromised patients and is frequently isolated from patients found in intensive care units suffering from severe burns or other traumas. It is the second most common pathogen in hospital-acquired pneumonia behind *Staphylococcus aureus.*[23] For cystic fibrosis patients, the onset of colonization by this bacterium is of great concern. Morbidity rates of patients who suffer from the disease are directly correlated to the virulence of *P. aeruginosa* biofilms.[24,25] The speed and prevalence with which multidrug resistant (MDR) strains are appearing puts pressure on the medical community to find ways to combat the aggressive nature of this bacterium.[26]

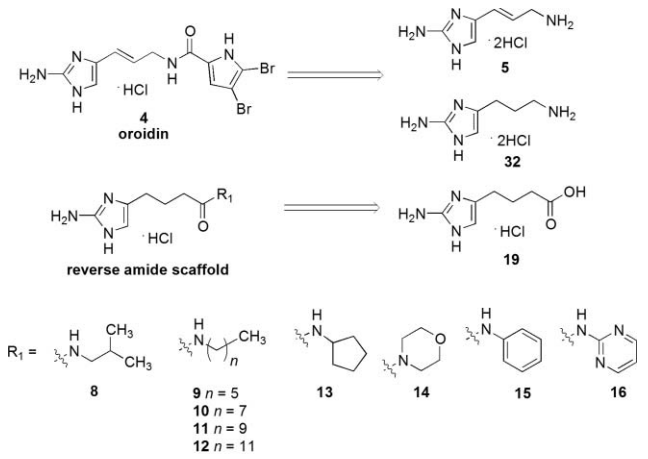
Table 1 Completion of the RA library



Amine	Conditions	Coupled product	Target
Isobutylamine	*a*	**23**	**8**
Hexylamine	*b*	**24**	**9**
Octylamine	*b*	**25**	**10**
Decylamine	*a*	**26**	**11**
Dododecylamine	*b*	**27**	**12**
Cyclopentylamine	*a*	**28**	**13**
Morpholine	*a*	**29**	**14**
Aniline	*a*	**30**	**15**
2-Aminopyrimidine	*a*	**31**	**16**

Reaction conditions: (*a*) $AlMe_3$, **18**, DCE, 0 °C to 60 °C (*b*) **22**, EDC, HOBt, DMF (*c*) TFA, CH_2Cl_2, (*d*) 2 M HCl in Et_2O

Members of the reverse amide library along with oroidin[16] were initially screened at 500 μM in a 96-well format using a crystal violet reporter assay to assess each compound's ability to inhibit the formation of PAO1 or PA14 biofilms (Fig. 4).[27] Compounds **5**, **19**, and **32** were used as controls in the assays and all showed only marginal inhibition. There was a remarkable range of activities among the RA compounds analyzed in the inhibition assay. Similar activities were observed between PAO1 and PA14, although most compounds were slightly more potent against PA14. Interestingly, this trend is opposite to our previously reported bromoageliferin analogues.[10] These screens also suggested that the aliphatic chain derivatives (**9–12**) and oroidin **4** were very potent inhibitors of *P. aeruginosa* biofilms.



Compound	% Inhibition vs. PAO1	% Inhibition vs. PA14
oroidin (**4**)	> 95	> 95
5	14 ± 3	10 ± 2
32	20 ± 2	15 ± 1
19	9 ± 4	12 ± 1
8	31 ± 2	50 ± 3
9	> 95	> 95
10	> 95	> 95
11	> 95	> 95
12	> 95	> 95
13	64 ± 5	70 ± 6
14	53 ± 3	67 ± 5
15	49 ± 6	63 ± 4
16	31 ± 4	55 ± 5

Fig. 4 Preliminary inhibition data at 500 μM for the RA library. All values are averages of at least three experiments.

Subsequently the aliphatic derivatives (**9–12**) and oroidin **4** were selected for IC_{50} value determination against PAO1 and PA14 (Table 2). The generation of dose–response curves for compounds **9–12** revealed a correlation between the length of the carbon chain and the potency of the compound (supporting information). This trend is apparent when the IC_{50} values are plotted as a function of chain length in both PAO1 and PA14 (Fig. 5). Increasing the

Table 2 PAO1 and PA14 IC_{50} values

Compound	PAO1 IC_{50}/μM	PA14 IC_{50}/μM
Oroidin (**4**)	190 ± 9	166 ± 23
9 ($n = 5$)	32.7 ± 6.5	39.9 ± 13.0
10 ($n = 7$)	18.4 ± 2.3	13.3 ± 1.8
11 ($n = 9$)	8 ± 1	6 ± 1
12 ($n = 11$)	2.84 ± 0.93	2.26 ± 0.83

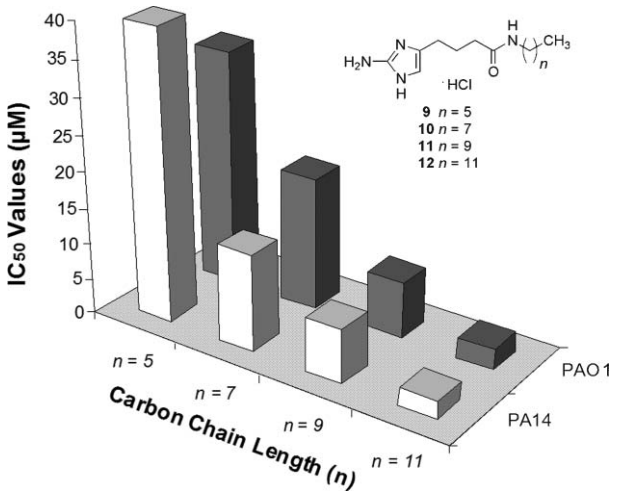


Fig. 5 Structure activity relationship of aliphatic chain RA analogues.

