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A nitroenolate approach to the synthesis of 4,5-disubstituted-2-aminoimidazoles. Pilot library assembly and screening for antibiotic and antibiofilm activity†

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A library of 4,5-disubstituted-2-aminoimidazoles was synthesized using a nitroenolate route and then screened for antibiofilm and antimicrobial activity. These compounds displayed notable biofilm dispersal and planktonic microbicidal activity against various Gram-positive and Gram-negative bacteria.

Introduction

The global rise of antibiotic resistant pathogens[1] has driven a revitalization of the field of anti-infective research. This research emphasis has been multi-faceted, including the obvious search for novel antibiotics. However, as bacteria have shown the ability to evolve resistance to any antibiotics placed in the human healthcare system,[2] additional approaches to controlling bacterial infections are being explored. These approaches include small molecules that target: (1) quorum sensing (QS),[3] (2) plasmids that harbor antibiotic resistance genes,[4] and (3) the formation/maintenance of bacteria within the biofilm state.[5]

In an effort to address these needs in biomedical science, our research group has spent a number of years developing molecules that inhibit/disperse bacterial biofilms.[6] Bacterial biofilms, defined as surface-attached communities of microorganisms encased in a protective extracellular matrix of biomolecules,[7] are specifically targeted because within a biofilm state, bacteria are upwards of 1000-fold more resistant to conventional antibiotics and are inherently insensitive to the host immune response in comparison to their free-floating (planktonic) counterparts.[8] The biofilm mode of existence is the preferred lifestyle for bacteria as approximately 80% of the world's microbial mass is in a biofilm at any given time and the NIH has estimated that *ca.* 75% of bacterial infections are driven by a biofilm.[9] To this end, we have developed a number of 2-aminoimidazole derivatives based upon the natural products bromoageliferin and oroidin that inhibit and disperse biofilms from both Gram-positive and Gram-negative bacteria.[10–18] We have also developed a class of 2-aminobenzimidazoles that inhibit and disperse gram-positive biofilms *via* a Zn(II)-dependent mechanism.[19] Both classes of molecules control biofilm development and maintenance *via* a non-microbicidal mechanism.

As a therapeutic strategy, molecules that inhibit/disperse biofilms through non-microbicidal mechanisms will most likely serve as adjuvants to conventional antibiotics.[20] The anti-biofilm molecules will keep the bacteria within their more sensitive planktonic state, while a combination of conventional antibiotics and the host immune response will serve to eliminate the bacterial threat. In this regard, we have developed new antibiotics based upon a 2-aminobenzimidazole/amide-conjugate framework that are active against MRSA and multi-drug resistant *Acinetobacter baumannii* and are not affected by current mechanisms of antibiotic resistance (multidrug efflux pumps, *etc.*).[21]

As the 2-aminoimidazole heterocycle is a key pharmacophore (isolated or fused to an aromatic ring) in all the aforementioned molecules developed by our group, methods to access 2-aminoimidazoles with pre-defined substitution patterns are necessary to systematically evaluate the impact these substitution patterns have upon both anti-biofilm and antibiotic activity. Recently, Looper and co-workers have developed an elegant solution to the synthesis of substituted 2-aminoimidazoles *via* an intramolecular La(III)-catalyzed addition/hydroamination sequence.[22] Parallel to this approach, we have developed a nitroenolate approach to access 4,5-disubstituted-2-aminoimidazoles. Herein, we detail the use of the nitroenolate reaction to access α-nitroketones, that following reduction and condensation with cyanamide yield 4,5-disubstituted-2-aminoimidazoles. Using this methodology, we have assembled a pilot library of 4,5-disubstituted-2-aminoimidazoles that have been subsequently screened for both anti-biofilm and antibiotic activity against *E. coli*, multi-drug resistant *Acinetobacter baumannii* (MDRAB), methicillin resistant *Staphylococcus aureus* (MRSA), methicillin sensitive *Staphylococcus aureus* (MSSA), *Staphylococcus epidermidis*, vancomycin resistant *Enterococcus facium* (VRE), *Vibrio cholerae*, *Vibrio vulnificus*, *Listonella anguillarum* and *Rhodospirillum salexigens*.

Results and discussion

Synthesis and pilot library assembly

Fundamentally, our approach to pilot library synthesis relies upon the use of readily available building blocks to rapidly access 2-aminoimidazoles with pre-defined substitution patterns

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† Electronic supplementary information (ESI) available: ^{1}H NMR and ^{13}C NMR spectra for all new compounds, synthetic protocols and characterization for all α-nitro ketones and 4,5-disubstituted-2-aminoimidazoles not presented in the experimental section, and biofilm dispersion and hemolysis graphs. See DOI: 10.1039/c001479f

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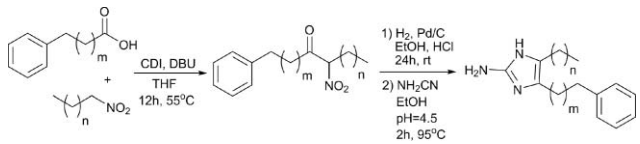
for biological evaluation. Therefore, we were interested in developing the synthesis of 4,5-disubstituted-2-aminoimidazoles *via* a nitroenolate approach (Scheme 1) because the starting building blocks, activated carboxylic acids and alkyl nitro derivatives, are either commercially available or available in one step from either carboxylic acids or alkyl halides, respectively. Once assembled, the target α-nitro ketones could simply be reduced and then condensed with cyanamide to yield 4,5-disubstituted-2-aminoimidazoles.

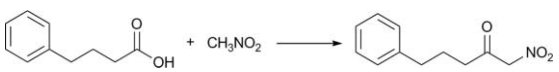


Scheme 1 4,5-Disubstituted-2-aminoimidazole synthetic route.

This synthetic approach[23] was first optimized by using 4-phenylbutyric acid and nitromethane as the building blocks. We sought to determine the optimal combination of enolization base, carboxylate activation and solvent that would deliver the highest yield of the targeted α-nitro ketone (Table 1). We screened THF and CH_2Cl_2 as potential solvents, DBU and *t*-BuO^-K^+ as enolization bases and CDI and ethyl chloroformate as carboxylate activators. From this initial screen, we determined that 2.5 equivalents of DBU as the enolization base, THF as the solvent and CDI as the activating agent generated the target α-nitro ketone in the highest yield (76%) when the reaction was run at 40 °C overnight.

