DOI: 10.1002/cbic.200700774

Synthesis and Screening of an Oroidin Library against *Pseudomonas aeruginosa* Biofilms

Justin J. Richards, T. Eric Ballard, Robert W. Huigens, III, and Christian Melander*[a]

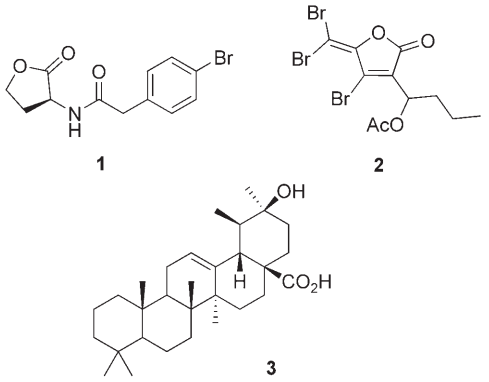
A 50-compound library based on the marine natural product oroidin was synthesized and assayed for anti-biofilm activity against PAO1 and PA14, two strains of the medically relevant γ-proteobacterium Pseudomonas aeruginosa*. Through structure–activity relationship (SAR) analysis of analogues based on the oroidin template, several conclusions can be drawn as to what structural properties of the synthetic derivatives are necessary to elicit a biological response. Notably, the most active analogues identified were those that contained a 2-aminoimidazole (2-AI) motif and a dibrominated pyrrolecarboxamide subunit. Here we disclose the synthesis and subsequently determined biological activity of this unique class of compounds as inhibitors of biofilm formation that have no direct antibiotic effect.*

Introduction

A bacterial biofilm is a community of surface-attached bacteria protected by an extracellular matrix of biomolecules. The formation of biofilms can be thought of as a developmental process in which planktonic bacteria adhere to a solid surface and initiate the formation of a complex sessile microcolony, which then exists as a community of bacteria.[1] It has been estimated that upwards of 80% of the world's microbial biomass resides within the biofilm state. Biofilm bacteria are resistant to many host immune responses and are also inherently insensitive to antiseptics or other antimicrobial compounds. Biofilms have been estimated to be upwards of 1000 times more resistant to conventional antibiotics,[2] which represents a significant impediment to antimicrobial therapy, because biofilms account for between 50–80% of microbial infections in the body.[3,4] In particular, persistent infections of indwelling medical devices remain a serious problem, since eradication of these infections is virtually impossible.[5,6] Other diseases in which biofilms are of importance include endocarditis, otitis media, chronic prostatitis, periodontal disease, chronic urinary tract infections, legionnaires' disease, and cystic fibrosis.[3,7]

Because of the breadth of detrimental effects that bacterial biofilms have on human healthcare, there has been a significant effort to develop molecules that will inhibit their formation,[1] the underlying principal being that if bacteria can be maintained in the planktonic state, then they will not attach to a target surface (lung tissue, implanted medical devices) and can then be subsequently killed with a low dose of microbicide (such as an antibiotic). Examples of molecular scaffolds that inhibit biofilm formation are scarce. They include the homoserine lactones (**1**), which are naturally occurring bacterial signaling molecules,[8] brominated furanones (**2**), originally isolated from the macroalga *Delisea pulchra*,[9,10] and ursene triterpenes (**3**) from the plant *Diospyros dendo*.[11]

Pseudomonas aeruginosa is a Gram-negative γ-proteobacterium that is one of the best-studied models in terms of biofilm formation and quorum sensing (QS).[12] This bacterium is also a

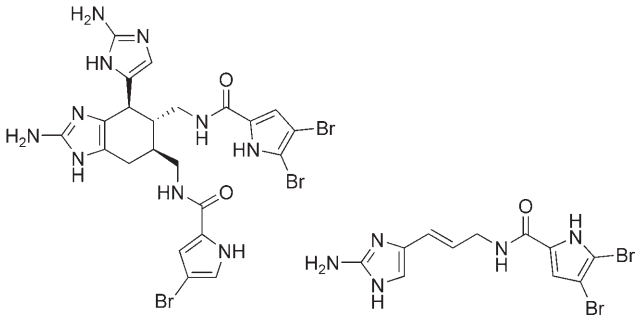


significant opportunistic pathogen that is responsible for a myriad of infections.[13] The virulence and persistence with which *P. aeruginosa* infections occur is known to be based in part upon the ability of the bacterium to form robust biofilms. For patients who suffer from cystic fibrosis (CF), the onset of colonization by this bacterium is of great concern. *P. aeruginosa* biofilms are directly correlated to the morbidity rates of CF patients.[14-17] Coupled with the speed and prevalence with which multi-drug resistant (MDR) strains are appearing,[18] there is a pressing need to develop novel small molecules that may play a role in *P. aeruginosa* remediation efforts.

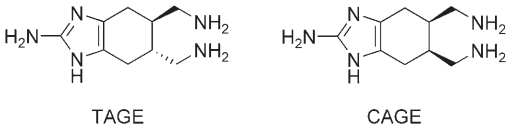
Given the paucity of molecular architectures that have been reported to inhibit the formation of bacterial biofilms, we have begun to investigate the effects that simple analogues of sponge-derived marine alkaloids have upon biofilm development. Two members of the oroidin family, bromoageliferin and

[a] *J. J. Richards, T. E. Ballard, R. W. Huigens, III, Prof. C. Melander*
Department of Chemistry, North Carolina State University
Raleigh, NC 27695 (USA)
Fax: (+1) 919-515-5079
E-mail: christian_melander@ncsu.edu

Supporting information for this article is available on the WWW under http://www.chembiochem.org or from the author.



oroidin, were documented as possessing anti-biofouling properties through inhibition of biofilm development in the marine α-proteobacterium *R. salexigens*.[19] Biofouling is initiated by a process termed microfouling, which is the formation of a bacterial biofilm on a surface submerged under water. We posited that architecture embedded within complex, marine alkaloids with anti-biofouling properties might provide a basis for novel molecules to inhibit bacterial biofilms. Recently we disclosed the synthesis of TAGE and CAGE, two derivatives of bromoageliferin that were shown to be effective inhibitors of *P. aeruginosa* biofilm formation.[20] Because of the success of these analogues, we opted to determine whether even simpler derivatives bearing resemblance to the ageliferin skeleton would possess anti-biofilm properties. Here we provide a full account of the structure–activity relationship (SAR) analysis from the synthesis and biological evaluation of a 50-compound oroidin library in the context of anti-biofilm activity against the medically relevant Gram-negative γ-proteobacterium *P. aeruginosa*.



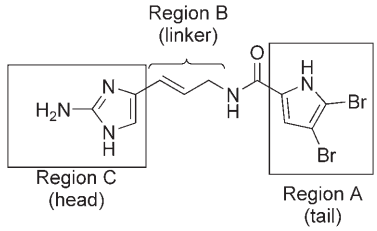
Results and Discussion

Design

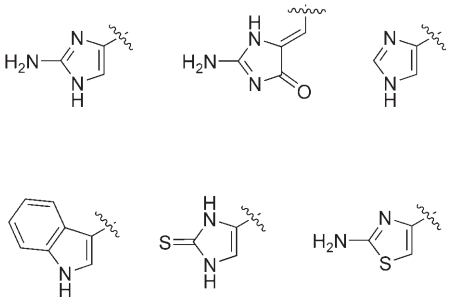
Marine natural products provide a diverse array of chemical structures and are known to possess a plethora of biological activities.[21] Most members of the oroidin alkaloid family have nitrogen-dense architectures that contain a 2-aminoimidazole (2-AI) subunit.[22,23] These compounds are typically found in sponges of the family *Agelasidae* and mainly serve as a chemical anti-feeding defense mechanism against predators.[24] Oroidin (**5**) is believed to be one of the main building blocks in the biosynthesis of other more complex family members including palau'amine and the stylissadines.[25,26] In addition to being documented as interfering with the biofouling process of *R. salexigens*, oroidin has also been observed to retard bacterial attachment and colonization in a limited number of studies.[27,28]

To gain a more thorough understanding of the activity profile of this unique class of molecules, we elected to synthesize a library of analogues based upon the oroidin template. The structure–activity relationships (SARs) were then delineated within the context of anti-biofilm activity. Molecules based on oroidin would require relatively short reaction sequences to access (two–six steps) and should be capable of rapid assembly from core scaffolds and screened for their anti-biofilm properties.

With this natural product serving as our base, a focused library was constructed by systematically varying three regions within the oroidin template (Scheme 1) to delineate what structural features of the molecule were essential for biological activity. These areas were designated as: the tail group (Region A), the linker chain (Region B), and the head group (Region C). The tail group was varied as: absent, an N–H pyrrole derivative, or an *N*-methyl pyrrole derivative. The linker between the head group and tail group was varied from two to four carbons, and the effect of chain unsaturation was also examined. The head groups considered for analysis included 2-aminoimidazole, 2-amino-4-oxoimidazole, imidazole, tryptophan, 2-thioimidazolone, and 2-aminothiazole (vide infra).



Scheme 1. Fragmentation of the oroidin template for SAR study.

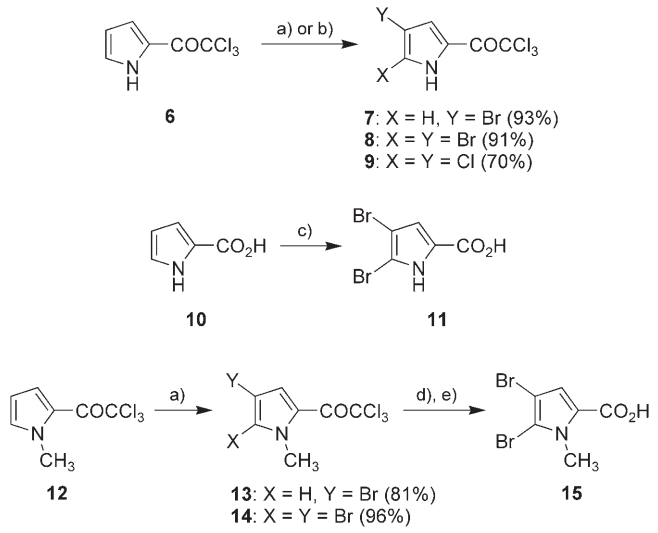


To examine each compound's ability to inhibit the formation of *Pseudomonas aeruginosa* biofilms, PAO1 and PA14 were employed as the target bacterial strains by using a crystal violet reporter assay.[29] All compounds were initially screened at 500 μM for anti-biofilm activity. IC_{50} values were then determined for compounds that displayed exceptional activity in the preliminary screening, followed by growth curve and colony count analysis to verify that the compounds were in

fact true inhibitors of bacterial biofilm formation and were not acting as microbicides, inducing cell death before biofilm development had begun.

Region A SAR: tail-group analogue synthesis and biological activity

Nearly all oroidin alkaloids are known to contain the pyrrolecarboxamide moiety with various degrees of bromination, and this provided the first structural element for investigation.[22] Each analogue was prepared by a convergent synthetic approach, with amide bond formation between the scaffold 4-(3-aminopropyl)-2-aminoimidazole dihydrochloride **16** (Table 1) and the appropriate (trichloroacetyl)pyrrole derivative serving as the final step. (Trichloroacetyl)pyrroles are known to undergo smooth amide bond formation in the presence of unprotected 2-aminoimidazoles and are among the most frequently used reagents in the total synthesis of many oroidin relatives.[30,31] The necessary (trichloroacetyl)pyrroles were synthesized as outlined in Scheme 2.[32] The corresponding N–H and

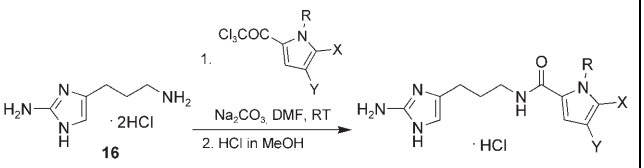


Scheme 2. Synthesis of pyrrole subunits for Region A SAR; Reaction conditions: a) Br_2, $CHCl_3$, 0 °C. b) SO_2Cl_2, $CHCl_3$, reflux. c) Br_2, HOAc, $CHCl_3$, 50 °C, 87%. d) K_2CO_3, MeOH, 92%, e) LiOH, MeOH/THF/H_2O, 94%.

N-methyl dibromo carboxylic acids **11** and **15** were also prepared. These simple compounds are frequently isolated in high concentrations in conjunction with the more complex oroidin alkaloids from the *Agelasidae* sponges and would serve as controls in the inhibition assay.[33] A compiled activity list of all compounds synthesized and assayed at 500 μM for Region A SAR is summarized in Table 1.