chain length from six to twelve carbons effectively increased the inhibition activity over a full order of magnitude in both strains. All linear carbon chain analogues were significantly more potent than oroidin (PAO1 IC_{50} = 190 μM, PA14 IC_{50} = 166 μM). The most active RA analogue identified was **12** (PAO1 IC_{50} = 2.84 μM, PA14 IC_{50} = 2.26 μM), effectively demonstrating that changes to certain portions of the natural product have the ability to dramatically increase biological activity.

To validate that our compounds were true inhibitors of biofilm formation and not acting as bactericidal agents, growth curves were performed at the determined IC_{50} values for PAO1 and PA14 with the dodecyl-based analogue **12** and oroidin **4**. Bacterial cell densities for both strains remained unchanged when grown in the presence or absence of either the natural product **4** or **12** throughout a 24 hour time period (supporting information).

While the focus has predominantly been on designing small molecules that inhibit the formation of bacterial biofilms, the more significant challenge is the development of a small molecule that will *disperse* established biofilms. Treatment of chronic infections is commonly hindered by the presence of established biofilms that impart increased resistance to conventional antibiotics.[28] Small molecules able to disperse established biofilms are, therefore, of great interest to the medical community. To test for the ability to disperse established biofilms, PAO1 and PA14 were allowed to form biofilms for 24 hours in the absence of compound. After this time the media was discarded. The wells were washed and fresh media was added containing varying concentrations of **12** and then incubated at 37 °C for 24 hours. RA analogue **12** displayed significant anti-biofilm activity, dispersing established PAO1 and PA14 biofilms with EC_{50} values of 32.8 ± 4.7 μM and 21.3 ± 3.9 μM respectively (Fig. 6).

Conclusion

We have identified several reverse amide (RA) analogues that possess potent anti-biofilm properties. These compounds are based on a reverse amide scaffold which switches the directionality of the amide bond frequently found in many members of the oroidin class of marine alkaloids. The path taken to access these

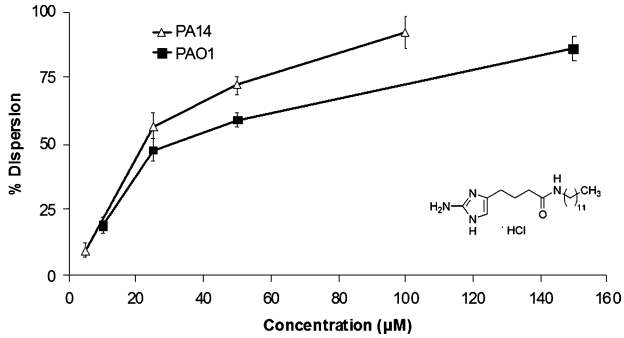


Fig. 6 Dispersion of established *P. aeruginosa* with **12**.

derivatives allows for rapid synthesis and simplified purification of all library members. Clearly, the most potent derivatives were those that contained linear carbon chains of various lengths from the amide nitrogen. The most active of these compounds, **12**, has also been shown to disperse established *P. aeruginosa* biofilms at low micromolar concentrations, making it a highly noteworthy addition to the limited number of small molecules known to possess such characteristics.[29–31] Current efforts are under way to develop a more versatile route which leads to improved yields during the amide bond formation step. It also remains a goal to identify even more potent compounds with structures similar to the most active analogues identified in this study while gaining mechanistic insight into how these compounds elicit their anti-biofilm effects.

Experimental

Stock solutions (100, 10, 1 mM) of all compounds assayed for biological activity were prepared in DMSO and stored at room temperature. The amount of DMSO used in both inhibition and dispersion screens did not exceed 1% (by volume). *P. aeruginosa* strains PAO1 and PA14 were graciously supplied by the Wozniak group at Wake Forest University School of Medicine.

General static inhibition assay protocol for *Pseudomonas aeruginosa*.

An overnight culture of the wild type strain was subcultured at an OD_{600} of 0.10 into LBNS along with a predetermined concentration of the small molecule to be tested for biofilm inhibition. Samples were then aliquoted (100 μL) into the wells of a 96-well PVC microtiter plate. The microtiter dishes were covered and sealed before incubation under stationary conditions at 37 °C for 24 hours. After that time, the medium was discarded and the plates thoroughly washed with water. The wells were then inoculated with a 0.1% aqueous solution of crystal violet (100 μL) and allowed to stand at ambient temperature for 30 minutes. Following another thorough washing with water the remaining stain was solubilized with 200 μL of 95% ethanol. Biofilm inhibition was quantitated by measuring the OD_{540} for each well by transferring 125 μL of the ethanol solution into a fresh polystyrene microtiter dish for analysis.

General static dispersion assay protocols for *Pseudomonas aeruginosa*.