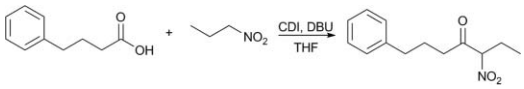
Once we had determined the optimized conditions of the nitroenolate approach, we sought to apply these conditions to the synthesis of 4,5-disubstituted-2-aminoimidazoles. We first applied the aforementioned reaction conditions to the coupling of 4-phenylbutyric acid and 1-nitropropane. Under these conditions, we were able access the target α-nitro ketone in 27% yield. Given this low yield, we sought to further optimize the reaction by studying the optimal reaction temperature and reaction time (Table 2). The temperature was first varied from room temperature to reflux (66 °C, entries 1–6) and we determined the optimal temperature was 55 °C. Heating the reaction to higher temperatures led to a decreased reaction yield, most likely from decomposition. We then sought to determine if reaction time would affect reaction yield by running the reaction for either 6, 12, 24, or 48 h. As predicted by our temperature studies, the longer the reaction is heated, the lower the yield of the target α-nitro ketone. At 6 h, the reaction yield was slightly lower. Under these optimized conditions (2.5 eq. DBU, 2 eq. CDI, THF, 55 °C, 12 h), we were able to achieve a 50% yield of the target α-nitro ketone.

Table 1 Effects of reaction solvent and base on product yield



Solvent	Enolization base	Activating base	Yield [%]
THF	DBU	Ethyl chloroformate	—
THF	DBU	CDI	76
THF	*t*-BuO^-K^+	CDI	—
CH_2Cl_2	*t*-BuO^-K^+	CDI	—
CH_2Cl_2	DBU	CDI	46

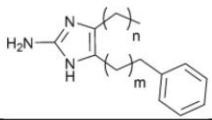
Table 2 Effects of reaction time and temperature on product yield



Entry	*T*/°C	Time/h	Yield [%]
1	RT	12	20
2	40	12	27
3	45	12	47
4	50	12	46
5	55	12	50
6	66	12	20
7	55	6	43
8	55	24	24
9	55	48	—

Once we had the α-nitro ketone, we verified that the reduction/condensation sequence would deliver the target 2-aminoimidazole. We reduced the nitro group to the corresponding amine using H_2, 5 eq. HCl and 5% Pd/C in ethanol. The resulting crude reaction was then simply filtered over Celite, and the ethanol and excess HCl removed *in vacuo* to deliver the crude α-amino ketone as its HCl salt. The salt was then redissolved in ethanol, 5 eq. cyanamide was then added, the pH adjusted to 4.5 and the resulting solution was heated to 95 °C for two hours. After purification, the target 4,5-disubstituted-2-aminoimidazole was isolated in 55% yield.

Using this approach, we assembled a 15-member pilot library where we varied both the length of the alkyl chain and the number of methylene units between the 2-aminoimidazole and phenyl group (Fig. 1).



Compound	n =	m =	Compound	n =	m =
1	0	2	**9**	3	3
2	1	2	**10**	4	3
3	2	2	**11**	0	4
4	3	2	**12**	1	4
5	4	2	**13**	2	4
6	0	3	**14**	3	4
7	1	3	**15**	4	4
8	2	3			

Fig. 1 Composition of pilot library.

Biological screening

We first assessed the ability of each member of the pilot library to inhibit either *E. coli* or MDRAB biofilm formation at 100 μM using a crystal violet reporter assay.[24] The results of this initial screen are summarized in Table 3. From this screen, 12 compounds inhibited *E. coli* biofilm formation >95%, while eight compounds inhibited MDRAB biofilm formation >95%.

When we subjected each compound that showed >80% activity to a dose–response study to determine the IC_{50} value for biofilm inhibition, we noted that biofilm inhibition dropped precipitously over a narrow concentration range. This is typically indicative of biofilm inhibition *via* a traditional microbicidal mechanism instead of a mechanism that modulates biofilm formation through non-microbicidal mechanisms. The microbicidal activity of

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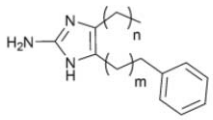
Table 3 Test compound inhibition of biofilm formation at 100 μM

Compound	*E. coli*	MDRAB
1	<5	<5
2	>99	>99
3	>99	>99
4	>99	>99
5	>99	>99
6	>99	>99
7	86.4 ± 7.9	72.9 ± 0.4
8	97.9 ± 0.6	96.5 ± 2.6
9	98.2 ± 0.03	>99
10	96.6 ± 2.9	94.3 ± 2.2
11	96.3 ± 0.08	>99
12	>99	94.5 ± 0.2
13	95.8 ± 1.5	87.8 ± 0.3
14	96.9 ± 1.1	85.4 ± 0.7
15	83.0 ± 0.8	80.3 ± 8.1

representative compound **15** was verified by conducting a growth curve analysis (A_{600}) against *E. coli* at its IC_{50} concentration (13 μM). At this concentration, we observed a 97% reduction in bacterial growth.

Once we determined that this specific 4,5-disubstitution pattern imparted microbicidal activity onto the 2-AI framework, we quantified this activity by measuring the MIC of each derivative against a variety of representative pathogenic bacterial strains using the microdilution protocol.[25] We chose *E. coli*, MDRAB, MRSA, MSSA, *S. epidermidis* and VRE for initial evaluation. The results of this study are outlined in Table 4. From this screen, compound **15** was determined to be our lead compound and had MIC values (μg mL^{-1}) of 2, 2, 1, 0.5, 0.25, and 1 against *E. coli*, MDRAB, MRSA, VRE, *S. Epidermidis* and MSSA. In general, we noted that activity correlated with the length of the alkyl chain, with 5 carbons being most active. We also observed that the linker between the 2-AI and phenyl ring also modulated activity, as the 5-carbon spacer was most active.

Once we noted that increasing chain length of both the alkyl chain and the linker correlated with increased activity, we synthesized five second generation compounds (Fig. 2) where we further varied these two parameters to maximize activity. The MIC values of the second generation compounds against each of the aforementioned bacterial strains are outlined in Table 5. Unfortunately, none of the second generation compounds displayed augmented activity in comparison to lead compound **15**; however, compound **19** had similar activity.



Compound	n =	m =
16	3	5
17	4	5
18	5	5
19	5	4
20	5	3

Fig. 2 Composition of second generation compounds.

Once we had determined the effect that these 4,5-disubstituted-2-aminoimidazoles had on representative terrestrial pathogens, we determined their activity against a representative set of marine

Table 4 Microdilution MIC of pilot library of compounds for representative pathogenic bacterial strains

Compound	*E. coli*[a]	MDRAB[a]	MRSA[a]	MSSA[a]	*S. epidermidis*[a]	VRE[a]
1	128	128	64	32	8	256
2	32	32	16	8	2	16
3	16	32	8	4	4	8
4	32	64	16	16	4	16
5	8	8	4	4	1	2
6	32	16	8	2	0.25	16
7	32	32	32	16	4	32
8	8	16	8	4	2	4
9	32	32	8	8	2	4
10	8	8	2	2	0.5	1
11	8	8	4	2	1	8
12	16	32	8	4	2	8
13	8	8	4	4	0.5	4
14	4	4	2	2	0.25	2
15	2	2	1	1	0.25	0.5

[a] MIC values are in μg mL^{-1}.