The N–H pyrrole subclass was the first group of analogues studied. The dihydro derivatives of the natural products clathrodin,[34] hymenidin,[35] and oroidin represent the various successive degrees of N–H pyrrole bromination and were synthesized and screened for their ability to inhibit the formation of *P. aeruginosa* biofilms (Table 1). As previously reported, scaffold **16** was relatively inactive against both strains (20% inhibition

Table 1. Region A SAR synthesis and biological evaluation.



	Yield [%]	Biofilm inhibition at 500 μM vs. PAO1 [%]	PA14 [%]
17 R=X=Y=H	63	<10	16
18 R=X=H, Y=Br	74	74	86
19 R=H, X=Y=Br	59	61	37
20 R=H, X=Y=Cl	65	37	48
21 R=CH_3, X=Y=H	58	25	–
22 R=CH_3, X=H, Y=Br	56	88	83
23 R=CH_3, X=Y=Br	63	>95	>95

against PAO1, 15% against PA14).[20] Dihydroclathrodin (DHC, **17**) showed similar activity to the base scaffold, with <10% inhibition of PAO1 and 16% inhibition of PA14 biofilm formation. Dihydrohymenidin (DHH, **18**), however, showed a remarkable increase in activity, inhibiting the formation of PAO1 and PA14 biofilms by 74% and 86%, respectively. Addition of a second bromine atom at the 5-position on the pyrrole ring yielded dihydrooroidin (DHO, **19**). It was hypothesized that activity would yet again be increased, but surprisingly DHO displayed a decrease in potency against both strains (PAO1 inhibition = 61%, PA14 inhibition = 37%).

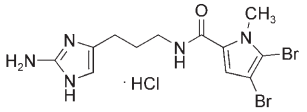
The requirement for a particular halogen identity on the pyrrole ring was also examined by replacing both bromine atoms with less sterically demanding and less electronegative chlorine atoms (**20**). No known oroidin family members possess chlorine substituents on the pyrrolecarboxamide subunit, yet some do contain chlorinated positions in other parts of the molecule.[26] This venture proved unfruitful, however, as no substantial benefit was gained with the dichloro derivative **20**, which inhibited the formation of PAO1 biofilms by 37% and PA14 biofilms by 48% at 500 μM.

Investigation into how introduction of a methyl substituent on the pyrrole nitrogen would affect activity was the next step in the SAR process for this region. This decision was based upon the observation that some naturally occurring members of the oroidin family—that is, sventrin (**24**; Table 3)—contain an *N*-methylated pyrrole instead of the more commonly seen N–H pyrrole moiety.[36] The non-brominated derivative **21** showed a slight increase in activity relative to DHC, as it was observed that **21** inhibited the formation of PAO1 biofilms by 25%. However, this derivative was found to be inactive against PA14. Analogously to what was observed with the N–H pyrrole subset, *N*-methyl dihydrohymenidin (**22**) showed a substantial increase in activity (88% and 83% inhibition for PAO1 and PA14, respectively). This time, addition of a second bromine was observed to enhance activity as this compound—dihydrosventrin (DHS, **23**)—inhibited the formation of both PAO1 and PA14 biofilms by >95% at 500 μM.

All compounds that revealed >70% biofilm inhibition activity during the preliminary 500 μM screening were selected for

further biological characterization. Dose response curves were generated for each compound to determine the analogue's IC_{50} value against both *Pseudomonas* strains. These results are summarized in Table 2. Of the tail-modified derivatives, dihydrosventrin (DHS) was the most active, with an IC_{50} of 51 ± 9 μM against PAO1 and 111 ± 8 μM against PA14. When the tail fragment **15** was tested alone, it displayed no activity at 500 μM. This evidence further reinforces the conjecture that the two fragments must be covalently linked to elicit their anti-biofilm activity. Growth curves and colony counts were also performed for both PAO1 and PA14 in the presence and absence of DHS **23** at its respective IC_{50} concentration. In each case, no reduction in bacterial density or viable colonies was observed, thus confirming that our compounds were true inhibitors of biofilm formation and not eliciting their activity through a microbicidal mechanism (see the Supporting Information).

Table 2. IC_{50} values for Region A analogues.



dihydrosventrin (**23**)

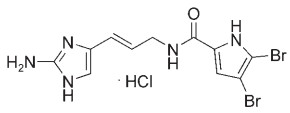
Compound	PAO1 IC_{50} [μM]	PA14 IC_{50} [μM]
dihydrohymenidin (**18**)	323 ± 30	266 ± 23
N-methyldihydrohymenidin (**22**)	348 ± 14	309 ± 16
dihydrosventrin (**23**)	51 ± 9	111 ± 8

The data gathered from this section of the SAR indicated a rough correlation between the degree of bromination on the pyrrole ring and increased anti-biofilm activity. It was also observed that compounds bearing the *N*-methylated pyrrole had better potential as biofilm inhibitors, illustrated by the remarkable difference in activity between dihydrosventrin **23** and dihydrooroidin **19**.

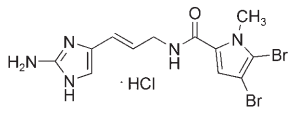
Region B SAR: linker analogue synthesis and biological activity

The double bond found in oroidin is proposed to have a profound impact on the ability of the sponge to synthesize a number of more complex chemical skeletons (that is, ageliferins, sceptrins) through dimerization-type reactions.[25] Discerning whether or not unsaturation was necessary for a biological response from an anti-biofilm standpoint could allow us to circumvent a low-yielding extra synthetic step needed to install the double bond between the 3- and the 4-positions in the dihydro scaffold **16**. Oroidin (**5**) was prepared as previously reported.[37] Sventrin (**24**) was synthesized by a synthetic approach identical to that used for oroidin, with the exception that **14** was employed in the amide bond formation step. Initial screens at 500 μM revealed that both natural products inhibited the formation of PAO1 and PA14 biofilms >95%. The activity of oroidin (**5**; PAO1 $IC_{50}=190\pm9$ μM, PA14 $IC_{50}=166\pm19$ μM) was exceptionally better than that of its dihydro congener, which was not even considered a candidate for IC_{50} value determination (vide supra). In contrast, the IC_{50} values of sventrin (**24**; $IC_{50}=75\pm5$ μM PAO1, $IC_{50}=115\pm3$ μM PA14) were very similar to those of its saturated counterpart (Table 3). This seemed to indicate that as we begin to fine tune our scaffolds to maximize anti-biofilm activity, unsaturation within the linker is not necessary to elicit maximum biological activity. As carried out with DHS, follow-up growth curve and colony count analysis of PAO1 and PA14 grown in the presence or absence of either natural product at their corresponding IC_{50} concentrations did not induce microbial cell death.

Table 3. IC_{50} values for the natural products oroidin (**5**) and sventrin (**24**).



oroidin (**5**)



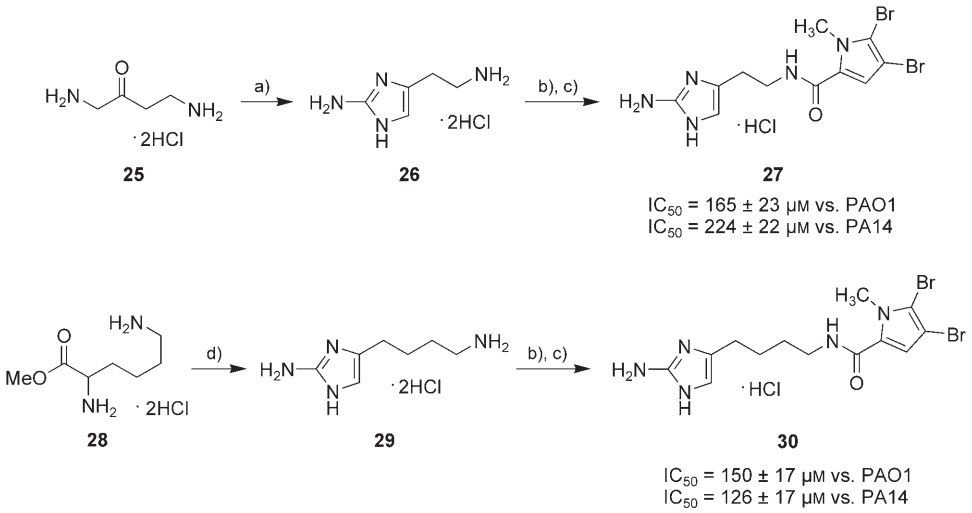
sventrin (**24**)

oroidin (**5**)	sventrin (**24**)
$IC_{50}=190\pm9$ μM vs. PAO1 $IC_{50}=166\pm19$ μM vs. PA14	$IC_{50}=75\pm5$ μM vs. PAO1 $IC_{50}=115\pm3$ μM vs. PA14

Given that a fully saturated chain, when coupled to the 4,5-dibromo-*N*-methylpyrrole subunit, yielded a compound (DHS) with the highest activity, we then elected to study the effect that linker length had upon biological activity. Homologues of DHS containing a 2-methylene or a 4-methylene spacer between the 2-AI head and the pyrrole tail were envisioned. These compounds were quickly accessed as outlined in Scheme 3. Briefly, commercially available 1,4-diaminobutan-2-one dihydrochloride (**25**) was condensed with cyanamide under pH-controlled conditions to yield the 2-methylene spacer **26** (2-AI),[38] which was subsequently coupled to fragment **14** to deliver target **27**. The 4-methylene spacer was generated through Akabori reduction of lysine methyl ester (**28**) to produce the corresponding α-amino aldehyde,[39,40] which, upon cyclization with cyanamide and ensuing amide bond formation, afforded the 2-AI **30**.

Initial screens at 500 μM revealed that each compound, like the parent compound DHS, inhibited the formation of PAO1 and PA14 biofilms by >95%. IC_{50} values for both **27** and **30** did, however, indicate the subtle effects that alkyl linker length had upon activity, with both modifications decreasing activity in relation to DHS. Increasing the alkyl chain length to four methylene units elicited a smaller drop in activity (PAO1 $IC_{50}=150\pm17$ μM, PA14 $IC_{50}=126\pm17$ μM), while the reduction in potency was slightly more pronounced when the alkyl chain length was decreased to two methylene units (PAO1 $IC_{50}=165\pm23$ μM, PA14 $IC_{50}=224\pm22$ μM). Colony counts and growth curves evaluated with these homologues revealed no microbicidal activity at their respective IC_{50} values.

Screening of these various linker analogues quickly revealed two important SAR features of the oroidin scaffold in terms of anti-biofilm activity. First, the optimum chain length between the 2-AI head and pyrrole tail was three carbon units. Second,

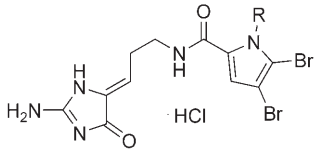


Scheme 3. Region B SAR linker synthesis; Reaction conditions: a) NH_2CN, H_2O, 95 °C, pH 4.3, 62%. b) **14**, Na_2CO_3, DMF, RT. c) HCl in MeOH, **27**: 64%, **30**: 54%. d) Na/Hg, H_2O, 5 °C, pH 1.5, then NH_2CN, H_2O, 95 °C, pH 4.3, 18%.

unsaturation was not necessary to elicit a biological response, thus eliminating the need for an additional synthetic step that would otherwise have been needed for analogue synthesis.

Region C SAR: head-group analogue synthesis and biological activity

Given the ubiquitous nature of the 2-aminoimidazole group in oroidin alkaloids, a substantial effort was made to delineate the importance of the 2-AI head group. We first focused on determining the ramifications of oxidizing the 2-AI ring at the 4-position. The natural product dispacamide[41] **31** and its *N*-methyl congener **32** were synthesized and subsequently assayed for inhibition of PAO1 and PA14 biofilms. Dispacamide was prepared as previously reported,[37] while dihydrosventrin (**23**) was also oxidized with molecular bromine in DMSO to afford its requisite *N*-methyl analogue. Each compound showed a substantial reduction in activity, with <20% PAO1 and PA14 biofilm formation inhibition at 500 μM.



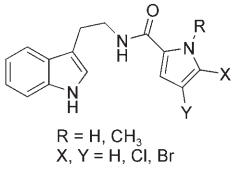
31: R = H (dispacamide)
32: R = CH_3 (*N*-methyl-dispacamide)

The repercussions of atomic deletion or full head group replacement within Region C were investigated next. This was examined by replacement of the 2-AI group with a tryptophan residue or an imidazole group lacking the 2-amino functionality. It was deemed unnecessary to delineate a synthesis for a 3-carbon linker of trytophan and imidazole when their 2-carbon homologues were commercially available and could be directly compared to the corresponding 2-AI derivative with a 2-methylene unit linker, which had already been characterized. Tryptamine hydrochloride or histamine dihydrochloride were coupled to all of the different (trichloroacetyl)pyrroles discussed in the Region A SAR portion of this report and assayed for biofilm inhibition activity (Table 4).