An overnight culture of the wild type strain was subcultured at an OD_{600} of 0.50 into LBNS and then aliquoted (100 μL) into the wells of a 96-well PVC microtiter plate. The microtiter dishes were covered and sealed before incubation under stationary conditions at room temperature to allow formation of the biofilms. After 24 hours the medium was discarded and the plates thoroughly washed with water. Fresh medium containing the appropriate concentration of compound was then added to the wells. The plates were again sealed and this time incubated under stationary conditions at 37 °C. After 24 hours, the media was discarded from the wells and the plates washed thoroughly with water. The wells were inoculated with a 0.1% aqueous solution of crystal violet (100 μL) and allowed to stand at ambient temperature for 30 minutes. Following another thorough washing with water the remaining stain was solubilized with 200 μL of 95% ethanol. Biofilm dispersion was quantitated by measuring the OD_{540} for each well by transferring 125 μL of the ethanol solution into a fresh polystyrene microtiter dish for analysis. Percent dispersion was calculated by comparison of the OD_{540} for established biofilm (untreated) *versus* treated established biofilm under identical conditions.

Chemistry

All reagents including anhydrous solvents used for the chemical synthesis of the library were purchased from commercially available sources and used without further purification unless otherwise noted. All reactions were run under either a nitrogen or argon atmosphere. Flash silica gel chromatography was performed with 60 Å mesh standard grade silica gel from Sorbtech. ^{1}H and ^{13}C NMR spectra were obtained using Varian 300 MHz or 400 MHz spectrometers. NMR solvents were purchased from Cambridge Isotope Labs and used as is. Chemical shifts are given in parts per million relative to DMSO-d_6 (δ 2.50) and $CDCl_3$ (δ 7.27) for proton spectra and relative to DMSO-d_6 (δ 39.51) and $CDCl_3$ (δ 77.21) for carbon spectra with an internal TMS standard. High-resolution mass spectra were obtained at the North Carolina State Mass Spectrometry Laboratory for Biotechnology. ESI experiments were carried out on an Agilent LC-TOF mass spectrometer.

6-Bromo-5-oxo-hexanoic acid methyl ester. Methyl glutaryl chloride (2.5 mL, 18.23 mmol) was dissolved into anhydrous dichloromethane (10 mL) and added drop-wise to a 0 °C solution of CH_2N_2 (55.0 mmol generated from Diazald®–KOH) in diethyl ether (150 mL). This solution was stirred at 0 °C for 1.5 h at which time the reaction was quenched *via* the drop-wise addition of 48% HBr (7.5 mL). The reaction mixture was diluted with dichloromethane (25 mL) and immediately washed with sat. $NaHCO_3$ (3 × 25 mL) and brine (2 × 25 mL) before being dried ($MgSO_4$), filtered and concentrated. The crude oil was purified *via* flash column chromatography (10–30% EtOAc–hexanes) to obtain the title compound (3.76 g, 93%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.91 (s, 2H), 3.68 (s, 3H), 2.76 (t, 2H, $J = 7.2$ Hz), 2.38 (t, 2H, $J = 7.2$ Hz), 1.95 (quint., 2H, $J = 7.2$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.36, 173.41, 51.67, 38.74, 34.16, 32.87, 19.13; HRMS (ESI) calcd for $C_7H_{12}O_3Br$ $(MH)^+$ 222.9964, found 222.9964.

6-Chloro-5-oxo-hexanoic acid methyl ester. Using the same general procedure as used above but instead quenching with conc. HCl afforded the chloro derivative (2.93 g, 90%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.13 (s, 2H), 3.67 (s, 3H), 2.69 (t, 2H, $J = 7.2$ Hz), 2.38 (t, 2H, $J = 7.2$ Hz), 1.94 (quint., 2H, $J = 7.2$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.85, 173.33, 51.57, 48.22, 38.47, 32.66, 18.61; HRMS (ESI) calcd for $C_7H_{12}O_3Cl$ $(MH)^+$ 179.0469, found 179.0476.

6-Bromo-5-oxo-hexanoic acid benzyl ester. Butanoic acid monobenzyl ester **20** (3.00 g, 13.6 mmol) was dissolved in anhydrous dichloromethane (70 mL) at 0 °C and a catalytic amount of DMF was added. To this solution was added oxalyl chloride (3.60 mL, 41.3 mmol) drop-wise and the solution was then warmed to room temperature. After 1 h, the solvent and excess oxalyl chloride were removed under reduced pressure. The resulting solid was dissolved into anhydrous dichloromethane (10 mL) and added drop-wise to a 0 °C solution of CH_2N_2 (42.0 mmol generated from Diazald®–KOH) in diethyl ether (120 mL). This solution was stirred at 0 °C for 1.5 h at which time the reaction was quenched *via* the drop-wise addition of 48% HBr (4.7 mL). The reaction mixture was diluted with dichloromethane (25 mL) and immediately washed with sat. $NaHCO_3$ (3 × 25 mL) and brine (2 × 25 mL) before being dried ($MgSO_4$), filtered and concentrated. The crude oil was purified by flash column chromatography (0–30% EtOAc–hexanes) to obtain the title compound (3.57 g, 88%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (m, 5H), 5.12 (s, 2H), 3.85 (s, 2H), 2.73 (t, 2H, $J = 6.8$ Hz), 2.42 (t, 2H, $J = 6.8$ Hz), 1.96 (quint., 2H, $J = 6.8$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.43, 172.88, 136.15, 128.79, 128.48, 128.45, 66.52, 38.74, 34.07, 33.20, 19.23; HRMS (ESI) calcd for $C_{13}H_{16}O_3Br$ $(MH)^+$ 299.0277, found 299.0279.