Table 5 Microdilution MIC values of second generation compounds

Compound	*E. coli*[a]	MDRAB[a]	MRSA[a]	MSSA[a]	*S. epidermidis*[a]	VRE[a]
16	8	8	1	2	0.5	1
17	32	16	1	—	—	1
18	8	16	1	2	0.5	1
19	2	4	1	1	0.5	0.5
20	8	8	1	2	0.5	1

[a] MIC values are in μg mL^{-1}.

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Table 6 Marine bacteria microdilution MIC data for both pilot and second generation libraries

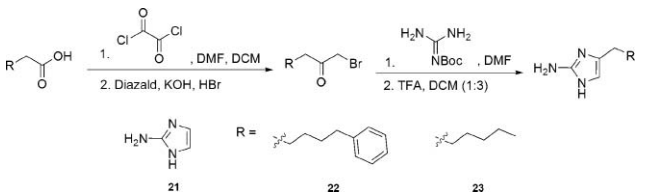
Compound	*V. cholerae*[a]	*V. vulnificus*[a]	*L. anguillarum*[a]	*R. salexigens*[a]
1	>50	50.4	50.4	50.4
2	53.2	26.6	26.6	13.3
3	27.9	27.9	13.9	13.9
4	58.8	58.8	58.8	29.4
5	15.4	15.4	7.70	3.85
6	26.6	26.6	13.9	13.3
7	55.9	13.9	13.9	13.9
8	29.4	14.7	14.7	7.35
9	30.8	30.8	30.8	7.70
10	16.1	8.05	8.05	2.01
11	27.9	13.9	13.9	7.00
12	29.4	29.4	14.7	14.7
13	15.4	3.85	7.70	3.85
14	8.05	4.02	4.02	8.05
15	4.20	2.10	2.10	2.10
16	8.40	8.40	4.20	2.10
17	69.9	69.9	69.9	69.9
18	18.2	18.2	18.2	2.27
19	2.19	2.19	2.19	1.09
20	4.20	4.20	4.20	2.10

[a] MIC values are in μg mL^{-1}.

Table 7 Marine bacteria biofilm dispersion data for lead compounds

Compound	**15**[a]	**19**[a]
E. coli	12.0 ± 1.6	42.5 ± 6.1
MDRAB	7.1 ± 1.3	11.1 ± 0.1
MRSA	33.9 ± 3.9	74.9 ± 9.8
MSSA	19.9 ± 3.3	26.1 ± 0.7
S. epidermidis	24.7 ± 5.8	17.7 ± 0.3
VRE	17.3 ± 2.3	14.1 ± 0.2
V. cholerae	7.12 ± 0.3	14.7 ± 1.8
V. vulnificus	18.1 ± 2.3	3.20 ± 1.3
L. anguillarum	14.7 ± 1.7	13.8 ± 1.5
R. salexigens	6.60 ± 1.1	17.6 ± 3.2

[a] EC_{50} values are in μM.



Scheme 2 Structure of **21** and the synthesis of **22** and **23**.

bacteria. This was driven by a number of factors, including our interest in the development of antibiofoulants[26] and the prevalence of vibrio infections in human populations.[27] *Vibrio cholerae*, *Vibrio vulnificus*, *Listonella anguillarum* and *Rhodospirillum salexigens* were chosen for evaluation. Given the microbicidal activity that was observed against terrestrial bacterial strains, we determined the MIC values of each compound against all the aforementioned strains. This data is summarized in Table 6. Paralleling our previous results, compounds **15** and **19** were the lead compounds. Compound **15** had MIC values of 4.2, 2.1, 2.1 and 2.1 μg mL^{-1} against *V. cholerae*, *V. vulnificus*, *L. anguillarum* and *R. salexigens*, respectively, while compound **19** had MIC values of 2.2 μg mL^{-1} against *V. cholerae*, *V. vulnificus* and *L. anguillarum*, and 1.1 μg mL^{-1} against *R. salexigens*.

We also determined the ability of **15** and **19** to disperse pre-formed biofilms against all the aforementioned bacterial strains (both marine and terrestrial). To quantify this effect, we determined the EC_{50} value of each compound against each bacterial strain, where EC_{50} is defined as the concentration of compound that elicits 50% dispersion of a pre-formed biofilm. This data is summarized in Table 7. Both compounds are able to disperse pre-formed biofilms at low micromolar concentrations, albeit these concentrations are microbicidal to planktonic bacteria.

To examine the necessity of the 4,5-disubstitution pattern for antimicrobial activity of the 2-aminoimidazole scaffold, two mono-substituted 2-aminoimidazole analogues of compound **19** were synthesized and screened alongside the parent 2-aminoimidazole for antibiotic activity (Scheme 2). Heptanoic acid and phenylhexanoic acid were treated with oxalyl chloride with catalytic dimethyl formamide in dichloromethane to produce the respective acid chlorides which were then reacted with diazomethane and quenched with hydrobromic acid to make the respective α-bromo ketones. These α-bromo ketones were separately cyclized with boc-guanidine, providing a boc-protected 2-aminoimidazole scaffold that was subsequently deprotected upon treatment with trifluoroacetic acid in dichloromethane to yield **22** and **23**. 2-Aminoimidazole **21** was found to have no antibiotic activity at the highest concentration tested (36 μg mL^{-1}) against every bacterial strain. Compound **22** demonstrated MIC values of greater than 41 μg mL^{-1} against all of the test strains except for *S. epidermidis* in which a remarkable MIC value of 0.04 μg mL^{-1} was found; making **22** the most potent antibiotic in this study. Lastly, compound **23** was also found to contain no notable antibiotic activity against any of the test strains except for *S. epidermidis*, for which an MIC value of 0.05 μg mL^{-1} was found. This demonstrates the necessity for substitution on the 2-AI scaffold to elicit the antibiotic response with broad spectrum activity being attributed to 4, 5-disubstitution.

Once we had evaluated both the anti-biofilm and antibacterial properties of each 2-AI derivative, we assessed the hemolytic potential of compounds **15**, **19** and **1**.[28] Compounds **15** and **19** are the leads from our first generation and second generation compounds, respectively, while compound **1** was chosen for comparison purposes to evaluate the activity of a bacterial inactive 2-AI. Hemolysis was measured using difibrinated sheep's blood. Hemolytic potential was quantified by determining the HD_{50} of compounds **15**, **19** and **1**, where HD_{50} is defined as the concentration that elicits 50% hemolysis. From this assay, we determined that the HD_{50} of compounds **15**, **19** and **1** were 66, 61 and >400 μM. This corresponds to 22 and 21 μg mL^{-1} for compounds **15** and **19**.