Replacement of the 2-AI subunit with a tryptophan abolished all activity at 500 μM, no matter what pyrrole derivative was appended to the tail. Removal of the 2-amino group was not as deleterious, as each compound we initially assayed at 500 μM showed varying degrees of anti-biofilm activity against both PAO1 and PA14. IC_{50} value determination of analogue **40** ($IC_{50}=277\pm35$ μM PAO1, $IC_{50}=203\pm25$ μM PA14) for comparison with its 2-AI 2-methylene spacer homologue **27** (PAO1 $IC_{50}=165\pm23$ μM, PA14 $IC_{50}=224\pm22$ μM) revealed that a substantial drop in activity against PAO1 in relation to **27** is noted, along with slightly better activity against PA14 (Table 5). Subsequent growth curves and colony counts indicated that **40** was not inhibiting biofilm development through microbicidal activity.

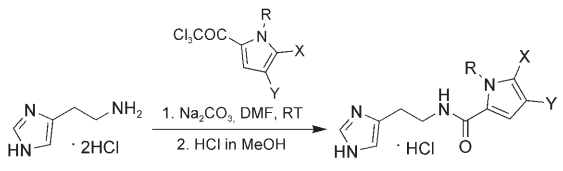
Finally, we investigated how single atom changes within the 2-AI subunit would affect anti-biofilm activity. To this end, we elected to synthesize the 2-thioimidazolone and 2-aminothi-

Table 4. Region C SAR synthesis and biological evaluation.



R = H, CH_3
X, Y = H, Cl, Br

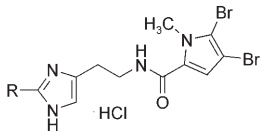
No activity for the inhibition of either PAO1 or PA14 biofilms



33

	Yield [%]	Biofilm inhibition at 500 μM vs. PAO1 [%]	PA14 [%]
34 R=X=Y=H	54	42	31
35 R=X=H, Y=Br	40	46	43
36 R=H, X=Y=Br	35	73	69
37 R=H, X=Y=Cl	62	33	38
38 R=CH_3, X=Y=H	55	29	34
39 R=CH_3, X=H, Y=Br	62	37	21
40 R=CH_3, X=Y=Br	60	73	82

Table 5. Direct comparison of an atom deletion effect in Region C.

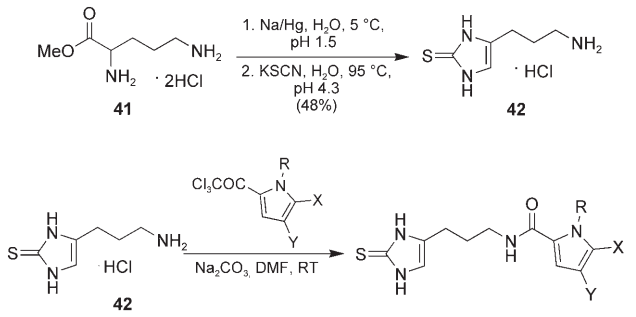


27: R = NH_2 **40**: R = H

IC_{50} = 165 ± 23 μM vs. PAO1	IC_{50} = 277 ± 35 μM vs. PAO1
IC_{50} = 224 ± 22 μM vs. PA14	IC_{50} = 203 ± 25 μM vs. PA14

azole (2-AT) scaffolds for SAR study. Condensation of an α-amino carbonyl compound with an isocyanate is well known,[42] and provided the basis for the synthesis of the 2-thioimidazolone scaffold **42** (Table 6). Similarly to the known route to access 2-AI scaffold **16**, Akabori reduction of ornithine

Table 6. Additional region C SAR synthesis and biological evaluation.



	Yield [%]	Biofilm inhibition at 500 μM vs. PAO1 [%]	PA14 [%]
43 R = X = Y = H	53	39	33
44 R = X = H, Y = Br	52	28	14
45 R = H, X = Y = Br	41	61	56
46 R = H, X = Y = Cl	65	41	38
47 R = CH_3, X = Y = H	57	27	36
48 R = CH_3, X = H, Y = Br	54	41	57
49 R = CH_3, X = Y = Br	71	46	38

methyl ester followed immediately by cyclization with KSCN under pH-controlled conditions afforded the 2-thioimidazolone **42**. Acylation of the terminal amine was accomplished with conditions adopted from the Region A SAR study to afford **43**–**49** in modest yields. All derivatives in this subset were able to inhibit biofilm formation throughout a range of values at 500 μM. However, none was deemed significant enough to be carried for further biological evaluation.

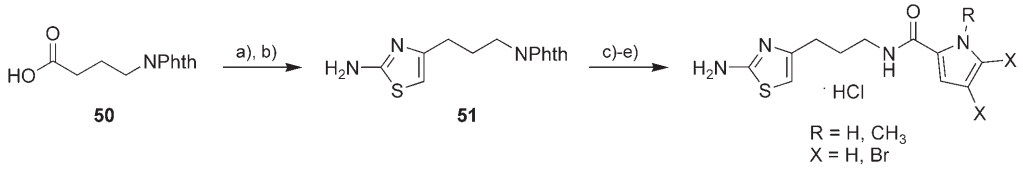
2-ATs are known to possess biological activity and were thus deemed a logical choice for head group study.[43,44] To affect the synthesis of the 2-AT scaffold, a new synthetic plan was necessary to install a sulfur atom selectively at the 1-position in the ring (Scheme 4). Synthesis commenced with formation of the acyl chloride of the known 4-phthalimidobutanoic acid (**50**).[45] This was followed by diazomethane homologation and concomitant quenching with concentrated HBr, which afforded the α-bromoketone. Cyclization of the α-bromoketone with thiourea under neutral conditions cleanly and regioselectively installed the 2-AT ring (**51**).[43,46] Removal of the phthlamide protecting group was accomplished with hydrazine in methanol to deliver the 2-AT scaffold. Again, acylation of the terminal amine was accomplished as previously outlined to afford the final target analogues. The 2-AT sublibrary was completely inactive at 500 μM, as seen with the tryptophan derivatives.

These single-atom replacements concluded the SAR study of Region C. Oxidation of the 2-AI ring in DHS proved detrimental, eliminating nearly all biological activity. In addition, the 2-AI remained of utmost importance in the ability of these compounds to inhibit the formation of *Pseudomonas* biofilms, despite the trading out of the 2-AI subunit for a variety of functionally unique moieties.

Conclusions

Through the generation of a 50-compound library, several trends become apparent when the SAR data are reviewed in the context of anti-biofilm activity. First, a three-methylene linker between the 2-AI head and the pyrrole tail elicits maximum biological activity. Second, unsaturation within the linker does not appear to be necessary to augment biological response once the other regions of the oroidin template are fine-tuned for maximum activity. Third, an imidazole or 2-AI head is necessary to maintain activity. Fourth, derivatives that contain dibrominated *N*-methylpyrroles have the tendency to be the most potent analogues within their corresponding sublibraries.

These trends culminated in the identification of a lead candidate, DHS **23**, as a very potent inhibitor of *Pseudomonas* biofilm formation with no direct antibiotic effect. Efforts to determine the mode of action of these compounds through generation of additional structural diversity on other 2-AI based scaffolds are currently underway. Most notably, we are focusing on exploring the effects that more advanced *N*-alkylated pyrroles



Scheme 4. Region C SAR synthesis; 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, 84%. b) Thiourea, DMF, 0–25 °C, 97%; c) N_2H_4, MeOH, 25–55 °C, 90%; d) K_2CO_3, **6**/**8**/**12**/**14**, DMF; *e*) HCl in MeOH.

have upon both biofilm development and maintenance of *Pseudomonas* and other medically relevant biofilm-forming bacteria.

Experimental Section

Biology: Stock solutions 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). Preliminary screens at 500 μM were performed in duplicate. IC_{50} dose response assays were performed in triplicate or more. *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 h. After that point, the medium was discarded and the plates were thoroughly washed with water. The wells were then inoculated with an aqueous solution of crystal violet (0.1%, 100 μL) and allowed to stand at ambient temperature for 30 min. Following another thorough washing with water, the remaining stain was solubilized with ethanol (95%, 200 μL). 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.

Chemistry: All reagents including anhydrous solvents used for the chemical synthesis of the library were purchased from commercially available sources and were used without further purification unless otherwise noted. All reactions were run under either nitrogen or argon. Flash silica gel chromatography was performed with standard grade silica gel (60 Å mesh) from Sorbtech. ^{1}H and ^{13}C NMR spectra were obtained with Varian 300 MHz or 400 MHz spectrometers. NMR solvents were purchased from Cambridge Isotope Labs and used as received. Chemical shifts are given in parts per million relative to [D_6]DMSO ($\delta = 2.50$ ppm), CD_3OD ($\delta = 3.31$ ppm), and $CDCl_3$ ($\delta = 7.27$ ppm) for proton spectra and relative to [D_6]DMSO ($\delta = 39.51$ ppm), CD_3OD ($\delta = 49.00$ ppm), and $CDCl_3$ ($\delta = 77.21$ ppm) for carbon spectra with an internal TMS standard. High-resolution mass spectra were obtained at the North Carolina State Mass Spectrometry Laboratory for Biotechnology. FAB experiments were carried with a JOEL HX110HF mass spectrometer while ESI experiments were carried out on Agilent LC-TOF mass spectrometer.

1-(4-Bromo-1*H*-pyrrol-2-yl)-2,2,2-trichloroethanone (7): 2-(Trichloroacetyl)pyrrole (**6**, 5.00 g, 23.3 mmol) was dissolved in anhydrous chloroform (20 mL). The solution was cooled to −10 °C before dropwise addition of bromine (1.20 mL, 23.3 mmol) to the flask. Once addition was complete the reaction mixture was allowed to warm to room temperature on its own accord while stirring for an additional 30 min. The reaction was poured into water (40 mL) and extracted with chloroform (3×20 mL). The combined organic layers were washed with sat. $NaHCO_3$ (2×30 mL) and brine (1×20 mL), dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. Purification of the residue by column chromatography (hexanes/diethyl ether, 95:5) yielded the title compound **7** (6.37 g, 93%) as an off-white solid: ^{1}H NMR (300 MHz, [D_6]DMSO): $\delta = 12.86$ (s, 1H), 7.54 (s, 1H), 7.32 ppm (s, 1H); ^{13}C NMR (100 MHz, [D_6]DMSO): $\delta = 171.67$, 129.06, 122.01, 121.54, 97.60, 94.56 ppm; HRMS (FAB) calcd for $C_6H_3BrCl_3NO$: 288.8464 $[M]^+$; found: 288.8479.

1-(4-Bromo-1-methylpyrrol-2-yl)-2,2,2-trichloroethanone (13): Through the same general procedure as used for the synthesis of 1-(4-bromo-1*H*-pyrrol-2-yl)-2,2,2-trichloroethanone (**7**), 2-trichloroacetyl-1-methylpyrrole (5.00 g) afforded the title compound **13** (5.46 g, 81%) as a white solid: ^{1}H NMR (300 MHz, [D_6]DMSO): $\delta =$ 7.66 (d, $J = 1.2$ Hz, 1H), 7.42 (d, $J = 1.8$ Hz, 1H), 3.91 ppm (s, 3H); ^{13}C NMR (100 MHz, [D_6]DMSO): $\delta = 171.48$, 134.40, 123.62, 121.19, 95.36, 95.12 ppm; HRMS (FAB) calcd for $C_7H_6BrCl_3NO$: 303.8698 $[M+H]^+$; found: 303.8678.

2,2,2-Trichloro-1-(4,5-dibromo-1*H*-pyrrol-2-yl)ethanone (8): 2-(Trichloroacetyl)pyrrole (**6**, 5.00 g, 23.3 mmol) was dissolved in anhydrous chloroform (20 mL). The solution was cooled to −10 °C before the dropwise addition of bromine (2.64 mL, 51.3 mmol) to the reaction mixture. Once addition was complete the reaction mixture was allowed to warm to room temperature on its own accord while stirring for an additional 30 min. The reaction was poured into water (40 mL) and extracted with chloroform (3× 20 mL). The combined organic layers were washed with sat. $NaHCO_3$ (2×30 mL) and brine (1×20 mL) and dried over anhydrous sodium sulfate. Filtration and evaporation afforded the crude product, which was recrystallized from hexanes to deliver the title compound **8** (7.93 g, 91%) as an off-white solid: ^{1}H NMR (300 MHz, [D_6]DMSO): $\delta = 13.75$ (s, 1H), 7.40 ppm (s, 1H); ^{13}C NMR (100 MHz, [D_6]DMSO): $\delta = 170.94$, 123.30, 122.45, 114.62, 100.88, 94.08 ppm; HRMS (FAB) calcd for $C_6H_2Br_2Cl_3NO$: 366.7569 $[M]^+$; found: 366.7556.