2-Amino-4-(3-methoxycarbonyl-propyl)-imidazole-1-carboxylic acid *tert*-butyl ester (18). 6-Bromo-5-oxo-hexanoic acid methyl ester (2.30 g, 10.3 mmol), Boc-guanidine (4.92 g, 30.9 mmol),[32] and NaI (3.07 g, 20.6 mmol) were dissolved in DMF (30 mL) and allowed to stir at room temperature. After 24 h the DMF was removed under reduced pressure and the residue was taken up in ethyl acetate (100 mL) and washed with water (3 × 50 mL) and brine (50 mL) before being dried (Na_2SO_4), filtered and evaporated to dryness. The resulting oil was purified by flash column chromatography (50–100% EtOAc–hexanes) to obtain a yellow oil. Trituration of the viscous oil with cold hexanes (20 mL) produced a precipitate, which upon filtration yielded **18** (1.89 g, 65%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.53 (s, 1H), 5.6 (br s, 2H), 2.41 (t, 2H, $J = 7.2$ Hz), 2.37 (t, 2H, $J = 7.2$ Hz), 1.93 (quint., 2H, $J = 7.2$ Hz), 1.58 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.09, 150.11, 149.61, 138.39, 107.15, 84.81, 51.56, 33.65, 28.18, 27.68, 23.82; HRMS (ESI) calcd for $C_{13}H_{22}N_3O_4$ $(MH)^+$ 284.1604, found 284.1606.

4-(2-Amino-1*H*-imidazol-4-yl)butyric acid hydrochloride (19). To 2-amino-4-(3-methoxycarbonyl-propyl)-imidazole-1-carboxylic acid *tert*-butyl ester **18** (50 mg, 0.176 mmol) was added methanol (0.60 mL), tetrahydrofuran (0.20 mL), and water (0.20 mL). Lithium hydroxide (9 mg, 0.352 mmol) was then added and the reaction was stirred at room temperature for 30 min. The pH of the solution was carefully adjusted to pH = 5 with a 1 N aqueous solution of HCl before being evaporated to dryness.

The crude product was purified *via* a silica gel plug (100% MeOH sat. NH_3) to deliver the product as its corresponding free base. The hydrochloride salt was obtained through addition of a single drop of concentrated HCl to a methanolic solution (2 mL) of the free base. Rotary evaporation of this solution afforded **19** (34 mg, 94%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.25 (s, 1H), 12.13 (br s, 1H), 11.77 (s, 1H), 7.33 (s, 2H), 6.54 (s, 1H), 2.43 (t, 2H, J = 7.2 Hz), 2.21 (t, 2H, J = 7.2 Hz), 1.73 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.04, 146.92, 126.01, 108.62, 32.81, 23.47, 23.05; HRMS (ESI) calcd for $C_7H_{12}N_3O_2$ $(MH)^+$ 170.0924, found 170.0927.

2-Amino-4-(3-benzyloxycarbonyl-propyl)-imidazole-1-carboxylic acid *tert*-butyl ester (21). 6-Bromo-5-oxo-hexanoic acid benzyl ester (3.42 g, 11.99 mmol) and Boc-guanidine (5.73 g, 35.97 mmol) were dissolved in DMF (35 mL) and allowed to stir at room temperature. After 48 h the DMF was removed under reduced pressure and the residue was taken up in ethyl acetate (100 mL) and washed with water (3 × 50 mL) and brine (50 mL) before being dried (Na_2SO_4), filtered and evaporated to dryness. The resulting oil was purified by flash column chromatography (30–100% EtOAc–hexanes) to obtain the title compound (2.79 g, 66%) as a colorless oil which solidified upon prolonged standing. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (m, 5H), 6.51 (s, 1H), 5.91 (s, 2H), 5.12 (s, 2H), 2.41 (m, 4H), 1.94 (quint., 2H, J = 7.2 Hz), 1.57 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.45, 150.31, 149.59, 138.27, 136.36, 128.67, 128.29, 128.27, 107.05, 84.73, 66.23, 33.82, 28.16, 27.62, 23.79; HRMS (ESI) calcd for $C_{19}H_{26}N_3O_4$ $(MH)^+$ 360.1917, found 360.1919.

2-Amino-4-(3-carboxy-propyl)-imidazole-1-carboxylic acid *tert*-butyl ester (22). To a solution of anhydrous THF (2 mL) and 10% Pd/C (12 mg) was charged 2-amino-4-(3-benzyloxycarbonyl-propyl)-imidazole-1-carboxylic acid *tert*-butyl ester **21** (101 mg, 0.281 mmol). Air was removed from the system and the reaction was back flushed with hydrogen. This process was repeated three times before setting the reaction under a hydrogen balloon at atmospheric pressure and temperature for 1 h. After that time the reaction was filtered through a Celite® pad and the filter cake was washed with THF (8 mL). The filtrate was concentrated under reduced pressure to afford the title compound **21** (75 mg, 98%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.52 (s, 1H), 6.42 (br s, 2H), 2.52 (t, 2H, J = 5.4 Hz), 2.18 (t, 2H, J = 5.4 Hz), 1.71 (m, 2H), 1.53 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.00, 149.99, 148.95, 138.28, 105.86, 84.09, 39.24, 38.85, 33.70, 27.52, 27.08, 23.52; HRMS (ESI) calcd for $C_{12}H_{20}N_3O_4$ $(MH)^+$ 270.1448, found 270.1452.