Finally, given that active compounds are characterized by a polar head group and a lipophilic tail, we probed whether compounds **15** and **19** were eliciting their activity *via* a pore-forming mechanism. As a control, we also included the inactive compound **1**. This was probed *via* a dye dispersion assay from synthetic vesicles.[29] Two types of vesicles were prepared, one containing 1-palmitoyl-2-oleoyl-sn-*glycero*-3-phosphocholine (POPC) and the other containing 1-palmitoyl-2-oleoyl-sn-*glycero*-3-phosphoglycerol (POPG). POPC mimics typical cell membranes encountered for mammalian red blood cells, while POPG mimics the negatively charged lipids encountered in bacterial membranes.

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In 5 min, at 25 μM, compounds **15** and **19** elicited 20% dye leakage from the POPG vesicles, while they elicited 10 and 15% dye leakage from the POPC vesicles, respectively. However, in 5 min at 500 μM, compounds **15** and **19** elicited 78 and 85% dye leakage from the POPG vesicles, respectively. In 5 min at 25 μM, inactive compound **1** elicited 9 and 18% dye leakage from the POPC and POPG vesicles, respectively, while in 5 min at 500 μM **1** elicited 15 and 50% leakage from the POPC and POPG vesicles. Given this differential response, it's probable that compounds **15** and **19** are inducing microbicidal activity through a simple pore-forming mechanism; however, further experiments are necessary to validate this.

Conclusions

In summary, we have developed a route to 4,5-disubstituted-2-aminoimidazoles that allows rapid assembly of 2-AI derivatives from readily available building blocks. Using this approach, we have identified two lead compounds, **15** and **19**, that are both microbicidal and have the ability to disperse pre-formed biofilms. Given this activity and the simple substituent patterns employed in this study, it is highly probable that more functionalized 4,5-disubstituted-2-aminoimidazoles, as well as 2-aminoimidzoles with alternative substitution patterns, will yield 2-AI derivatives with either enhanced antibiotic or anti-biofilm activity. These studies are ongoing in our lab and will be reported in due course.

Experimental

General experimental

All reagents used for chemical synthesis were purchased from commercially available sources and used without further purification. Chromatography was performed using 60 Å mesh standard grade silica gel from Sorbtech (Atlanta, GA, USA). NMR solvents were obtained from Cambridge Isotope Labs and used as is. ^{1}H NMR (300 MHz or 400 MHz) and ^{13}C NMR (75 MHz or 100 MHz) spectra were recorded at 25 °C on Varian Mercury spectrometers. Chemical shifts (δ) are given in ppm relative to tetramethylsilane or the respective NMR solvent; coupling constants (J) are in Hertz (Hz). Abbreviations used are s = singlet, bs = broad singlet, d = doublet, dd = doublet of doublets, t = triplet, dt = doublet of triplets, td = triplet of doublets, bt = broad triplet, q = quartet, m = multiplet, bm = broad multiplet and br = broad. Mass spectra were obtained at the NCSU Department of Chemistry Mass Spectrometry Facility.

S. aureus (ATCC # 29213), *S. epidermidis* (ATCC # 29886), MRSA (ATCC # BAA-44), MDRAB (ATCC # BAA-1605), vancomycin resistant *Enterococcus faecium* (VRE) (ATCC # 51559) and *E. coli* (ATCC # 35695) were obtained from the ATCC. Mechanically difibrinated sheep blood (DSB100) was obtained from Hemostat Labs. Mueller-Hinton medium was purchased from Fluka (# 70192).

Synthesis of 4,5-disubstituted 2-aminoimidazoles

General procedure for the preparation of α-nitro ketones. To a vial (23 × 85 mm) was added appropriate phenyl carboxylic acids and 1,1′-carbonyldiimidazole in THF (3 mL) and stirred at room temperature for 20 min. A mixture of appropriate nitroalkanes and 1,8-diazabicyclo[5.4.0]undec-7-ene, dissolved in THF (2 mL) that was pre-stirred for 20 min, was then added dropwise to the resulting solution. The resulting mixture was stirred for 12 h at 55 °C. The mixture was cooled down to 0 °C and 1 N HCl (2 mL) was added, then extracted with ethyl acetate (3 × 2 mL). The combined organic extracts were washed with water (1 × 3 mL), brine (1 × 3 mL), dried over Na_2SO_4 and the solvent evaporated under reduced pressure. Purification of the residue took place on a silica-gel column eluted with dichloromethane to give the target α-nitro ketones. Resulting α-nitro ketones judged to be >85% pure by ^{1}H NMR were then subjected to hydrogenation/cyclization (detailed below). Representative α-nitro ketones and 4,5-disubstituted-2-aminoimidazoles are detailed below. All other characterization is supplied in the supplementary information.

2-Nitro-8-phenyloctan-3-one. 6-Phenylhexanoic acid (0.154 g, 0.80 mmol) and CDI (0.261 g, 1.60 mmol) were added together and then reacted with nitroethane (0.090 g, 1.21 mmol) and DBU (0.306 g, 2.00 mmol) according to the general procedure. Purification by column chromatography gave 0.060 g (30%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (m, 2H), 7.21(m, 3H), 5.24 (q, J = 7.2 Hz, 1H), 2.64 (t, J = 7.6 Hz, 2H), 2.58 (td, J = 3.2, 6.8 Hz, 2H), 1.69 (d, J = 7.2 Hz, 3H), 1.68 (m, 4H), 1.37 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.1, 142.6, 128.7, 128.6, 126.0, 89.1, 39.3, 35.9, 31.4, 28.7, 23.3, 15.2 ppm; IR ν_{max}/cm^{-1} 3026, 2933, 2858, 1732, 1559, 1453, 1362, 1030, 749, 701; HRMS (FAB) calcd for $C_{14}H_{19}NO_3$ (MNa^+) 272.1257, found 272.1255.

3-Nitro-9-phenylnonan-4-one. 6-Phenylhexanoic acid (0.100 g, 0.52 mmol) and CDI (0.169 g, 1.04 mmol) were added together and then reacted with nitropropane (0.070 g, 0.78 mmol) and DBU (0.198 g, 1.30 mmol) according to the general procedure. Purification by column chromatography gave 0.059 g (43%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (m, 2H), 7.17 (m, 3H), 5.04 (dd, J = 4.4, 9.6 Hz, 1H), 2.60 (m, 4H), 1.62 (m, 6H), 1.31 (m, 2H) 1.02 (t, J = 7.2 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.3, 142.5, 128.6, 128.5, 126.0, 95.8, 39.5, 35.9, 31.3, 28.7, 23.5, 23.3, 10.6 ppm; IR ν_{max}/cm^{-1} 2922, 2855, 1730, 1559, 1454, 1363, 1030, 747, 699; HRMS (FAB) calcd for $C_{15}H_{21}NO_3$ (MNa^+) 286.1414, found 286.1410.