2,2,2-Trichloro-1-(4,5-dibromo-1-methylpyrrol-2-yl)ethanone (14): Through the same general procedure as used for the synthesis of 2,2,2-trichloro-1-(4,5-dibromo-1*H*-pyrrol-2-yl)ethanone (**8**), 2-trichloroacetyl-1-methylpyrrole (**12**, 5.00 g) gave the title compound **14** (8.14 g,96%) as white needles. ^{1}H NMR (300 MHz, [D_6]DMSO): $\delta = 7.60$ (s, 1H), 3.96 ppm (s, 3H); ^{13}C NMR (100 MHz, [D_6]DMSO): $\delta = 170.86$, 123.81, 122.68, 120.58, 99.58, 94.89, 37.60 ppm; HRMS (FAB) calcd for $C_7H_4Br_2Cl_3NO$: 380.7725 $[M]^+$; found: 380.7744.

2,2,2-Trichloro-1-(4,5-dichloro-1*H*-pyrrol-2-yl)ethanone (9): 2-(Trichloroacetyl)pyrrole (**6**, 5.00 g, 23.5 mmol) was dissolved in anhydrous chloroform (10 mL), and the reaction flask was covered in aluminum foil to exclude light. Sulfuryl chloride (4.20 mL, 51.8 mmol) was then added, and the reaction mixture was heated at reflux for 16 h before being cooled to room temperature and poured into cold water (100 mL). The aqueous layer was removed and washed with dichloromethane (2×25 mL). The combined organic layers were then washed with sat. $NaHCO_3$ (3×35 mL), dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by column chromatography (hexanes/diethyl ether 95:5) to afford the desired compound **9** (4.61 g, 70%) as a white solid: ^{1}H NMR (300 MHz, [D_6]DMSO): $\delta = 13.84$ (s, 1H), 7.41 ppm (s, 1H); ^{13}C NMR (100 MHz, [D_6]DMSO): $\delta = 171.2$, 123.6, 119.9, 119.7, 110.8, 94.8 ppm; HRMS (FAB) calcd for $C_6H_2Cl_5NO$: 278.8579 $[M]^+$; found: 278.8573.

4,5-Dibromo-1*H*-pyrrole-2-carboxylic acid (11): Pyrrole-2-carboxylic acid (**10**, 1.00 g, 9.00 mmol), was dissolved in anhydrous chloroform (10 mL) and glacial HOAc (2 mL). Bromine (0.971 mL, 18.9 mmol) was slowly added at room temperature to the resulting cloudy solution, and once addition was complete the reaction mixture was heated at 50 °C for 5 h. After cooling to ambient temper-

ature the reaction mixture was partitioned between water (30 mL) and chloroform (40 mL). The organic layer was rinsed with water (2×30 mL) and K_2CO_3 (10%, 40 mL). The K_2CO_3 extract was then washed with chloroform (2×20 mL) and acidified to pH 3 with an aqueous solution of HCl (4 N). The precipitate was collected by vacuum filtration, and the filter cake was rinsed with water (15 mL) to afford the target compound **11** (2.10 g, 87%) as a white solid: ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 12.80 (br s, 1H), 6.82 ppm (s, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 160.43, 125.37, 116.73, 106.50, 98.70 ppm; HRMS (FAB) calcd for $C_5H_3Br_2NO$: 266.8531 $[M]^+$; found: 266.8525.

4,5-Dibromo-1-methylpyrrole-2-carboxylic acid methyl ester: 2,2,2-Trichloro-1-(4,5-dibromo-1-methyl-1*H*-pyrrol-2-yl)ethanone (**14**, 1.00 g, 2.60 mmol), anhydrous potassium carbonate (0.719 g, 5.20 mmol), and anhydrous methanol (20 mL) were placed in a reaction flask. The resulting suspension was stirred for 16 h at room temperature, after which the reaction was quenched with water (10 mL). The methanol was removed under reduced pressure, and the residue was partitioned between ethyl acetate (100 mL) and water (20 mL). The organic layer was subsequently washed with sat. $NaHCO_3$ (2×30 mL) and brine (2×20 mL), dried over anhydrous sodium sulfate, and filtered. Evaporation of the filtrate yielded the title compound (0.710 g, 92%) as a white solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 7.05 (s, 1H), 3.90 (s, 3H), 3.76 ppm (s, 3H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 159.38, 123.62, 118.51, 113.93, 98.06, 51.58, 35.78 ppm; HRMS (FAB) calcd for $C_7H_7Br_2NO_2$: 294.8844 $[M]^+$; found: 294.8861.

4,5-Dibromo-1-methylpyrrole-2-carboxylic acid (15): 4,5-Dibromo-1-methyl-1*H*-pyrrole-2-carboxylic acid methyl ester (0.675 g, 2.27 mmol), lithium hydroxide (0.436 g, 18.19 mmol), methanol (12 mL), tetrahydrofuran (4 mL), and water (4 mL) were stirred for 16 h at ambient temperature. The pH was then adjusted to 7.0 with an aqueous solution of HCl (4 N). The organics were removed by rotary evaporation, and the resulting residue was diluted with water (15 mL). Acidification of the aqueous layer to pH 3 with HCl (4 N) afforded a white solid, which was collected by vacuum filtration. The filter cake was rinsed with water (10 mL) to give the title compound **15** (0.601 g, 94%) as a white solid: ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 12.83 (s, 1H), 7.00 (s, 1H), 3.90 ppm (s, 3H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 160.51, 124.74, 118.41, 113.07, 97.72, 35.64 ppm; HRMS (FAB) calcd for $C_6H_5Br_2NO_2$: 280.8687 $[M]^+$; found: 280.8676.

4-(3-Aminopropyl)-1*H*-imidazol-2-ylamine dihydrochloride (16): This compound was prepared as previously reported.[37] ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.04 (br s, 1H), 8.25 (br s, 2H), 7.41 (s, 2H), 6.65 (s, 1H), 2.75 (t, J = 7.2 Hz, 2H), 2.52 (m, 2H), 1.85 ppm (tt, J = 7.5, 14.7 Hz, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 146.9, 125.4, 108.9, 37.7, 25.5, 21.1 ppm; HRMS (FAB) calcd for $C_6H_{12}N_3S$: 158.0752 $[M+H]^+$; found: 158.0743.

1*H*-Pyrrole-2-carboxylic acid [3-(2-amino-1*H*-imidazol-4-yl)propyl]amide hydrochloride (17): 4-(3-Aminopropyl)-1*H*-imidazol-2-ylamine dihydrochloride (**16**, 0.100 g, 0.458 mmol), 2-(trichloroacetyl)pyrrole (**6**, 0.103 g, 0.488 mmol), and anhydrous sodium carbonate (0.172 g, 1.63 mmol) were dissolved in anhydrous *N*,*N*-dimethylformamide (5 mL). The reaction was stirred at ambient temperature for 16 h. Evaporation of the reaction mixture under reduced pressure and purification of the residue by column chromatography (CH_2Cl_2/MeOH sat. NH_3 85:15) afforded the desired compound in its free base form. Addition of a single drop of concentrated hydrochloric acid to a methanol solution (8 mL) and evaporation under reduced pressure yielded the title compound **17** (0.078 g, 63%) as a white solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.07 (s, 1H), 11.59 (s, 1H), 11.45 (s, 1H), 8.14 (t, J = 5.1 Hz, 1H), 7.30 (s, 2H), 6.81 (m, 2H), 6.74 (s, 1H), 6.59 (s, 1H), 6.04 (s, 1H), 3.19 (dt, J = 6.6, 12.6 Hz, 2H), 2.42 (t, J = 6.9 Hz, 2H), 1.75 ppm (tt, J = 6.6, 13.8 Hz, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 160.74, 146.76, 126.43, 126.40, 121.13, 110.28, 108.65, 108.50, 37.59, 28.14, 21.60 ppm; HRMS (ESI) calcd for $C_{11}H_{16}N_5O$: 234.1349 $[M+H]^+$; found: 234.1354.

4-Bromo-1*H*-pyrrole-2-carboxylic acid [3-(2-amino-1*H*-imidazol-4-yl)propyl]amide hydrochloride (18): Through the same general procedure as used for the synthesis of 1*H*-pyrrole-2-carboxylic acid [3-(2-amino-1*H*-imidazol-4-yl)propyl]amide hydrochloride (**17**), 4-(3-aminopropyl)-1*H*-imidazol-2-ylamine dihydrochloride (**16**, 0.132 g) gave the target compound **18** (0.159 g, 74%) as an off-white solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.20 (s, 1H), 11.85 (s, 1H), 11.57 (s, 1H), 8.23 (m, 1H), 7.31 (s, 2H), 6.97 (d, J = 1.5 Hz, 1H), 6.86 (d, J = 1.5 Hz, 1H), 6.61 (s, 1H), 3.21 (m, 2H), 2.44 (t, 2H, J = 7.2 Hz), 1.73 ppm (m, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 159.63, 146.68, 126.99, 126.40, 121.06, 111.61, 108.70, 94.90, 37.67, 27.91, 21.56 ppm; HRMS (FAB) calcd for $C_{11}H_{15}BrN_5O$: 312.0460 $[M+H]^+$; found: 312.0475.

4,5-Dibromo-1*H*-pyrrole-2-carboxylic acid [3-(2-amino-1*H*-imidazol-4-yl)propyl]amide hydrochloride (19): Through the same general procedure as used for the synthesis of 1*H*-pyrrole-2-carboxylic acid [3-(2-amino-1*H*-imidazol-4-yl)propyl]amide hydrochloride (**17**), 4-(3-aminopropyl)-1*H*-imidazol-2-ylamine dihydrochloride (**16**, 0.100 g) afforded the title compound **19** (0.117 g, 59%) as an off-white solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.33 (t, J = 5.4 Hz, 1H), 7.07 (s, 2H), 6.95 (s, 1H), 6.56 (s, 1H), 3.22 (dt, J = 6.0, 12.3 Hz, 2H), 2.43 (t, J = 7.2 Hz, 2H), 1.73 ppm (tt, J = 6.9, 13.8 Hz); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 158.92, 146.87, 128.31, 126.75, 112.88, 108.82, 104.33, 97.76, 37.74, 27.89, 21.74 ppm; HRMS (FAB) calcd. for $C_{11}H_{14}Br_2N_5O$: 389.9565 $[M+H]^+$; found: 389.9570.

4,5-Dichloro-1*H*-pyrrole-2-carboxylic acid [3-(2-amino-1*H*-imidazol-4-yl)propyl]amide hydrochloride (20): Through the same general procedure as used for the synthesis of 1*H*-pyrrole-2-carboxylic acid [3-(2-amino-1*H*-imidazol-4-yl)propyl]amide hydrochloride (**17**), 4-(3-aminopropyl)-1*H*-imidazol-2-ylamine dihydrochloride (**16**, 0.200 g) afforded the title compound **20** (0.204 g, 65%) as a white solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.32 (t, J = 4.8 Hz, 1H), 6.91 (s, 2H), 6.53 (s, 1H), 3.21 (dt, J = 6.6, 12.6 Hz, 2H), 2.42 (t, J = 7.2 Hz, 2H), 1.72 (tt, J = 7.5, 14.1 Hz, 2H) ppm; ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 159.15, 147.11, 127.31, 125.14, 114.75, 109.82, 108.99, 107.77, 37.83, 28.03, 22.04 ppm; HRMS (ESI) calcd for $C_{11}H_{14}Cl_2N_5O$: 302.0569 $[M+H]^+$; found: 302.0569.

1-Methylpyrrole-2-carboxylic acid [3-(2-amino-1*H*-imidazol-4-yl)-propyl]amide hydrochloride (21): Through the same general procedure as used for the synthesis of 1*H*-pyrrole-2-carboxylic acid [3-(2-amino-1*H*-imidazol-4-yl)propyl]amide hydrochloride (**17**), 4-(3-aminopropyl)-1*H*-imidazol-2-ylamine dihydrochloride (**16**, 0.300 g) delivered the target compound **21** (0.229 g, 58%) as a pale yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.03 (t, J = 5.1 Hz, 1H), 6.86 (m, 1H), 6.75 (m, 1H), 6.32 (s, 1H), 5.98 (m, 1H), 5.86 (br s, 2H), 3.82 (s, 3H), 3.17 (dt, J = 6.3, 13.2 Hz, 2H), 2.36 (t, J = 7.2 Hz, 2H), 1.75 ppm (tt, J = 7.2, 14.1 Hz, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 161.39, 148.11, 129.74, 127.47, 125.72, 112.09, 109.63, 106.53, 38.00, 36.15, 28.65, 23.30 ppm; HRMS (ESI) calcd for $C_{12}H_{18}N_5O$: 248.1506 $[M+H]^+$; found: 248.1514.