General aminolysis procedure

To a stirring 0 °C solution of amine (0.704 mmol) in anhydrous 1,2-dichloroethane (1 mL) was added drop-wise a 2 M solution of $AlMe_3$ in $PhCH_3$ (0.351 mL, 0.704 mmol). The solution was stirred for 10 min before the addition of 2-amino-4-(3-methoxycarbonyl-propyl)-imidazole-1-carboxylic acid *tert*-butyl ester **18** (100 mg, 0.352 mmol) in several portions. Once dissolution was complete, the reaction was warmed to 60 °C and stirred until completion as evident by TLC analysis. The reaction was then cooled back down to 0 °C before being diluted with dichloromethane (5 mL) and quenched with water (1 mL). The resulting viscous solution was warmed to ambient temperature and Celite® was added. After stirring for 5 min, the mixture was filtered and the filtrate washed with brine (2 × 3 mL), dried (Na_2SO_4), and evaporated to dryness. The crude product was purified *via* flash column chromatography (2–10% MeOH–CH_2Cl_2) to afford pure product.

2-Amino-4-(3-isobutylcarbamoyl-propyl)-imidazole-1-carboxylic acid *tert*-butyl ester (23). White solid (46 mg, 40%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.75 (m, 1H), 6.51 (s, 1H), 6.37 (br s, 2H), 2.85 (t, 2H, J = 6.4 Hz), 2.22 (t, 2H, J = 6.8 Hz), 2.07 (t, 2H, J = 7.2 Hz), 1.61–1.73 (m, 3H), 1.53 (s, 9H), 0.81 (d, 6H, J = 6.4 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.82, 149.92, 148.95, 138.39, 105.84, 84.04, 45.94, 34.88, 28.09, 27.52, 27.23, 24.10, 20.14; HRMS (ESI) calcd for $C_{16}H_{29}N_4O_3$ $(MH)^+$ 325.2234, found 325.2238.

2-Amino-4-(3-decylcarbamoyl-propyl)-imidazole-1-carboxylic acid *tert*-butyl ester (26). Tan solid (24 mg, 16%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.74 (m, 1H), 6.52 (s, 1H), 6.49 (br s, 2H), 3.00 (q, 2H, J = 6.8 Hz), 2.22 (t, 2H, J = 6.8 Hz), 2.04 (t, 2H, J = 6.8 Hz), 1.71 (quint., 2H, J = 6.8 Hz), 1.53 (s, 9H), 1.36 (m, 2H), 1.23 (s, 14H), 0.85 (t, 3H, J = 6.8 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.64, 149.82, 148.86, 137.90, 105.90, 84.16, 38.33, 34.88, 31.31, 29.16, 29.03, 28.97, 28.75, 28.73, 28.02, 27.51, 27.05, 26.40, 24.04, 22.12, 13.97; HRMS (ESI) calcd for $C_{22}H_{41}N_4O_3$ $(MH)^+$ 409.3173, found 409.3175.

2-Amino-4-(3-cyclopentylcarbamoyl-propyl)-imidazole-1-carboxylic acid *tert*-butyl ester (28). White solid (54 mg, 45%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.72 (d, 1H, J = 6.4 Hz), 6.5 (s, 1H), 6.38 (s, 2H), 3.97 (m, 1H), 2.21 (t, 2H, J = 7.2 Hz), 2.02 (t, 2H, J = 7.2 Hz), 1.73 (m, 4H), 1.61 (m, 2H), 1.53 (s, 9H), 1.47 (m, 2H), 1.32 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.27, 149.92, 148.96, 138.37, 105.88, 84.02, 50.04, 34.79, 32.32, 28.05, 27.53, 27.17, 24.00, 23.43; HRMS (ESI) calcd for $C_{17}H_{29}N_4O_3$ $(MH)^+$ 337.2234, found 337.2235.

2-Amino-4-(4-morpholin-4-yl-4-oxo-butyl)-imidazole-1-carboxylic acid *tert*-butyl ester (29). Tan solid (33 mg, 27%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.52 (s, 1H), 6.39 (s, 2H), 3.52 (m, 4H), 3.41 (m, 4H), 2.28 (m, 4H), 1.42 (quint., 2H, J = 7.2 Hz), 1.53 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.43, 150.56, 149.64, 139.17, 106.58, 84.78, 66.85, 46.14, 32.23, 28.23, 27.89, 24.19; HRMS (ESI) calcd for $C_{16}H_{27}N_4O_4$ $(MH)^+$ 339.2026, found 339.2027.

2-Amino-4-(3-phenylcarbamoyl-propyl)-imidazole-1-carboxylic acid *tert*-butyl ester (30). White solid (66 mg, 55%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.88 (s, 1H), 7.59 (d, 2H, J = 8.1 Hz), 7.27 (t, 2H, J = 7.5 Hz), 7.00 (t, 1H, J = 7.2 Hz), 6.55 (s, 1H), 6.44 (br s, 2H), 2.97 (m, 4H), 1.82 (m, 2H), 1.53 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.95, 149.71, 148.86, 139.25, 138.16, 128.47, 122.78, 118.98, 105.92, 84.00, 38.42, 35.71, 27.44, 27.01, 23.72; HRMS (ESI) calcd for $C_{18}H_{25}N_4O_3$ $(MH)^+$ 345.1921, found 345.1920.