4-Nitro-10-phenyldecan-5-one. 6-Phenylhexanoic acid (0.100 g, 0.52 mmol) and CDI (0.169 g, 1.04 mmol) were added together and then reacted with nitrobutane (0.080 g, 0.78 mmol) and DBU (0.198 g, 1.30 mmol) according to the general procedure. Purification by column chromatography gave 0.084 g (59%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (m, 2H), 7.20 (m, 3H), 5.17 (dd, J = 4.4, 10.0 Hz, 1H), 2.63 (m, 4H), 1.65 (m, 6H), 1.36 (m, 4H) 1.00 (t, J = 7.2 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.5, 142.6, 128.7, 128.6, 126.0, 94.3, 39.5, 35.9, 31.8, 31.4, 28.7, 23.3, 19.5, 13.6 ppm; IR ν_{max}/cm^{-1} 3027, 2935, 2858, 1730, 1560, 1454, 1373, 1031, 749, 700; HRMS (FAB) calcd for $C_{16}H_{23}NO_3$ (MNa^+) 300.1570, found 300.1575.

7-Nitro-1-phenylundecan-6-one. 6-Phenylhexanoic acid (0.100 g, 0.52 mmol) and CDI (0.169 g, 1.04 mmol) were added together and then reacted with nitropentane (0.091 g, 0.78 mmol) and DBU (0.198 g, 1.30 mmol) according to the general procedure. Purification by column chromatography gave 0.049 g (32%) as a

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yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (m, 2H), 7.17 (m, 3H), 5.10 (dd, $J = 4.4$, 10.0 Hz, 1H), 2.61 (m, 4H), 1.63 (m, 6H), 1.35 (m, 6H) 0.92 (t, $J = 6.8$ Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.5, 142.5, 128.6, 128.5, 126.0, 94.5, 39.5, 35.9, 31.3, 29.6, 28.7, 28.1, 23.3, 22.2, 13.9 ppm; IR ν_{max}/cm^{-1} 3027, 2931, 2859, 1731, 1559, 1454, 1363, 1030, 748, 700; HRMS (FAB) calcd for $C_{17}H_{25}NO_3$ (MNa^+) 314.1727, found 314.1722.

7-Nitro-1-phenyldodecan-6-one. 6-Phenylhexanoic acid (0.150 g, 0.78 mmol) and CDI (0.253 g, 1.56 mmol) were added together and then reacted with nitrohexane (0.153 g, 1.17 mmol) and DBU (0.297 g, 1.95 mmol) according to the general procedure. Purification by column chromatography gave 0.090 g (38%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (m, 2H), 7.17 (m, 3H), 5.11 (dd, $J = 4.8$, 10.4 Hz, 1H), 2.61 (m, 4H), 1.63 (m, 6H), 1.33 (m, 8H) 0.90 (t, $J = 6.8$ Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.5, 142.5, 128.6, 128.5, 126.0, 94.6, 39.5, 35.9, 31.3, 31.2, 29.9, 28.7, 25.7, 23.3, 22.4, 14.1 ppm; IR ν_{max}/cm^{-1} 3027, 2930, 2858, 1731, 1559, 1454, 1363, 1030, 748, 699; HRMS (FAB) calcd for $C_{18}H_{27}NO_3$ (MNa^+) 328.1883, found 328.1881.

7-Nitro-1-phenyltridecan-6-one. 6-Phenylhexanoic acid (0.150 g, 0.78 mmol) and CDI (0.253 g, 1.56 mmol) were added together and then reacted with nitroheptane (0.170 g, 1.17 mmol) and DBU (0.297 g, 1.95 mmol) according to the general procedure. Purification by column chromatography gave 0.104 g (42%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (m, 2H), 7.17 (m, 3H), 5.11 (dd, $J = 4.8$, 10.0 Hz, 1H), 2.61 (m, 4H), 1.63 (m, 6H), 1.33 (m, 10H) 0.89 (t, $J = 7.2$ Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.5, 142.5, 128.6, 128.5, 126.0, 94.6, 39.4, 35.9, 31.5, 31.3, 29.9, 28.8, 28.7, 26.0, 23.3, 22.7, 14.2 ppm; IR ν_{max}/cm^{-1} 3027, 2931, 2859, 1731, 1559, 1454, 1363, 1031, 748, 700; HRMS (FAB) calcd for $C_{19}H_{29}NO_3$ (MNa^+) 342.2040, found 342.2038.

General procedure for 2-aminoimidazole synthesis

The appropriate α-nitro ketone (>85% pure, judged by ^{1}H NMR) was dissolved in ethanol (3 mL), concentrated HCl and 5% palladium on carbon (0.2 equivalents) were added and the reaction was stirred under H_2 for 24 h. The mixture was filtered through Celite and the solvent was evaporated under reduced pressure. The residue was dissolved in ethanol (3 mL) and the pH was adjusted to 4.5 with 0.1 N NaOH. To the solution was added cyanamide and heated at 95 °C for 2 h. The ethanol was then evaporated under reduced pressure and the resulting residue was purified by column chromatography (CH_2Cl_2–MeOH sat. NH_3 80 : 20) to afford the desired compound in its free base form. Addition of concentrated HCl to a methanol solution (2 mL) of the free base followed by solvent evaporation under reduced pressure delivered the corresponding 2-aminoimidazole as its HCl salt.

5-Methyl-4-(5-phenylpentyl)-1*H*-imidazol-2-amine (11). 2-Nitro-8-phenyloctan-3-one (0.025 g, 0.10 mmol) reacted with concentrated HCl (0.50 mmol) and palladium, 5 wt.% on activated carbon (0.043 g, 0.020 mmol) under H_2, then reacted with cyanamide (0.021 g, 0.50 mmol) according to the general procedure. Purification by column chromatography gave 0.010 g (41%) over two steps as a yellow oil: ^{1}H NMR (300 MHz, CD_3OD) δ 7.22 (m, 2H), 7.14 (m, 3H), 2.59 (t, $J = 7.5$ Hz, 2H), 2.42 (t, $J = 6.9$ Hz, 2H), 2.01 (s, 3H) 1.60 (m, 4H), 1.31 (m, 2H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 146.2, 142.5, 128.3, 128.1, 125.5, 121.9, 117.5, 35.5, 31.1, 28.6, 28.1, 22.9, 7.5 ppm; IR ν_{max}/cm^{-1} 3169, 2929, 2857, 1680, 1453, 1020, 749, 700; HRMS (FAB) calcd for $C_{15}H_{21}N_3$ (MH^+) 244.1808, found 244.1814.

5-Ethyl-4-(5-phenylpentyl)-1*H*-imidazol-2-amine (12). 3-Nitro-9-phenylnonan-4-one (0.050 g, 0.19 mmol) reacted with concentrated HCl (0.95 mmol) and palladium, 5 wt.% on activated carbon (0.081 g, 0.038 mmol) under H_2, then reacted with cyanamide (0.040 g, 0.95 mmol) according to the general procedure. Purification by column chromatography gave 0.014 g (29%) over two steps as a yellow oil: ^{1}H NMR (300 MHz, CD_3OD) δ 7.25 (m, 2H), 7.14 (m, 3H), 2.59 (t, $J = 7.5$ Hz, 2H), 2.42 (m, 4H), 1.63 (m, 4H), 1.28 (m, 2H), 1.13 (t, $J = 7.5$ Hz, 3H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 146.2, 142.4, 128.2, 128.1, 125.5, 123.6, 121.3, 35.5, 31.1, 28.7, 28.1, 22.9, 16.5, 13.1 ppm; IR ν_{max}/cm^{-1} 3327, 2934, 1680, 1453, 1016, 749; HRMS (FAB) calcd for $C_{16}H_{23}N_3$ (MH^+) 258.1965, found 258.1973.