4-Bromo-1-methylpyrrole-2-carboxylic acid [3-(2-amino-1*H*-imidazol-4-yl)propyl]amide hydrochloride (22): Through the same general procedure as used for the synthesis of 1*H*-pyrrole-2-carboxylic acid [3-(2-amino-1*H*-imidazol-4-yl)propyl]amide hydrochloride

(**17**), 4-(3-aminopropyl)-1*H*-imidazol-2-ylamine dihydrochloride (**16**, 0.150 g) afforded the desired compound **22** (0.142 g, 56%) as a pale yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=8.17 (t, J=5.7 Hz, 1H), 7.08 (d, J=1.2 Hz, 1H), 6.91 (s, 2H), 6.85 (d, J=1.5 Hz, 1H), 6.52 (s, 1H), 3.80 (s, 3H), 3.17 (dt, J=6.3, 12.9 Hz, 2H), 2.41 (t, J=7.2 Hz, 2H), 1.71 ppm (m, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=160.29, 147.23, 127.39, 126.87, 126.43, 113.60, 108.94, 92.89, 37.75, 36.33, 28.02, 22.08 ppm; HRMS (ESI) calcd for $C_{12}H_{17}BrN_5O$: 326.0610 [M+H]$^+$; found: 326.0613.

4,5-Dibromo-1-methylpyrrole-2-carboxylic acid [3-(2-amino-1*H*-imidazol-4-yl)propyl]amide hydrochloride (23): Through the same general procedure as used for the synthesis of 1*H*-pyrrole-2-carboxylic acid [3-(2-amino-1*H*-imidazol-4-yl)propyl]amide hydrochloride (**17**), 4-(3-aminopropyl)-1*H*-imidazol-2-ylamine dihydrochloride (**16**, 0.200 g) gave the title compound **23** (0.258 g, 63%) as a white solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=12.06 (s, 1H), 11.59 (s, 1H), 8.31 (t, J=5.4 Hz, 1H), 7.32 (s, 2H), 7.03 (s, 1H), 6.60 (s, 1H), 3.87 (s, 3H), 3.18 (dt, J=6.3, 12.3 Hz, 2H), 2.45 (t, 2H, J=7.8 Hz), 1.73 ppm (m, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=159.77, 147.30, 127.99, 127.77, 114.00, 110.43, 109.06, 96.86; 37.94, 35.38, 27.96, 22.28 ppm; HRMS (FAB) calcd for $C_{12}H_{16}Br_2N_5O$: 403.9722 [M+H]$^+$; found: 403.9728.

Oroidin hydrochloride (5): This compound was prepared as previously reported.[37] ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=12.78 (s, 1H), 12.54 (s, 1H), 11.89 (s, 1H), 8.55 (t, J=6.0 Hz, 1H), 7.47 (s, 2H), 6.99 (d, J=3.0 Hz, 1H), 6.90 (s, 1H), 6.17 (m, 2H), 3.95 ppm (m, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=158.73, 147.46, 127.99, 126.85, 124.84, 116.15, 112.81, 111.15, 104.74, 97.91, 39.83 ppm; HRMS (FAB) calcd for $C_{11}H_{12}Br_2N_5O$: 387.9409 [M+H]$^+$; found: 387.9402.

Sventrin hydrochloride (24): Through the same general procedure as used for the synthesis of 1*H*-pyrrole-2-carboxylic acid [3-(2-amino-1*H*-imidazol-4-yl)propyl]amide hydrochloride (**17**), 4-(3-aminopropenyl)-1*H*-imidazol-2-ylamine dihydrochloride (0.050 g) afforded sventrin hydrochloride (**24**, 0.062 g, 61%) as a pale yellow solid: ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=8.49 (t, J=5.6 Hz, 1H), 7.06 (s, 1H), 6.77 (s, 2H), 6.75 (s, 1H), 6.19 (d, J=15.6 Hz, 1H), 6.02 (dt, J=5.6, 11.2 Hz, 1H), 3.94 (m, 2H), 3.89 ppm (s, 3H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=159.57, 147.44, 127.59, 126.75, 124.85, 116.25, 114.17, 111.20, 110.88, 96.98, 35.45 ppm; HRMS (ESI) calcd for $C_{12}H_{14}Br_2N_5O$: 401.9560 [M+H]$^+$; found: 401.9560.

4-(2-Aminoethyl)-1*H*-imidazol-2-ylamine dihydrochloride (26): 1,4-Diaminobutan-2-one dihydrochloride (**25**, 0.300 g, 1.71 mmol) and cyanamide (0.753 g, 17.9 mmol) were dissolved in water (10 mL). The pH of the solution was adjusted to pH 4.3, after which the reaction mixture was heated at 95 °C for 3.5 h while open to the atmosphere. After the systems had cooled to ambient temperature, ethanol (10 mL) was added to the flask, and the solution was evaporated to dryness. Purification of the residue by column chromatography (MeOH sat. with NH_3/CH_2Cl_2 90:10) yielded the product as its corresponding free base. Addition of methanol (10 mL) and concentrated hydrochloric acid followed by evaporation in vacuo afforded the target compound **26** (0.211 g, 62%) as a yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=6.21 (s, 1H), 5.14 (br s, 2H), 2.81 (m, 2H), 2.47 ppm (m, 2H); HRMS (ESI) calcd for $C_5H_{11}N_4$: 127.0978 [M+H]$^+$; found: 127.0977.

4,5-Dibromo-1-methylpyrrole-2-carboxylic acid [2-(2-amino-1*H*-imidazol-4-yl)ethyl]amide hydrochloride (27): Through the same general procedure as used for the synthesis of 1*H*-pyrrole-2-carboxylic acid [3-(2-amino-1*H*-imidazol-4-yl)propyl]amide hydrochloride (**17**), 4-(2-aminoethyl)-1*H*-imidazol-2-ylamine dihydrochloride (**26**, 0.150 g) afforded the title compound **27** (0.206 g, 64%) as an off-white solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=8.24 (t, J=5.1 Hz, 1H), 6.95 (s, 1H), 6.20 (s, 1H), 5.02 (s, 2H), 3.87 (s, 3H), 3.31 ppm (m, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=159.54, 149.20, 128.14, 113.86, 113.69, 110.33, 99.14, 96.83, 35.33, 27.45 ppm; HRMS (ESI) calcd for $C_{11}H_{14}Br_2N_5O$: 389.9559 [M+H]$^+$; found: 389.9574.

4-(4-Aminobutyl)-1*H*-imidazol-2-ylamine dihydrochloride (29): Through the same general procedure as used for the synthesis of 4-(3-aminopropyl)-1*H*-imidazol-2-ylamine dihydrochloride (**16**), lysine methyl ester dihydrochloride (**28**, 12.5 g) afforded the target compound **29** (2.25 g, 18%) as a yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=6.09 (s, 1H), 4.96 (s, 2H), 2.56 (t, J=6.3 Hz, 2H), 2.27 (t, J=6.9 Hz, 2H), 1.35–1.51 ppm (m, 4H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=149.11, 132.29, 110.59, 40.75, 31.41, 26.62, 26.12 ppm; HRMS (ESI) calcd for $C_7H_{15}N_4$: 155.1291 [M+H]$^+$; found: 155.1293.

4,5-Dibromo-1-methylpyrrole-2-carboxylic acid [4-(2-amino-1*H*-imidazol-4-yl)butyl]amide hydrochloride (30): Through the same general procedure as used for the synthesis of 1*H*-pyrrole-2-carboxylic acid [3-(2-amino-1*H*-imidazol-4-yl)propyl]amide hydrochloride (**17**), 4-(4-aminobutyl)-1*H*-imidazol-2-ylamine dihydrochloride (**29**, 0.200 g) delivered the target compound **30** (0.216 g, 54%) as a pale yellow solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=8.20 (t, J=5.1 Hz, 1H), 6.99 (s, 1H), 6.35 (s, 1H), 6.29 (br s, 2H), 3.86 (s, 3H), 3.17 (m, 2H), 2.35 (m, 2H), 1.49 ppm (m, 4H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=159.66, 147.48, 128.39, 128.06, 113.86, 110.37, 109.17, 96.85, 38.23, 35.35, 28.51, 25.48, 24.53 ppm; HRMS (ESI) calcd for $C_{13}H_{18}Br_2N_5O$: 417.9873 [M+H]$^+$; found: 417.9870.

2-Amino-5-(3-aminopropylidene)-1,5-dihydroimidazol-4-one dihydrochloride: 4-(3-aminopropyl)-1*H*-imidazol-2-ylamine (**16**, 0.200 g, 0.930 mmol) was dissolved in anhydrous dimethyl sulfoxide (6 mL). Bromine (0.047 mL, 0.930 mmol) was added dropwise, and the solution was stirred at room temperature for 1 h. Diethyl ether (7 mL) was added, and the organics were then decanted. The residue was purified by column chromatography (MeOH sat. with NH_3) to yield the desired product as its free base. Addition of concentrated hydrochloric acid to a methanol solution (8 mL) of the free base, followed by evaporation under reduced pressure, afforded the target compound (*Z* isomer exclusively, 0.141 g, 67%) as a tan solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=12.10 (br s, 1H), 9.20 (br s, 2H), 8.18 (br s, 2H), 5.92 (t, J=7.8 Hz, 1H), 2.96 (m, 2H), 2.66 ppm (m, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=164.13, 156.63, 130.88, 113.70, 37.57, 24.94 ppm; HRMS (FAB) calcd for $C_6H_{10}N_4O$: 155.0933 [M+H]$^+$; found: 155.0943.

Dispacamide hydrochloride (31): Through the same general procedure as used for 2-amino-5-(3-aminopropylidene)-1,5-dihydroimidazol-4-one dihydrochloride, dihydrooroidin hydrochloride (**19**, 0.185 g) gave dispacamide hydrochloride (**31**, 8:1 *Z*/*E* isomers, 0.120 g, 63%) as a tan solid: ^{1}H NMR (300 MHz, CD_3OD, *Z* isomer): δ=6.79 (s, 1H), 6.14 (t, J=7.8 Hz, 1H), 3.46 (t, J=6.9 Hz, 2H), 2.58 ppm (dt, J=6.9, 14.7 Hz, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=164.32, 162.06, 157.58, 130.90, 128.75, 119.09, 114.51, 106.47, 100.15, 39.11, 28.81 ppm; HRMS (ESI) calcd for $C_{11}H_{12}Br_2N_5O_2$: 403.9352 [M+H]$^+$; found: 403.9350.

4,5-Dibromo-1-methylpyrrole-2-carboxylic acid [3-(2-amino-5-oxo-3,5-dihydroimidazol-4-ylidene)propyl]amide hydrochloride (32): Through the same general procedure as used for 2-amino-5-(3-aminopropylidene)-1,5-dihydroimidazol-4-one dihydrochloride, dihydrosventrin hydrochloride (**23**, 0.100 g) gave the title compound **32** (0.048 g, 47%) as a tan solid (*Z* isomer exclusively). ^{1}H NMR (300 MHz, CD_3OD): δ=6.84 (s, 1H), 6.16 (t, J=7.8 Hz, 1H), 3.91 (s, 3H), 3.46 (t, J=6.9 Hz, 2H), 2.59 ppm (dt, J=6.9, 14.7 Hz,

2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 163.80, 163.00, 157.19, 130.63, 129.04, 119.42, 115.92, 112.67, 99.09, 39.06, 36.28, 28.73 ppm; HRMS (ESI) calcd for $C_{12}H_{14}Br_2N_5O_2$: 417.9509 $[M+H]^+$; found: 417.9511.

General procedure for the synthesis of tryptophan-based Region C SAR analogues: Tryptamine hydrochloride (0.150 g, 0.763 mmol), the desired appropriately substituted (trichloroacetyl)pyrrole (0.915 mmol), and anhydrous sodium carbonate (0.162 g, 1.53 mmol), were dissolved in anhydrous *N,N*-dimethylformamide (5 mL). The reaction mixture was stirred at ambient temperature for 8 h, after which it was partitioned between ethyl acetate (75 mL) and water (35 mL). The organic layer was successively washed with water (3×20 mL), an aqueous solution of HCl (1 N, 2× 35 mL), and brine (20 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. Purification of the crude residue by column chromatography (ethyl acetate/hexanes) yielded the final targets in the sublibrary.