2-Amino-4-[3-(pyrimidin-2-ylcarbamoyl)-propyl]-imidazole-1-carboxylic acid *tert*-butyl ester (31). Tan solid (19 mg, 11%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.51 (s, 1H), 8.63 (d, 2H, J = 4.8 Hz), 7.15 (t, 1H, J = 4.8 Hz), 6.54 (s, 1H), 6.40 (s, 2H), 2.49 (t, 2H, J = 7.2 Hz), 2.29 (t, 2H, J = 7.2 Hz), 1.80 (quint., 2H, J = 7.2 Hz), 1.53 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.34,

158.11, 157.63, 149.76, 148.85, 138.31, 116.37, 105.81, 83.98, 35.75, 27.44, 27.05, 23.37; HRMS (ESI) calcd for $C_{16}H_{23}N_6O_3$ $(MH)^+$ 347.1826, found 347.1827.

General EDC–HOBt procedure

2-Amino-4-(3-carboxy-propyl)-imidazole-1-carboxylic acid *tert*-butyl ester **22** (100 mg, 0.371 mmol), 1-hydroxybenzotriazole (100 mg, 0.742 mmol) and *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (142 mg, 0.742 mmol) were dissolved in anhydrous DMF (3 mL). The appropriate amine coupling partner (1.48 mmol) was then added and the solution was stirred at ambient temperature until completion was evident by TLC analysis. The reaction was concentrated under reduced pressure and the residue partitioned between ethyl acetate (20 mL) and water (10 mL). The organic layer was successively washed with water (3 × 10 mL), a 10% aqueous solution of citric acid (2 × 10 mL), sat. $NaHCO_3$ (2 × 10 mL), and brine (10 mL) before being dried (Na_2SO_4) and evaporated to dryness. The crude product was purified *via* flash column chromatography (2–10% MeOH–CH_2Cl_2) to afford the target compound.

2-Amino-4-(3-hexylcarbamoyl-propyl)-imidazole-1-carboxylic acid *tert*-butyl ester (24). Pale yellow solid (41 mg, 32%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.73 (m, 1H), 6.50 (s, 1H), 6.39 (s, 2H), 2.99 (q, 2H, $J = 6.3$ Hz), 2.21 (t, 2H, $J = 7.5$ Hz), 2.04 (t, 2H, $J = 7.2$ Hz), 1.70 (m, 2H), 1.53 (s, 9H), 1.31 (m, 3H), 1.23 (br s, 7H), 0.85 (t, 3H, $J = 5.1$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.68, 149.93, 148.95, 138.37, 105.81, 84.04, 38.35, 34.91, 31.01, 29.14, 27.52, 27.22, 26.10, 24.06, 22.09, 13.93; HRMS (ESI) calcd for $C_{18}H_{33}N_4O_3$ $(MH)^+$ 353.2547, found 353.2549.

2-Amino-4-(3-octylcarbamoyl-propyl)-imidazole-1-carboxylic acid *tert*-butyl ester (25). White solid (48 mg, 34%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.73 (m, 1H), 6.50 (s, 1H), 6.38 (s, 2H), 2.99 (q, 2H, $J = 5.4$ Hz), 2.21 (t, 2H, $J = 7.5$ Hz), 2.04 (t, 2H, $J = 7.2$ Hz), 1.73 (m, 2H), 1.53 (s, 9H), 1.36 (m, 4H), 1.23 (br s, 10H), 0.85 (t, 3H, $J = 5.1$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.72, 149.95, 148.95, 138.43, 105.83, 84.06, 38.35, 34.92, 31.26, 29.16, 28.71, 27.53, 27.22, 26.43, 24.10, 22.12, 13.98; HRMS (ESI) calcd for $C_{20}H_{37}N_4O_3$ $(MH)^+$ 381.2860, found 381.2861.

2-Amino-4-(3-dodecylcarbamoyl-propyl)-imidazole-1-carboxylic acid *tert*-butyl ester (27). White solid (44 mg, 28%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.73 (t, 1H, $J = 5.6$ Hz), 6.50 (s, 1H), 6.38 (s, 2H), 3.00 (q, 2H, $J = 5.6$ Hz), 2.21 (t, 2H, $J = 7.6$ Hz), 2.04 (t, 2H, $J = 7.6$ Hz), 1.71 (quint., 2H, $J = 7.6$ Hz), 1.53 (s, 9H), 1.36 (m, 2H), 1.23 (s, 18H), 0.85 (t, 3H, $J = 6.0$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.58, 149.77, 148.88, 138.42, 105.76, 83.98, 38.28, 34.90, 31.18, 29.06, 28.88, 28.58, 27.47, 27.18, 26.29, 24.04, 21.96, 13.81, 13.27; HRMS (ESI) calcd for $C_{24}H_{45}N_4O_3$ $(MH)^+$ 437.3486, found 437.3487.