4-(5-Phenylpentyl)-5-propyl-1*H*-imidazol-2-amine (13). 4-Nitro-10-phenyldecan-5-one (0.084 g, 0.30 mmol) reacted with concentrated HCl (1.50 mmol) and palladium, 5 wt.% on activated carbon (0.129 g, 0.060 mmol) under H_2, then reacted with cyanamide (0.064 g, 1.50 mmol) according to the general procedure. Purification by column chromatography gave 0.025 g (30%) over two steps as a yellow oil: ^{1}H NMR (300 MHz, CD_3OD) δ 7.20 (m, 2H), 7.15 (m, 3H), 2.60 (t, $J = 7.2$ Hz, 2H), 2.40 (m, 4H), 1.56 (m, 6H), 1.30 (m, 2H), 0.92 (t, $J = 7.2$ Hz, 3H) ppm; ^{13}C NMR (100 MHz, CD_3OD) δ 146.4, 142.5, 128.3, 128.1, 125.6, 122.1, 121.9, 35.5, 31.1, 28.8, 28.2, 25.0, 23.0, 22.3, 12.6 ppm; IR ν_{max}/cm^{-1} 3165, 2931, 1680, 1453, 1031, 747, 699; HRMS (FAB) calcd for $C_{17}H_{25}N_3$ (MH^+) 272.2121, found 272.2127.

5-Butyl-4-(5-phenylpentyl)-1*H*-imidazol-2-amine (14). 7-Nitro-1-phenylundecan-6-one (0.049 g, 0.17 mmol) reacted with concentrated HCl (0.85 mmol) and palladium, 5 wt.% on activated carbon (0.072 g, 0.034 mmol) under H_2, then reacted with cyanamide (0.035 g, 0.84 mmol) according to the general procedure. Purification by column chromatography gave 0.025 g (52%) over two steps as a yellow oil: ^{1}H NMR (400 MHz, CD_3OD) δ 7.22 (m, 2H), 7.15 (m, 3H), 2.60 (t, $J = 7.2$ Hz, 2H), 2.43 (m, 4H), 1.64 (m, 6H), 1.34 (m, 4H), 0.94 (t, $J = 7.6$ Hz, 3H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 146.4, 142.4, 128.2, 128.1, 125.6, 122.1, 121.9, 35.5, 31.2, 31.1, 28.7, 28.2, 23.0, 22.8, 21.9, 12.9 ppm; IR ν_{max}/cm^{-1} 3166, 2930, 2857, 1680, 1453, 1030, 748, 699; HRMS (FAB) calcd for $C_{18}H_{27}N_3$ (MH^+) 286.2278, found 286.2288.

5-Pentyl-4-(5-phenylpentyl)-1*H*-imidazol-2-amine (15). 7-Nitro-1-phenyldodecan-6-one (0.090 g, 0.29 mmol) reacted with concentrated HCl (1.45 mmol) and palladium, 5 wt.% on activated carbon (0.125 g, 0.060 mmol) under H_2, then reacted with cyanamide (0.062 g, 1.47 mmol) according to the general procedure. Purification by column chromatography gave 0.071 g (81%) over two steps as a yellow oil: ^{1}H NMR (300 MHz, CD_3OD) δ 7.20 (m, 2H), 7.14 (m, 3H), 2.59 (t, $J = 7.5$ Hz, 2H), 2.42 (m, 4H), 1.56 (m, 6H), 1.32 (m, 6H), 0.90 (t, $J = 7.2$ Hz, 3H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 146.3, 142.5, 128.3, 128.1, 125.6, 122.1, 121.8, 35.5, 31.1, 31.1, 28.7, 28.7, 28.2, 23.1, 23.0, 22.3, 13.3 ppm; IR ν_{max}/cm^{-1} 3169, 2928, 2857, 1680, 1453,

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1202, 1030, 746, 699; HRMS (FAB) calcd for $C_{19}H_{29}N_3$ (MH^+) 300.2434, found 300.2441.

5-Hexyl-4-(5-phenylpentyl)-1*H*-imidazol-2-amine (19). 7-Nitro-1-phenyltridecan-6-one (0.104 g, 0.33 mmol) reacted with concentrated HCl (1.65 mmol) and palladium, 5 wt.% on activated carbon (0.139 g, 0.065 mmol) under H_2, then reacted with cyanamide (0.068 g, 1.63 mmol) according to the general procedure. Purification by column chromatography gave 0.049 g (48%) over two steps as a yellow oil: ^{1}H NMR (400 MHz, CD_3OD) δ 7.23 (m, 2H), 7.15 (m, 3H), 2.60 (t, $J = 7.6$ Hz, 2H), 2.42 (m, 4H), 1.63 (m, 6H), 1.30 (m, 8H), 0.89 (t, $J = 6.8$ Hz, 3H) ppm; ^{13}C NMR (100 MHz, CD_3OD) δ 146.4, 142.4, 128.2, 128.1, 125.6, 122.1, 121.8, 35.5, 31.5, 31.1, 29.0, 28.7, 28.6, 28.2, 23.1, 23.0, 22.5, 13.3 ppm; IR ν_{max}/cm^{-1} 3165, 2929, 2857, 1680, 1453, 1202, 1029, 747, 699; HRMS (FAB) calcd for $C_{20}H_{31}N_3$ (MH^+) 314.2591, found 314.2596.