1*H*-Pyrrole-2-carboxylic acid [2-(1*H*-indol-3-yl)ethyl]amide: White solid (80%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.41 (s, 1H), 10.80 (s, 1H), 8.11 (m, 1H), 7.58 (d, J = 7.5 Hz, 1H), 7.33 (d, J = 7.8 Hz, 1H), 7.16 (s, 1H), 7.08 (t, J = 6.6 Hz, 1H), 6.97 (t, J = 7.2 Hz, 1H), 6.83 (s, 1H), 6.74 (s, 1H), 6.06 (s, 1H) 3.47 (dt, J = 7.2, 14.1 Hz, 2H), 2.90 ppm (t, J = 7.5 Hz, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 160.69, 136.27, 127.31, 126.53, 122.58, 121.13, 120.95, 118.36, 118.25, 112.00, 111.40, 109.66, 108.52, 39.47, 25.59 ppm; HRMS (FAB) calcd for $C_{15}H_{16}N_3O$: 254.1293 $[M+H]^+$; found: 254.1281.

4-Bromo-1*H*-pyrrole-2-carboxylic acid [2-(1*H*-indol-3-yl)ethyl]amide: White solid (81%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.81 (s, 1H), 10.81 (s, 1H), 8.23 (t, J = 6.0 Hz, 1H), 7.56 (d, J = 7.8 Hz, 1H), 7.33 (d, J = 8.1 Hz, 1H), 7.16 (s, 1H), 7.06 (t, J = 6.6 Hz, 1H), 6.97 (m, 2H), 6.82 (s, 1H), 3.48 (dt, J = 6.9, 13.5 Hz, 2H), 2.90 ppm (t, J = 7.8 Hz, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 159.59, 136.25, 127.26, 127.14, 122.65, 121.06, 120.95, 118.31, 118.25, 111.84, 111.41, 111.26, 94.12, 25.39 ppm; HRMS (FAB) calcd for $C_{15}H_{15}BrN_3O$: 332.0398 $[M+H]^+$; found: 332.0388.

4,5-Dibromo-1*H*-pyrrole-2-carboxylic acid [2-(1*H*-indol-3-yl)ethyl]amide: White solid (60%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.67 (s, 1H), 10.81 (s, 1H), 8.25 (t, J = 5.1 Hz, 1H), 7.57 (d, J = 8.1 Hz, 1H), 7.33 (d, J = 8.4 Hz, 1H), 7.15 (s, 1H), 7.07 (t, J = 6.9 Hz, 1H), 6.97 (t, J = 7.2 Hz, 1H), 6.90 (d, J = 2.7 Hz, 1H), 3.47 (dt, J = 6.6, 13.2 Hz, 2H), 2.89 ppm (t, J = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 158.87, 136.25, 128.41, 127.25, 122.70, 120.96, 118.29, 118.27, 112.42, 111.76, 111.41, 104.39, 97.79, 39.57, 25.31 ppm; HRMS (ESI) calcd for $C_{15}H_{14}Br_2N_3O$: 409.9498 $[M+H]^+$; found: 409.9501.

4,5-Dichloro-1*H*-pyrrole-2-carboxylic acid [2-(1*H*-indol-3-yl)ethyl]amide: White solid (73%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.71 (s, 1H), 10.81 (s, 1H), 8.27 (m, 1H), 7.56 (d, J = 7.8 Hz, 1H), 7.32 (d, J = 8.1 Hz, 1H), 7.15 (s, 1H), 7.03 (t, J = 6.9 Hz, 1H), 6.96 (t, J = 6.9 Hz, 1H), 6.86 (s, 1H), 3.47 (dt, J = 6.3, 13.2 Hz, 2H), 2.90 ppm (t, J = 7.5 Hz, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 159.63, 136.91, 127.91, 125.80, 123.34, 121.61, 118.94, 118.91, 115.33, 112.41, 112.07, 110.09, 108.54, 25.98 ppm; HRMS (FAB) calcd for $C_{15}H_{13}Cl_2N_3O$: 321.0436 $[M]^+$; found: 321.0429.

1-Methylpyrrole-2-carboxylic acid [2-(1*H*-indol-3-yl)ethyl]amide: White solid (63%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 10.81 (s, 1H), 8.10 (t, J = 5.4 Hz, 1H), 7.57 (d, J = 7.8 Hz, 1H), 7.33 (d, J = 8.1 Hz, 1H), 7.16 (s, 1H), 7.06 (t, J = 7.2 Hz, 1H), 7.00 (t, J = 7.8 Hz, 1H) 6.87 (s, 1H), 6.73 (d, J = 2.1 Hz, 1H), 6.00 (s, 1H), 3.84 (s, 3H), 3.45 (dt, J = 6.9, 14.1 Hz, 2H), 2.89 ppm (t, J = 7.8 Hz, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 161.31, 136.23, 127.46, 127.28, 125.78, 122.57, 120.91, 118.32, 118.21, 111.98, 111.94, 111.36, 106.51, 36.16, 25.49 ppm; HRMS (FAB) calcd for $C_{16}H_{18}N_3O$: 268.1450 $[M+H]^+$; found: 268.1434.

4-Bromo-1-methylpyrrole-2-carboxylic acid [2-(1*H*-indol-3-yl)ethyl]amide: White solid (72%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 10.81 (s, 1H), 8.20 (t, J = 5.4 Hz, 1H), 7.57 (d, J = 7.8 Hz, 1H), 7.33 (d, J = 8.1 Hz, 1H), 7.16 (d, J = 1.8 Hz, 1H), 7.07 (m, 2H), 6.97 (t, J = 6.9 Hz, 1H), 6.80 (d, J = 1.8 Hz, 1H), 3.82 (s, 3H), 3.45 (dt, J = 6.9, 14.1 Hz, 2H), 2.89 ppm (t, J = 7.8 Hz, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 159.94, 136.04, 127.08, 126.63, 126.42, 122.48, 120.75, 118.12, 118.06, 113.24, 111.69, 111.23, 92.74, 36.31, 25.29 ppm; HRMS (FAB) calcd for $C_{16}H_{16}BrN_3O$: 345.0477 $[M]^+$; found: 345.0483.

4,5-Dibromo-1-methylpyrrole-2-carboxylic acid [2-(1*H*-indol-3-yl)ethyl]amide: White solid (77%). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 10.81 (s, 1H), 8.32 (t, J = 5.7 Hz, 1H), 7.55 (d, J = 7.5 Hz, 1H), 7.33 (d, J = 8.1 Hz, 1H), 7.16 (s, 1H,) 7.06 (t, J = 7.2 Hz, 1H), 7.00 (t, J = 7.5 Hz, 1H), 6.95 (s, 1H), 3.88 (s, 3H), 3.45 (dt, J = 6.3, 13.5 Hz, 2H), 2.89 ppm (t, J = 7.8 Hz, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 159.66, 136.22, 128.15, 127.25, 122.67, 120.91, 118.24, 118.21, 113.78, 111.75, 111.37, 110.31, 96.81, 39.66, 35.32, 25.13 ppm; HRMS (ESI) calcd for $C_{16}H_{16}Br_2N_3O$: 423.9654 $[M+H]^+$; found: 423.9655.

General procedure for the synthesis of imidazole-based Region C SAR analogues (34–40): Histamine dihydrochloride **33** (0.100 g, 1.36 mmol), the desired appropriately substituted (trichloroacetyl)pyrrole (1.43 mmol), and anhydrous sodium carbonate (0.432 g, 4.08 mmol), were dissolved in anhydrous *N,N*-dimethylformamide (7 mL). The reaction mixture was stirred at ambient temperature for 6 h, after which it was partitioned between ethyl acetate (75 mL) and water (35 mL). The organic layer was washed successively with water (3×20 mL), sat. $NaHCO_3$ (2×35 mL), and brine (20 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. Purification of the crude residue by column chromatography (CH_2Cl_2/methanol, 85:15) delivered the desired targets in their free base forms. Addition of concentrated HCl to methanolic solutions (8 mL) of the free bases, followed by rotary evaporation, afforded the final analogues in this series as their corresponding hydrochloride salts.

1*H*-Pyrrole-2-carboxylic acid [2-(1*H*-imidazol-4-yl)ethyl]amide hydrochloride (34): White solid (54%); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 11.46 (s, 1H), 9.01 (s, 1H), 8.23 (t, 1H, J = 5.6 Hz), 7.46 (s, 1H), 6.82 (m, 1H), 6.73 (m, 1H), 6.05 (dd, J = 2.8, 6.0 Hz, 1H), 3.50 (dt, J = 6.8, 12.8 Hz, 2H), 2.87 ppm (t, J = 6.8 Hz, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 161.40, 134.28, 131.82, 126.75, 122.01, 116.71, 110.80, 109.18, 37.98, 25.40 ppm; HRMS (FAB) calcd for $C_{10}H_{13}N_4O$: 205.1089 $[M+H]^+$; found: 205.1083.

4-Bromo-1*H*-pyrrole-2-carboxylic acid [2-(1*H*-imidazol-4-yl)ethyl]amide hydrochloride (35): White solid (40%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.80 (s, 1H), 8.18 (t, J = 5.4 Hz, 1H), 7.53 (s, 1H), 6.96 (s, 1H), 6.80 (s, 2H), 3.41 (m, 2H), 2.70 ppm (t, J = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 159.55, 134.72, 127.06, 121.10, 116.67, 111.28, 94.92, 38.83, 27.18 ppm; HRMS (FAB) calcd for $C_{10}H_{12}BrN_4O$: 283.0194 $[M+H]^+$; found: 283.0198.

4,5-Dibromo-1*H*-pyrrole-2-carboxylic acid [2-(1*H*-imidazol-4-yl)ethyl]amide hydrochloride (36): White solid (35%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 12.64 (br s, 1H), 8.20 (t, J = 5.7 Hz, 1H), 7.59 (s, 1H), 6.89 (s, 1H), 6.83 (s, 1H), 3.41 (dt, J = 7.2, 13.2 Hz, 2H), 2.71 ppm (t, J = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 158.88, 133.83, 131.81, 128.10, 116.27, 112.97, 104.45, 97.84, 37.82,

25.10 ppm; HRMS (FAB) calcd for $C_{10}H_{11}Br_2N_4O$: 360.9300 $[M+H]^+$; found: 360.9295.

4,5-Dichloro-1*H*-pyrrole-2-carboxylic acid [2-(1*H*-imidazol-4-yl)-ethyl]amide hydrochloride (37): White solid (62%); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.82$ (s, 1H), 9.02 (s, 1H), 8.56 (t, $J=5.6$ Hz, 1H), 7.46 (s, 1H), 6.94 (d, $J=2.8$ Hz, 1H), 3.50 (dt, $J=6.8$, 12.8 Hz, 2H), 2.89 ppm (t, $J=6.4$ Hz, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=159.04$, 133.53, 130.96, 124.76, 116.10, 114.73, 110.16, 107.95, 37.49, 24.43 ppm; HRMS (ESI) calcd for $C_{10}H_{11}Cl_2N_4O$: 273.0304 $[M+H]^+$; found: 273.0309.

1-Methylpyrrole-2-carboxylic acid [2-(1*H*-imidazol-4-yl)ethyl]-amide hydrochloride (38): White solid (55%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=14.60$ (br s, 1H), 14.32 (br s, 1H), 9.03 (s, 1H), 8.17 (s, 1H), 7.50 (s, 1H), 6.87 (s, 1H), 6.74 (s, 1H), 5.98 (d, $J=2.7$ Hz, 1H), 3.80 (s, 3H), 3.48 (m, 2H), 2.88 ppm (m, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=161.32$, 134.68, 127.51, 125.75, 116.69, 111.98, 106.57, 38.77, 36.14, 27.17 ppm; HRMS (ESI) calcd for $C_{11}H_{15}N_4O$: 219.1240 $[M+H]^+$; found: 219.1245.

4-Bromo-1-methylpyrrole-2-carboxylic acid [2-(1*H*-imidazol-4-yl)ethyl]amide hydrochloride (39): White solid (54%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=11.86$ (br s, 1H), 8.15 (m, 1H), 7.53 (s, 1H), 7.06 (d, $J=1.5$ Hz, 1H), 6.79 (m, 2H), 3.81 (s, 3H), 3.38 (m, 2H), 2.69 ppm (t, $J=7.8$ Hz, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=$ 160.12, 134.66, 126.83, 126.52, 113.36, 92.85, 38.80, 36.29, 27.00 ppm; HRMS (ESI) calcd for $C_{11}H_{14}BrN_4O$: 297.0345 $[M+H]^+$; found: 297.0348.