4-(2-Amino-1*H*-imidazol-4-yl)-*N*-isobutyl-butyramide hydrochloride (8). A solution of 2-amino-4-(3-isobutylcarbamoyl-propyl)-imidazole-1-carboxylic acid *tert*-butyl ester **23** (76 mg, 0.234 mmol) in anhydrous dichloromethane (1 mL) was cooled to 0 °C. TFA (1 mL) was charged into the flask and the reaction stirred for 5 h. After that time the reaction was evaporated to dryness and toluene (2 mL) was added. Again the mixture was concentrated and the process repeated. The resulting TFA salt was dissolved in dichloromethane (1 mL) and 2 M HCl in diethyl ether (0.50 mL) was added followed by cold diethyl ether (8 mL). The precipitate was collected by filtration and washed with diethyl ether (3 mL) to yield the target compound **8** (59 mg, 97%) as a tan solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.14 (s, 1H), 11.70 (s, 1H), 7.89 (m, 1H), 7.34 (br s, 2H), 6.55 (s, 1H), 2.84 (t, 2H, $J = 6.6$ Hz), 2.38 (t, 2H, $J = 7.5$ Hz), 2.10 (t, 2H, $J = 7.5$ Hz), 1.60–1.79 (m, 3H), 0.82 (d, 6H, $J = 6.3$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.52, 146.78, 126.31, 108.68, 46.00, 34.41, 28.09, 23.94, 23.64, 20.18; HRMS (ESI) calcd for $C_{11}H_{21}N_4O$ $(MH)^+$ 225.1709, found 225.1711.

4-(2-Amino-1*H*-imidazol-4-yl)-*N*-hexyl-butyramide hydrochloride (9). Using the same general procedure as used for the synthesis of **8**, 2-amino-4-(3-hexylcarbamoyl-propyl)-imidazole-1-carboxylic acid *tert*-butyl ester **24** (90 mg, 0.255 mmol) gave **9** (70 mg, 96%) as a pale yellow foam. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.96 (s, 1H), 11.54 (s, 1H), 7.81 (m, 1H), 7.29 (br s, 2H), 6.56 (s, 1H), 3.01 (m, 2H), 2.40 (t, 2H, $J = 7.8$ Hz), 2.07 (t, 2H, $J = 7.2$ Hz), 1.73 (m, 2H), 1.23–1.36 (m, 8H), 0.85 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.35, 146.72, 126.37, 108.71, 38.43, 34.41, 31.00, 29.12, 26.12, 23.87, 23.62, 22.09, 13.96; HRMS (ESI) calcd for $C_{13}H_{25}N_4O$ $(MH)^+$ 253.2022, found 253.2025.

4-(2-Amino-1*H*-imidazol-4-yl)-*N*-octyl-butyramide hydrochloride (10). Using the same general procedure as used for the synthesis of **8**, 2-amino-4-(3-octylcarbamoyl-propyl)-imidazole-1-carboxylic acid *tert*-butyl ester **25** (50 mg, 0.131 mmol) gave **10** (39 mg, 93%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.13 (s, 1H), 11.69 (s, 1H), 7.87 (m, 1H), 7.33 (br s, 2H), 6.55 (s, 1H), 2.99 (q, 2H, $J = 6.3$ Hz), 2.38 (t, 2H, $J = 7.5$ Hz), 2.07 (t, 2H, $J = 7.5$ Hz), 1.73 (m, 2H), 1.35 (m, 2H), 1.23 (m, 10H), 0.85 (t, 3H, $J = 6.3$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.30, 146.80, 126.32, 108.57, 38.40, 34.39, 31.15, 29.06, 28.62, 28.56, 26.37, 23.86, 23.57, 21.99, 13.85; HRMS (ESI) calcd for $C_{15}H_{29}N_4O$ $(MH)^+$ 281.2335, found 281.2339.

4-(2-Amino-1*H*-imidazol-4-yl)-*N*-decyl-butyramide hydrochloride (11). Using the same general procedure as used for the synthesis of **8**, 2-amino-4-(3-decylcarbamoyl-propyl)-imidazole-1-carboxylic acid *tert*-butyl ester **26** (32 mg, 0.078 mmol) gave **11** (27 mg, 99%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.07 (s, 1H), 11.64 (s, 1H), 7.85 (s, 1H), 7.32 (br s, 2H), 6.56 (s, 1H), 3.00 (q, 2H, $J = 6.4$ Hz), 2.38 (t, 2H, $J = 7.2$ Hz), 2.07 (t, 2H, $J = 7.2$ Hz), 1.73 (quint., 2H, $J = 7.2$ Hz), 1.36 (m, 2H), 1.23 (s, 14H), 0.85 (t, 3H, $J = 7.2$ Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.33, 146.72, 126.36, 108.70, 38.42, 34.41, 31.32, 29.15, 29.04, 28.99, 28.77, 28.73, 26.45, 23.89, 23.62, 22.12, 13.99; HRMS (ESI) calcd for $C_{17}H_{33}N_4O$ $(MH)^+$ 309.2648, found 309.2647.