Control 2-aminoimidazole synthesis

22 and **23** were synthesized from their corresponding carboxylic acids as outlined below, using established literature protocols.[12]

1-Bromooctan-2-one. To a 100 mL round bottomed flask, heptanoic acid (1.41 g, 10.81 mmol) was added and then dissolved in dichloromethane and a stir bar was added and allowed to stir. Then, three drops of dimethylformamide was added and then the reaction mixture was cooled to 0 °C. Oxalyl chloride (4.12 g, 32.43 mmol) was added dropwise and left to continue stirring for one hour. Separately in a diazomethane kit, a stir bar, KOH (10.00 g, 178.2 mmol), 24 mL of ethanol, 17 mL of water was added to the top of the diazomethane apparatus and was heated to 65 °C and stirred. To a diazomethane kit liquid addition funnel diazald (10.00 g, 46.67 mmol) and 100 mL of diethyl ether was added. The diethyl ether/diazald mixture was allowed to add dropwise to the KOH–water–ethanol mixture so that the diazomethane was generated and distilled over to the collection flask that was cooled to 0 °C. Once the diazomethane had been completely collected, and the heptanoic acid had reacted with the oxalyl chloride for one hour, the heptanoic acid reaction mixture was concentrated *in vacuo* without heating in excess of 25 °C, dissolved in 3 mL of dichloromethane and was added slowly to the flask containing the diazomethane while still being cooled to 0 °C. The reaction mixture was allowed to stir for one hour at 0 °C. Then, 4 mL of concentrated hydrobromic acid was added slowly to the reaction mixture and allowed to stir for 20 min. Then, 100 mL of a saturated sodium bicarbonate solution was added slowly to the reaction mixture and allowed to stir for 30 min. The resulting mixture was then extracted with ethyl acetate, washed twice with a brine solution, concentrated *in vacuo* and then purified *via* column chromatography with a 10% ethyl acetate–hexanes solution providing 1-bromooctan-2-one as a light yellow oil (2.19 g, 97% yield) ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.79 (s, 2H), δ 2.49 (t, $J = 7.2$ Hz, 2H), δ 1.44 (m, 2H), δ 1.13 (bs, 6H), δ 0.72 (t, $J = 4.5$ Hz, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.15, 39.9, 34.9, 31.6, 28.8, 23.9, 22.6, 14.1 ppm; IR ν_{max}/cm^{-1} 3423, 306, 3025, 2933, 2856, 1716, 1495, 1453, 1030, 748; HRMS (ESI) calcd for $C_8H_{15}{}^{79}BrO$ (M+) 206.0306, found 206.0301.

1-Bromo-7-phenylhexan-2-one. To a 100 mL round bottomed flask, phenyl hexanoic acid (2.08 g, 10.81 mmol) was added and then dissolved in dichloromethane and a stir bar was added and allowed to stir. Then, three drops of dimethylformamide was added and then the reaction mixture was cooled to 0 °C. Oxalyl chloride (4.12 g, 32.43 mmol) was added dropwise and left to continue stirring for one hour. Separately in a diazomethane kit, a stir bar, KOH (10.00 g, 178.2 mmol), 24 mL of ethanol, 17 mL of water was added to the top of the diazomethane apparatus and was heated to 65 °C and stirred. To a diazomethane kit liquid addition funnel diazald (10.00 g, 46.67 mmol) and 100 mL of diethyl ether was added. The diethyl ether/diazald mixture was allowed to add dropwise to the KOH–water–ethanol mixture so that the diazomethane was generated and distilled over to the collection flask that was cooled to 0 °C. Once the diazomethane had been completely collected, and the heptanoic acid had reacted with the oxalyl chloride for one hour, the heptanoic acid reaction mixture was concentrated *in vacuo* without heating in excess of 25 °C, dissolved in 3 mL of dichloromethane and was added slowly to the flask containing the diazomethane while still being cooled to 0 °C. The reaction mixture was allowed to stir for one hour at 0 °C. Then 4 mL of concentrated hydrobromic acid was added slowly to the reaction mixture and allowed to stir for 20 min. Then, 100 mL of a saturated sodium bicarbonate solution was added slowly to the reaction mixture and allowed to stir for 30 min. The resulting mixture was then extracted with ethyl acetate, washed twice with a brine solution, concentrated *in vacuo* and then purified *via* column chromatography with a 10% ethyl acetate–hexanes solution providing 1-bromo-7-phenylheptan-2-one as a light yellow oil (2.53 g, 87% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (t, $J = 7.5$ Hz, 2H), δ 7.20 (d, $J = 7.5$ Hz, 3H), δ 3.86 (s, 2H), δ2.63 (t, $J = 7.2$ Hz, 4H), δ 1.65 (q, $J = 7.8$, 7.2 Hz, 4H), δ 1.34 (m, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.3, 142.7, 128.7, 128.6, 126.0, 39.9, 35.9, 34.9, 31.5, 28.9, 23.8 ppm; IR ν_{max}/cm^{-1} 3434, 2930, 2848, 2113, 1641, 1393; HRMS (ESI) calcd for $C_{13}H_{17}{}^{79}BrO$ (M+) 268.0462, found 268.0467.

4-Hexyl-1*H*-imidazol-2-amine hydrochloride. 1-Bromooctan-2-one (1.00 g, 4.83 mmol) was placed in a 50 mL round bottomed flask with a stir bar and dissolved in 10 mL of DMF. Boc guanidine (2.31 g, 14.89 mmol) was then added to the reaction mixture and it was allowed to stir for 48 h. Water was then added to the reaction mixture and it was placed in a separating funnel. The mixture was then extracted twice with ethyl acetate, washed twice with water, washed twice with brine, concentrated *in vacuo* and then purified *via* column chromatography (5% methanol–95% dichloromethane) to provide *tert*-butyl 2-amino-4-hexyl-1*H*-imidazole-1-carboxylate as a hygroscopic light yellow solid (0.74 g, 57% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.69 (s, 2H), δ 6.34 (s, 1H), δ 2.20 (t, $J = 7.5$ Hz, 2H), δ 1.44 (bs, 11H), 1.17 (s, 6H), δ 0.76 (s, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.1, 149.6, 139.3, 105.7, 84.1, 31.8, 29.2, 28.4, 28.3, 28.0, 22.7, 14.2 ppm; IR ν_{max}/cm^{-1} 3421, 2109, 1644, 1442, 1204, 1142; HRMS (ESI) calcd for $C_{14}H_{25}N_3O_2$ (M+) 267.1946, found 267.1940. Then, *tert*-butyl 2-amino-4-hexyl-1*H*-imidazole-1-carboxylate was dissolved in a 1 : 4 mixture of trifluoroacetic acid–dichloromethane and allowed to stir for five hours and then concentrated *in vacuo*. The sample was then dissolved with a dilute HCl–methanol (5 drops HCl/50 mL of methanol) and then concentrated *in vacuo* to

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provide 4-(5phenylpentyl)-1H)-imidazol-2-amine hydrochloride as a hygroscopic light yellow solid (0.57 g, 57% overall yield). ^{1}H NMR (300 MHz, CD_3OD) δ 6.34 (bs, 3H), δ 2.41 (t, J = 7.5 Hz, 2H), δ1.56 (m, 2H), δ 1.28 (bs, 6H), δ 0.82 (t, J = 6.6 Hz, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.4, 131.9, 111.9, 35.3, 32.4, 31.9, 28.3, 26.3, 17.1 ppm; IR ν_{max}/cm^{-1} 3411, 2099, 1638; HRMS (ESI) calcd for $C_9H_{17}N_3$ (M+) 167.1422, found 167.1416.