4,5-Dibromo-1-methylpyrrole-2-carboxylic acid [2-(1*H*-imidazol-4-yl)ethyl]amide hydrochloride (40): White solid (60%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=11.85$ (br s, 1H), 8.26 (t, $J=5.4$ Hz, 1H), 7.54 (s, 1H), 6.95 (s, 1H), 6.80 (s, 1H), 3.87 (s, 3H), 3.39 (dt, $J=6.9$, 13.8 Hz, 2H), 2.70 ppm (t, $J=7.2$ Hz, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=159.72$, 133.67, 131.48, 127.78, 116.20, 114.14, 110.62, 96.88, 37.75, 35.33, 24.71 ppm; HRMS (FAB) calcd for $C_{11}H_{13}Br_2N_4O_2$: 374.9456 $[M+H]^+$; found: 374.9458.

4-(3-Aminopropyl)-1,3-dihydroimidazole-2-thione hydrochloride (42): A solution of L-ornithine methyl ester hydrochloride (10.50 g, 47.9 mmol) in water (125 mL) was prepared in an Erlenmeyer flask. The solution was cooled to 5 °C, and the pH was adjusted to a value of 1.5 with concentrated HCl. While care was taken to maintain the above temperature and pH, Na(Hg) (5%, 250 g) was added slowly to the solution over a period of 35 min. After the addition was complete and bubbling had calmed, the Hg was decanted from the solution. The remaining aqueous portion was drained into a separate flask, and potassium thiocyanate (14.0 g, 144 mmol) and water (75 mL) were added. The pH of the solution was adjusted to a value of 4.30, and the flask was then heated at 95 °C for 1.5 h while open to the atmosphere. After the system had cooled to room temperature, ethanol (75 mL) was added, and the reaction mixture was evaporated to dryness. The residue was taken up in methanol and filtered to remove NaCl. After all of the NaCl had been removed, the crude residue was purified by column chromatography (CH_2Cl_2/MeOH sat. with NH_3 80:20) to afford the desired compound in its free base form. Addition of concentrated hydrochloric acid to a methanol solution (50 mL) of the free base, followed by evaporation to dryness, gave the title compound **42** (4.51 g, 48%) as a tan solid: ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=$ 11.92 (s, 1H), 11.69 (s, 1H), 7.77 (s, 2H), 6.58 (s, 1H), 2.73 (m, 2H), 2.42 (t, $J=6.6$ Hz, 2H), 1.76 ppm (tt, $J=7.5$, 13.8 Hz, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=160.23$, 128.03, 111.69, 38.08, 25.75, 21.27 ppm; HRMS (FAB) calcd for $C_6H_{12}N_3S$: 158.0752 $[M+H]^+$; found: 158.0743.

General procedure for the synthesis of thioimidazol-2-one Region C SAR analogues (43–49): 4-(3-Aminopropyl)-1,3-dihydroimidazole-2-thione hydrochloride (**42**; 0.150 g, 0.774 mmol), the desired appropriately substituted (trichloroacetyl)pyrrole (0.852 mmol), and anhydrous sodium carbonate (0.246 g, 2.32 mmol) were dissolved in anhydrous *N,N*-dimethylformamide (5 mL). The reaction mixture was stirred at ambient temperature for 12 h, after which it was partitioned between ethyl acetate (75 mL) and water (35 mL). The organic layer was successively washed with water (3×20 mL), aqueous HCl (1 N, 2×35 mL), and brine (20 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. Purification of the crude residue by column chromatography (CH_2Cl_2/methanol) afforded the final analogues in this series.

1*H*-Pyrrole-2-carboxylic acid [3-(2-thioxo-2,3-dihydro-1*H*-imidazol-4-yl)propyl]amide (43): Pale yellow solid (53%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=11.40$ (s, 1H), 8.20 (t, $J=5.7$ Hz, 1H), 6.97 (s, 1H), 6.82 (m, 1H), 6.75 (m, 1H), 6.06 (dd, $J=2.1$, 5.4 Hz, 1H), 3.23 (dt, $J=6.6$, 12.9 Hz, 2H), 2.52 (m, 2H), 1.75 ppm (tt, $J=7.2$, 14.7 Hz, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=160.80$, 160.71, 130.67, 126.31, 121.14, 113.24, 109.77, 108.47, 37.67, 28.32, 22.01 ppm; HRMS (FAB) calcd for $C_{11}H_{15}N_4OS$: 251.0967 $[M+H]^+$; found: 251.0961.

4-Bromo-1*H*-pyrrole-2-carboxylic acid [3-(2-thioxo-2,3-dihydro-1*H*-imidazol-4-yl)propyl]amide (44): Pale yellow solid (52%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=11.87$ (s, 1H), 11.81 (s, 1H), 11.65 (s, 1H), 8.10 (t, $J=5.1$ Hz, 1H), 6.96 (s, 1H), 6.83 (s, 1H), 6.57 (s, 1H), 3.18 (dt, $J=6.3$, 12.3 Hz, 2H), 2.37 (t, $J=7.5$ Hz, 2H), 1.71 (tt, $J=6.9$, 13.8 Hz, 2H) ppm; ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=160.35$, 160.30, 130.05, 127.63, 121.74, 112.76, 112.04, 95.56, 38.43, 28.77, 22.52 ppm; HRMS (ESI) calcd for $C_{11}H_{14}BrN_4OS$: 329.0066 $[M+H]^+$; found: 329.0062.

4,5-Dibromo-1*H*-pyrrole-2-carboxylic acid [3-(2-thioxo-2,3-dihydro-1*H*-imidazol-4-yl)propyl]amide (45): White solid (41%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=12.68$ (s, 1H), 11.86 (s, 1H), 11.65 (s, 1H), 8.13 (t, $J=5.1$ Hz, 1H), 6.91 (d, $J=2.7$ Hz, 1H), 6.57 (s, 1H), 3.17 (m, 2H), 2.36 (t, $J=6.9$ Hz, 2H), 1.70 ppm (tt, $J=6.9$, 13.8 Hz, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=160.04$, 158.91, 128.85, 128.02, 112.45, 111.30, 104.43, 97.76, 37.78, 27.95, 21.72 ppm; HRMS (ESI) calcd for $C_{11}H_{13}Br_2N_4OS$: 406.9171 $[M+H]^+$; found: 406.9174.

4,5-Dichloro-1*H*-pyrrole-2-carboxylic acid [3-(2-thioxo-2,3-dihydro-1*H*-imidazol-4-yl)propyl]amide (46): Yellow solid (65%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=12.71$ (s, 1H), 11.86 (s, 1H), 11.65 (s, 1H), 8.15 (m, 1H), 6.86 (s, 1H), 6.56 (s, 1H), 3.17 (m, 2H), 2.36 (t, $J=7.5$ Hz, 2H), 1.71 ppm (m, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=160.73$, 159.70, 129.53, 125.60, 115.40, 111.97, 110.16, 108.55, 38.47, 28.62, 22.40 ppm; HRMS (FAB) calcd for $C_{11}H_{12}Cl_2N_4OS$: 318.0109 $[M]^+$; found: 318.0099.

1-Methylpyrrole-2-carboxylic acid [3-(2-thioxo-2,3-dihydro-1*H*-imidazol-4-yl)propyl]amide (47): Pale yellow solid (57%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=7.99$ (m, 1H), 6.99 (s, 1H), 6.86 (s, 1H), 6.74 (m, 1H), 5.98 (m, 1H), 3.20 (dt, $J=6.0$, 12.3 Hz, 2H), 2.54 (t, $J=$ 7.2 Hz, 2H), 1.77 ppm (tt, $J=6.9$, 14.1 Hz, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=161.38$, 160.95, 127.51, 125.64, 115.10, 112.09, 106.50, 97.60, 37.71, 36.11, 28.32, 22.15 ppm; HRMS (ESI) calcd for $C_{12}H_{17}N_4OS$: 265.1118 $[M+H]^+$; found: 265.1120.

4-Bromo-1-methylpyrrole-2-carboxylic acid [3-(2-thioxo-2,3-dihydro-1*H*-imidazol-4-yl)propyl]amide (48): Yellow solid (54%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=11.86$ (s, 1H), 11.64 (s, 1H), 8.07

(m, 1H), 7.07 (s, 1H), 6.81 (s, 1H), 6.56 (s, 1H), 3.80 (s, 3H), 3.13 (dt, $J=6.3$, 12.9 Hz, 2H), 2.36 (t, $J=7.8$ Hz, 2H), 1.69 ppm (m, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=160.25$, 160.00, 128.95, 126.85, 126.45, 113.45, 111.34, 92.87, 37.70, 36.30, 27.96, 21.78 ppm; HRMS (ESI) calcd for $C_{12}H_{16}BrN_4OS$: 343.0222 $[M+H]^+$; found: 343.0223.

4,5-Dibromo-1-methylpyrrole-2-carboxylic acid [3-(2-thioxo-2,3-dihydro-1*H*-imidazol-4-yl)propyl]amide (49): White solid (71%); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=11.85$ (s, 1H), 11.64 (s, 1H), 8.19 (t, $J=5.2$ Hz, 1H), 6.97 (s, 1H), 6.55 (s, 1H), 3.86 (s, 3H), 3.15 (dt, $J=6.4$, 12.4 Hz, 2H), 2.36 (t, $J=7.2$ Hz, 2H), 1.70 ppm (tt, $J=7.2$, 14.0 Hz, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=160.00$, 159.76, 128.89, 128.10, 113.84, 111.33, 110.42, 96.84, 37.86, 35.35, 27.85, 21.77 ppm; HRMS (ESI) calcd for $C_{12}H_{15}Br_2N_4OS$: 420.9328 $[M+H]^+$; found: 420.9327.

1-Bromo-5-phthalimidopentan-2-one: 4-Phthalimidobutanoic acid (**50**, 4.64 g, 19.9 mmol) was dissolved in CH_2Cl_2 (100 mL) at 0 °C, and a catalytic amount of DMF was added. Oxalyl chloride (5.2 mL, 59.6 mmol) was added dropwise, 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 in CH_2Cl_2 (10 mL) and added slowly to a 0 °C solution of CH_2N_2 (~60 mmol generated from Diazald®/KOH) in Et_2O (170 mL). This solution was stirred at 0 °C for 1.5 h, at which point the reaction was quenched with the dropwise addition of HBr (48%, 7.0 mL). The reaction mixture was diluted with CH_2Cl_2 (50 mL) and immediately washed with sat. $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and concentrated. The resulting white solid was filtered and washed with Et_2O (100 mL) to obtain the title compound (4.77 g, 84%) as a fine white powder: ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=7.85$ (m, 4H), 4.32 (s, 2H), 3.57 (t, $J=6.9$ Hz, 2H), 2.65 (t, $J=6.9$ Hz, 2H), 1.82 ppm (quint., $J=6.9$ Hz, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=200.94$, 168.05, 134.34, 131.68, 123.01, 36.94, 36.64, 36.37, 22.30 ppm; HRMS (ESI) calcd for $C_{13}H_{13}BrNO_3$: 310.0073 $[M+H]^+$; found: 310.0072.

2-Amino-4-(3-phthalimidopropyl)thiazole (51): 1-Bromo-5-phthalimidopentan-2-one (0.500 g, 1.61 mmol) was dissolved in DMF (3.5 mL) at 0 °C, and thiourea (0.135 g, 1.77 mmol) was added dropwise as a solution in DMF (0.50 mL). The solution was allowed to warm to room temperature, and stirring was continued for 2 h, at which point the DMF was removed under reduced pressure and the resulting slurry was made alkaline with K_2CO_3 (10%, 100 mL). The aqueous solution was then extracted with EtOAc (3×40 mL), and the organic layer was washed with brine (50 mL), dried (Na_2SO_4), filtered, and concentrated to obtain **51** (448 mg, 97%) as a fine white powder in its free base form: ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=7.85$ (m, 4H), 6.78 (s, 2H), 6.13 (s, 1H), 3.56 (t, $J=6.9$ Hz, 2H), 2.42 (t, $J=7.5$ Hz, 2H), 1.88 ppm (quint., $J=7.5$ Hz, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=168.01$, 167.87, 151.20, 134.25, 131.61, 122.89, 100.17, 37.27, 28.70, 27.09 ppm; HRMS (ESI) calcd for $C_{14}H_{14}N_3O_2S$: 288.0801 $[M+H]^+$; found: 288.0799.