4-(2-Amino-1*H*-imidazol-4-yl)-*N*-dodecyl-butyramide hydrochloride (12). Using the same general procedure as used for the synthesis of **8**, 2-amino-4-(3-dodecylcarbamoyl-propyl)-imidazole-1-carboxylic acid *tert*-butyl ester **27** (20 mg, 0.046 mmol) gave **12** (16 mg, 94%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.03 (s, 1H), 11.60 (s, 1H), 7.83 (t, 1H, $J = 6.4$ Hz), 7.31 (s, 2H), 6.56 (s, 1H), 3.00 (q, 2H, $J = 6.4$ Hz), 2.38 (t, 2H, $J = 7.2$ Hz), 2.07 (t, 2H, $J = 7.2$ Hz), 1.73 (quint., 2H $J = 7.2$ Hz), 1.36 (m, 2H), 1.23 (s, 18H), 0.85 (t, 2H, $J = 6.4$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.20, 146.66, 126.34, 108.59, 38.34, 34.32, 34.32, 31.15, 29.02, 28.86, 28.6,

28.55, 26.31, 23.78, 23.52, 21.94, 13.79; HRMS (ESI) calcd for $C_{19}H_{37}N_4O$ $(MH)^+$ 337.2961, found 337.2964.

4-(2-Amino-1*H*-imidazol-4-yl)-*N*-cyclopentyl-butyramide hydrochloride (13). Using the same general procedure as used for the synthesis of **8**, 2-amino-4-(3-cyclopentylcarbamoyl-propyl)-imidazole-1-carboxylic acid *tert*-butyl ester **28** (100 mg, 0.297 mmol) gave **13** (78 mg, 96%) as a pale yellow foam. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.99 (s, 1H), 11.57 (s, 1H), 7.79 (d, 1H, $J = 7.2$ Hz), 7.30 (s, 2H), 6.56 (s, 1H), 3.97 (m, 1H), 2.37 (t, 2H, $J = 7.2$ Hz), 2.05 (t, 2H, $J = 7.2$ Hz), 1.69–1.79 (m, 4H), 1.59 (m, 2H), 1.47 (m, 2H), 1.31 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.97, 146.74, 126.38, 108.72, 50.11, 34.36, 32.31, 23.81, 23.45, 23.62; HRMS (ESI) calcd for $C_{12}H_{21}N_4O$ $(MH)^+$ 237.1709, found 237.1711.

4-(2-Amino-1*H*-imidazol-4-yl)-1-morpholin-4-yl-butan-1-one hydrochloride (14). Using the same general procedure as used for the synthesis of **8**, 2-amino-4-(4-morpholin-4-yl-4-oxo-butyl)-imidazole-1-carboxylic acid *tert*-butyl ester **29** (44 mg, 0.133 mmol) gave **14** (25 mg, 70%) as a tan solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.1 (s, 1H), 11.64 (s, 1H), 7.33 (s, 2H), 6.58 (s, 1H), 3.54 (m, 4H), 3.42 (m, 4H), 2.43 (t, 2H, $J = 7.2$ Hz), 2.33 (t, 2H, $J = 7.2$ Hz), 1.75 (quint., 2H, $J = 7.2$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.27, 146.72, 126.39, 108.61, 66.04, 45.28, 31.05, 23.56, 23.17; HRMS (ESI) calcd for $C_{11}H_{19}N_4O_2$ $(MH)^+$ 239.1502, found 239.1503.

4-(2-Amino-1*H*-imidazol-4-yl)-*N*-phenyl-butyramide hydrochloride (15). Using the same general procedure as used for the synthesis of **8**, 2-amino-4-(3-phenylcarbamoyl-propyl)-imidazole-1-carboxylic acid *tert*-butyl ester **30** (80 mg, 0.232 mmol) gave **15** (64 mg, 99%) as a tan solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.03 (s, 1H), 11.60 (s, 1H), 9.98 (s, 1H), 7.59 (d, 2H, $J = 8.0$ Hz), 7.33 (br s, 2H), 7.28 (t, 2H, $J = 8.0$ Hz), 7.02 (t, 1H, $J = 7.6$ Hz), 6.61 (s, 1H), 2.44 (m, 2H), 2.32 (t, 2H, $J = 6.8$ Hz), 1.85 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.65, 146.77, 139.25, 137.24, 128.59, 126.40, 123.00, 119.13, 108.83, 35.33, 23.60, 23.51; HRMS (ESI) calcd for $C_{13}H_{17}N_4O$ $(MH)^+$ 245.1396, found 245.1401.

4-(2-Amino-1*H*-imidazol-4-yl)-*N*-pyrimidin-2-yl-butyramide hydrochloride (16). Using the same general procedure as used for the synthesis of **8**, 2-amino-4-[3-(pyrimidin-2-ylcarbamoyl)-propyl]-imidazole-1-carboxylic acid *tert*-butyl ester **31** (50 mg, 0.144 mmol) gave **16** (41 mg, 99%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.06 (s, 1H), 11.64 (s, 1H), 10.59 (s, 1H), 8.64 (d, 2H, $J = 4.8$ Hz), 7.34 (s, 2H), 7.17 (t, 1H, $J = 4.8$ Hz), 6.60 (s, 1H), 2.51 (m, 2H), 2.46 (t, 2H, $J = 7.2$ Hz), 1.83 (quint., 2H, $J = 7.2$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 158.17, 157.54, 146.73, 129.02, 126.33, 116.5, 108.71, 35.35, 23.48, 23.09; HRMS (ESI) calcd for $C_{11}H_{15}N_6O$ $(MH)^+$ 247.1301, found 247.1304.

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