4-(5-Phenylpentyl)-1*H*)-imidazol-2-amine hydrochloride. 1-Bromo-7-phenylheptan-2-one (1.00 g, 3.71 mmol) was placed in a 50 mL round bottomed flask with a stir bar and dissolved in 10 mL of DMF. Boc guanidine (1.77 g, 11.13 mmol) was then added to the reaction mixture and it was allowed to stir for 48 h. Water was then added to the reaction mixture and it was placed in a separating funnel. The mixture was then extracted twice with ethyl acetate, washed twice with water, washed twice with brine, concentrated *in vacuo* and then purified *via* column chromatography (5% methanol–95% dichloromethane) to provide *tert*-butyl 2-amino-4-(5-phenylpentyl)-1*H*-imidazole-1-carboxylate as a hygroscopic light yellow solid (0.77 g, 63% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (m, 2H), δ 7.26 (m, 3H), δ 6.66 (s, 2H), δ 6.51 (s, 1H), δ 2.63 (t, J = 7.5 Hz, 2H), δ 2.39 (t, J = 7.5 Hz, 2H), δ 1.67 (m, 4H), δ 1.53 (s, 9H), δ 1.40 (m, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.1, 149.8, 143.0, 130.3, 128.7, 128.5, 125.8, 106.2, 84.5, 36.1, 31.6, 29.2, 28.5, 28.4, 28.2 ppm; IR ν_{max}/cm^{-1} 3433, 2098, 1639, 1454, 1204; HRMS (ESI) calcd for $C_{19}H_{27}N_3O_2$ (M+) 329.2103, found 329.2109. Then, *tert*-butyl 2-amino-4-(5-phenylpentyl)-1*H*-imidazole-1-carboxylate was dissolved in a 1 : 4 mixture of trifluoroacetic acid–dichloromethane and allowed to stir for five hours and then concentrated *in vacuo*. The sample was then dissolved with a dilute HCl–methanol (5 drops HCl/50 mL of methanol) and then concentrated *in vacuo* to provide 4-(5phenylpentyl)-1H)-imidazol-2-amine hydrochloride as a hygroscopic light yellow solid (0.62 g, 63% overall yield). ^{1}H NMR (300 MHz, CD_3OD) δ 7.20 (t, J = 7.2 Hz, 2H), δ 7.12 (d, J = 7.2 Hz, 3H), δ 6.37 (s, 1H), δ 2.56 (t, J = 7.5 Hz, 2H), δ 2.41 (t, J = 7.5 Hz, 2H), δ 1.57 (m, 4H), δ 1.34 (m, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.3, 142.5, 128.3, 128.1, 127.7, 125.6, 108.2, 35.5, 31.1, 28.3, 27.0, 24.2 ppm; IR ν_{max}/cm^{-1} 3385, 2933, 2857, 1679, 1536, 1495, 1453, 1335, 1172, 748, 700; HRMS (ESI) calcd for $C_{14}H_{19}N_3$ (M+) 229.1578, found 229.1571.

Biological screening experimental

Broth microdilution method for MIC determination. Overnight cultures of bacterial strain were subcultured to 5×10^5 CFU/mL in Mueller-Hinton medium (Fluka # 70192). The resulting bacterial suspension was aliquoted (1.0 mL) into culture tubes. Samples were prepared from these culture tubes containing either 256 μg mL^{-1} of specified antibiotic or no test compound as a control. Samples were then aliquoted (200 μL) into the first row of wells of a 96-well microtiter plate in which subsequent wells were prefilled with 100 μL of Mueller-Hinton medium based 5×10^5 CFU/mL bacterial subculture. Using the multichannel pipettor set at 100 μL, row one wells were mixed 8–10 times. Then, 100 μL were withdrawn and transferred to row two. Row two wells were mixed 8–10 times followed by a 100 μL transfer from row two to row three. This procedure was used to serial dilute the rest of the rows of the microtier plate. The microtiter plate sample was then covered with a microtiter plate lid and then placed in a covered plastic container. The chamber was incubated under stationary conditions at 37° C. After 16 h, the lid was removed and MIC values were recorded.

Red blood cell hemolysis assay. Hemolysis assays were performed on mechanically difibrinated sheep blood (Hemostat Labs: DSB100). 1.5 mL of blood was placed into a microcentrifuge tube and centrifuged at 10000 rpm for ten minutes. The supernatant was removed and then the cells were resuspended with 1 mL of phosphate-buffered saline (PBS). The suspension was centrifuged, the supernatant was removed and cells resuspended two more times. The final cell suspension was then diluted tenfold. Test compound solutions were made in PBS and then added to aliquots of the tenfold suspension dilution. PBS alone was used as a negative control and as a zero hemolysis marker, whereas a 1% Triton X sample was used as a positive control and the 100% lysis marker. Samples were then placed in an incubator at 37 °C while being shaken at 200 rpm for one hour. After one hour, the samples were transferred to microcentrifuge tubes and then centrifuged at 10000 rpm for ten minutes. The resulting supernatant was diluted by a factor of 40 in distilled water. The absorbance of the supernatant was measured with a UV spectrometer at a 540 nm wavelength.

Procedure to determine the dispersal effect of test compounds on *E. faecium* (VRE), MRSA, *S. aureus*, *S. epidermidis*, *E. coli*, *R. salexigens*, *V. cholerae*, *V. vulnificus* and *L. anguillarum* preformed biofilms. Dispersion assays were performed by taking an overnight culture of bacterial strain and subculturing it at an OD_{600} of 0.01 into the necessary medium (brain heart infusion for *E. faecium*, tryptic soy broth with a 0.5% glucose supplement (TSBG) for MRSA, *S. aureus* and *S. epidermidis*, Luria-Bertani (LB) medium for MDRAB and *E. coli*, and tryptic soy broth with a 0.5% glucose supplement and a 3.0% NaCl supplement (TGN) for *R. salexigens*, *V. cholerae*, *V. vulnificus* and *L. anguillarum*). The resulting bacterial suspension was aliquoted (100 μL) into the wells of a 96-well PVC microtiter plate. Plates were then wrapped in GLAD Press n' Seal® followed by an incubation under stationary conditions at 37 °C to establish the biofilms. After 24 h, the medium was discarded from the wells and the plates were washed thoroughly with water. Stock solutions of predetermined concentrations of the test compound were then made in the necessary medium. These stock solutions were aliquoted (100 μL) into the wells of the 96-well PVC microtiter plate with the established biofilms. Medium alone was added to a subset of the wells to serve as a control. Sample plates were then incubated for 24 h at 37 °C. After incubation, the medium was discarded from the wells and the plates were washed thoroughly with water. Plates were then stained with 100 μL of 0.1% solution of crystal violet (CV) and then incubated at ambient temperature for 30 min. Plates were washed with water again and the remaining stain was solubilized with 200 μL of 95% ethanol. A sample of 125 μL of solubilized CV stain from each well was transferred to the corresponding wells of a polystyrene microtiter dish. Biofilm dispersion was quantitated by measuring the OD_{540} of each well in which a negative control lane wherein no biofilm was formed served as a background and was subtracted out.

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