2-Amino-4-(3-aminopropyl)thiazole dihydrochloride: Thiazole **51** (0.300 g, 1.04 mmol) was dissolved in MeOH (4.5 mL), and N_2H_4 (0.10 mL, 3.20 mmol) was added dropwise to the stirring solution. The solution was stirred at room temperature for 1 h, warmed to 55 °C for 0.5 h, and then cooled to room temperature. The slurry was filtered, and the filtrate was concentrated under reduced pressure. The resulting residue was purified by flash column chromatography (MeOH/CH_2Cl_2, 50–100%, followed by TEA/MeOH 5–7%) to obtain the corresponding free base (0.148 g, 90%) as a fine white powder. Addition of concentrated HCl to a cold methanolic solution (8 mL) of the free base, followed by evaporation under reduced pressure, delivered the title compound as its dihydrochloride salt. ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=9.07$ (br s, 2H), 8.06 (br s, 3H), 6.57 (s, 1H), 2.78 (m, 2H), 2.62 (t, $J=7.2$ Hz, 2H), 1.87 ppm (quint., $J=7.2$ Hz, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=169.90$, 139.21, 102.13, 37.68, 25.10, 24.47 ppm; HRMS (ESI) calcd for $C_6H_{12}N_3S$: 158.0746 $[M+H]^+$; found: 158.0745.

General procedure for the synthesis of 2-AT Region C SAR analogues: 2-Amino-4-(3-aminopropyl)thiazole (0.200 mmol), the appropriately substituted (trichloroacetyl)pyrrole (0.210 mmol), and anhydrous potassium carbonate (0.300 mmol) were dissolved in anhydrous *N,N*-dimethylformamide (1.5 mL) and allowed to stir for 16 h at room temperature. The mixture was then concentrated under reduced pressure, and the resulting residue was dissolved in EtOAc (40 mL) and washed with H_2O (3×20 mL) and brine (20 mL), dried (Na_2SO_4), filtered, and concentrated. The crude residue was purified by flash column chromatography (EtOAc/hexanes 30–100%, followed by MeOH/EtOAc 5–10%) to obtain pure product. Addition of concentrated HCl to methanolic solutions (5 mL) of the free bases, followed by concentration under reduced pressure, afforded the requisite analogues for this series as their hydrochloride salts.

1*H*-Pyrrole-2-carboxylic acid [3-(2-aminothiazol-4-yl)propyl]-amide hydrochloride: Tan solid (64%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=11.51$ (s, 1H), 9.26 (s, 2H), 8.21 (s, 1H), 6.84 (s, 1H), 6.79 (s, 1H), 6.59 (s, 1H), 6.07 (d, $J=2.7$ Hz, 1H), 3.24 (q, $J=5.7$ Hz, 2H), 2.57 (t, $J=7.2$ Hz, 2H), 1.79 ppm (quint., $J=7.2$ Hz, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=169.90$, 160.68, 139.79, 126.28, 121.02, 110.02, 108.34, 101.61, 37.49, 27.59, 24.93 ppm; HRMS (ESI) calcd for $C_{11}H_{15}N_4OS$: 251.0961 $[M+H]^+$; found: 251.0960.

4,5-Dibromo-1*H*-pyrrole-2-carboxylic acid [3-(2-aminothiazol-4-yl)propyl]amide hydrochloride: Tan solid (56%); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=12.76$ (s, 1H), 9.19 (s, 2H), 8.36 (t, $J=5.6$ Hz, 1H), 6.96 (d, $J=2.0$ Hz, 1H), 6.58 (s, 1H), 3.23 (q, $J=7.2$ Hz, 2H), 2.56 (t, $J=7.2$ Hz, 2H), 1.78 ppm (quint., $J=7.2$ Hz, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=169.91$, 158.87, 139.93, 128.23, 112.81, 104.20, 101.70, 97.76, 37.67, 27.35, 25.00 ppm; HRMS (ESI) calcd for $C_{11}H_{13}Br_2N_4OS$: 406.9171 $[M+H]^+$; found: 406.9165.

1-Methylpyrrole-2-carboxylic acid [3-(2-aminothiazol-4-yl)propyl]amide hydrochloride: Tan solid (56%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta=9.19$ (br s, 2H), 8.08 (m, 2H), 6.88 (t, $J=2.1$ Hz, 1H), 6.78 (dd, $J=3.9$, 2.1 Hz, 1H), 6.57 (s, 1H), 5.99 (dd, $J=3.9$, 2.7 Hz, 1H), 3.82 (s, 3H), 3.20 (q, $J=7.2$ Hz, 2H), 2.55 (t, $J=7.2$ Hz, 2H), 1.78 ppm (quint., $J=7.2$ Hz, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=169.83$, 161.37, 139.93, 127.40, 125.54, 112.04, 106.40, 101.54, 37.44, 35.94, 27.52, 24.98 ppm; HRMS (ESI) calcd for $C_{12}H_{17}N_4OS$: 265.1118 $[M+H]^+$; found: 265.1117.

4,5-Dibromo-1-methylpyrrole-2-carboxylic acid [3-(2-aminothiazol-4-yl)propyl]amide hydrochloride: Tan solid (62%); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=9.02$ (br s, 2H), 8.29 (m, 1H), 7.02 (s, 1H), 6.55 (s, 1H), 3.87 (s, 3H), 3.19 (q, $J=6.8$ Hz, 2H), 2.53 (m, 2H), 1.77 ppm (t, $J=6.8$ Hz, 2H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta=169.92$, 159.83, 140.23, 128.03, 114.03, 110.40, 101.71, 96.86, 37.78, 35.34, 27.31, 25.14 ppm; HRMS (ESI) calcd for $C_{12}H_{15}Br_2N_4OS$: 420.9328 $[M+H]^+$; found: 420.9321.

Acknowledgements

Financial support from NCSU, Burroughs–Wellcome (pre-doctoral fellowship to J.J.R.) and GSK (pre-doctoral fellowship to T.E.B.) is

gratefully acknowledged. We thank Dr. Reza Ghiladi (NCSU) for spectroscopic assistance.

Keywords: biological activity · drug design · medicinal chemistry · natural products · structure–activity relationships

[1] D. J. Musk, Jr., P. J. Hergenrother, *Curr. Med. Chem.* **2006**, *13*, 2163.
[2] T. B. Rasmussen, M. Givskov, *Int. J. Med. Microbiol.* **2006**, *296*, 149.
[3] J. W. Costerton, P. S. Stewart, E. P. Greenberg, *Science* **1999**, *284*, 1318.
[4] D. Davies, *Nat. Rev. Drug Discovery* **2003**, *2*, 114.
[5] R. M. Donlan, J. W. Costerton, *Clin. Microbiol. Rev.* **2002**, *15*, 167.
[6] P. S. Stewart, J. W. Costerton, *Lancet* **2001**, *358*, 135.
[7] M. R. Parsek, P. K. Singh, *Annu. Rev. Microbiol.* **2003**, *57*, 677.
[8] G. D. Geske, R. J. Wezeman, A. P. Siegel, H. E. Blackwell, *J. Am. Chem. Soc.* **2005**, *127*, 12762.
[9] M. Manefield, R. de Nys, N. Kumar, R. Read, M. Givskov, P. Steinberg, S. Kjelleberg, *Microbiology* **1999**, *145*, 283.
[10] M. Manefield, T. B. Rasmussen, M. Henzter, J. B. Andersen, P. Steinberg, S. Kjelleberg, M. Givskov, *Microbiology* **2002**, *148*, 1119.
[11] J. F. Hu, E. Garo, M. G. Goering, M. Pasmore, H. D. Yoo, T. Esser, J. Sestrich, P. A. Cremin, G. W. Hough, P. Perrone, Y. S. L. Lee, N. T. Le, M. O'Neil-Johnson, J. W. Costerton, G. R. Eldridge, *J. Nat. Prod.* **2006**, *69*, 118.
[12] T. Bjarnsholt, M. Givskov, *Anal. Bioanal. Chem.* **2007**, *387*, 409.
[13] J. A. Driscoll, S. L. Brody, M. H. Kollef, *Drugs* **2007**, *67*, 351.
[14] J. W. Costerton, *Trends Microbiol.* **2001**, *9*, 50.
[15] R. B. Fick, Jr., F. Sonoda, D. B. Hornick, *Semin. Respir. Infect.* **1992**, *7*, 168.
[16] T. F. C. Mah, G. A. O'Toole, *Trends Microbiol.* **2001**, *9*, 34.
[17] R. T. Sadikot, T. S. Blackwell, J. W. Christman, A. S. Prince, *Am. J. Respir. Crit. Care Med.* **2005**, *171*, 1209.
[18] M. E. Falagas, E. A. Karveli, *Clin. Microbiol. Infect.* **2007**, *13*, 117.
[19] A. Yamada, H. Kitamura, K. Yamaguchi, S. Fukuzawa, C. Kamijima, K. Yazawa, M. Kuramoto, G. Y. S. Wang, Y. Fujitani, D. Uemura, *Bull. Chem. Soc. Jpn.* **1997**, *70*, 3061.
[20] R. W. Huigens, J. J. Richards, G. Parise, T. E. Ballard, W. Zeng, R. Deora, C. Melander, *J. Am. Chem. Soc.* **2007**, *129*, 6966.
[21] M. D. Lebar, J. L. Heimbegner, B. J. Baker, *Nat. Prod. Rep.* **2007**, *24*, 774.
[22] H. Hoffmann, T. Lindel, *Synthesis* **2003**, 1753.
[23] S. M. Weinreb, *Nat. Prod. Rep.* **2007**, *24*, 931.
[24] J. C. Braekman, D. Daloze, C. Stoller, R. W. M. Vansoest, *Biochem. Syst. Ecol.* **1992**, *20*, 417.
[25] A. Al Mourabit, P. Potier, *Eur. J. Org. Chem.* **2001**, 237.
[26] M. Köck, A. Grube, I. B. Seiple, P. S. Baran, *Angew. Chem.* **2007**, *119*, 6706; *Angew. Chem. Int. Ed.* **2007**, *46*, 6586.
[27] S. R. Kelly, E. Garo, P. R. Jensen, W. Fenical, J. R. Pawlik, *Aquat. Microb. Ecol.* **2005**, *40*, 191.
[28] S. R. Kelly, P. R. Jensen, T. P. Henkel, W. Fenical, J. R. Pawlik, *Aquat. Microb. Ecol.* **2003**, *31*, 175.
[29] G. A. O'Toole, R. Kolter, *Mol. Microbiol.* **1998**, *28*, 449.
[30] V. B. Birman, X. T. Jiang, *Org. Lett.* **2004**, *6*, 2369.
[31] D. P. O'Malley, K. Li, M. Maue, A. L. Zografos, P. S. Baran, *J. Am. Chem. Soc.* **2007**, *129*, 4762.
[32] D. M. Bailey, R. E. Johnson, *J. Med. Chem.* **1973**, *16*, 1300.
[33] A. E. Wright, S. A. Chiles, S. S. Cross, *J. Nat. Prod.* **1991**, *54*, 1684.
[34] J. J. Morales, A. D. Rodriguez, *J. Nat. Prod.* **1991**, *54*, 629.
[35] J. Kobayashi, Y. Ohizumi, H. Nakamura, Y. Hirata, *Experientia* **1986**, *42*, 1176.
[36] M. Assmann, S. Zea, M. Kock, *J. Nat. Prod.* **2001**, *64*, 1593.
[37] A. Olofson, K. Yakushijin, D. A. Horne, *J. Org. Chem.* **1998**, *63*, 1248.
[38] T. Vitali, M. Impicciatore, P. V. Plazzi, F. Bordi, M. Vitto, *Farmaco Ed. Sci.* **1984**, *39*, 70.
[39] S. Akabori, *Ber. Dtsch. Chem. Ges. A* **1933**, 66, 151.
[40] G. C. Lancini, E. Lazzari, *J. Heterocycl. Chem.* **1966**, *3*, 152.
[41] F. Cafieri, E. Fattorusso, A. Mangoni, O. Taglialatela Scafati, *Tetrahedron Lett.* **1996**, *37*, 3587.
[42] A. C. Barrios Sosa, K. Yakushijin, D. A. Horne, *Org. Lett.* **2000**, *2*, 3443.
[43] J. C. Eriks, H. Vandergoot, G. J. Sterk, H. Timmerman, *J. Med. Chem.* **1992**, *35*, 3239.
[44] J. L. Kane, B. H. Hirth, B. Liang, B. B. Gourlie, S. Nahill, G. Barsomian, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4463.
[45] W. J. Kruper, P. R. Rudolf, C. A. Langhoff, *J. Org. Chem.* **1993**, *58*, 3869.
[46] A. Hantzsch, V. Traumann, *Ber. Dtsch. Chem. Ges.* **1888**, *21*, 938.

Received: December 21, 2007
Published online on April 22, 2008

