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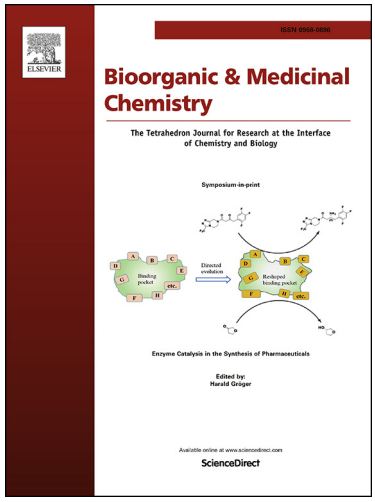

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Tryptamine Derivatives Disarm Colistin Resistance in Polymyxin-Resistant Gram-negative Bacteria

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Abstract

The last three decades have seen a dwindling number of novel antibiotic classes approved for clinical use and a concurrent increase in levels of antibiotic resistance, necessitating alternative methods to combat the rise of multi-drug resistant bacteria. A promising strategy employs antibiotic adjuvants, non-toxic molecules that disarm antibiotic resistance. When co-dosed with antibiotics, these compounds restore antibiotic efficacy in drug-resistant strains. Herein we identify derivatives of tryptamine, a ubiquitous biochemical scaffold containing an indole ring system, capable of disarming colistin resistance in the Gram-negative bacterial pathogens *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Escherichia coli* while having no inherent bacterial toxicity. Resistance was overcome in strains carrying endogenous chromosomally-encoded colistin resistance machinery, as well as resistance conferred by the *mobile colistin resistance – 1* (*mcr-1*) plasmid-borne gene. These compounds restore a colistin minimum inhibitory concentration (MIC) below the Clinical Laboratory Sciences Institute (CLSI) breakpoint in all resistant strains.

1. Introduction

The proliferation of antibiotic resistant bacteria threatens a collapse of modern medicine. As multi-drug resistant (MDR) bacterial strains continue to grow in both strength and number

while the antibiotic clinical arsenal remains stagnate, a "Post-Antibiotic World" has become increasingly tangible. Antibiotic resistant infections currently claim more than 700,000 lives world-wide each year.[1] These infections are most often caused by one or more of the *ESKAPE* pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species). Efforts to combat these six agents have been met with limited success, as only two novel antibiotic classes, lipopeptides and oxazolidinones, have been deployed clinically in the last two decades. Both are active only against Gram-positive bacteria, and offer a transient solution as bacterial resistance to each has been documented.[2] Given the lack of treatment options to combat MDR Gram-negative bacteria, colistin (polymyxin E), a decades-old cationic polypeptide, has reemerged as salvage therapy for MDR Gram-negative bacterial infections despite causing severe nephrotoxicity.[3] This "last resort" antibiotic is not exempt from resistance development, as numerous primary clinical isolates have shown resistance that far exceeds the Clinical Laboratory Standards Institute (CLSI) breakpoint for safe and effective treatment.[4]

Colistin elicits bacterial cell lysis by displacing divalent cations chelated by phosphate moieties on lipid A, a component of lipopolysaccharide (LPS), which destabilizes the outer membrane leading to envelope rupture and cell death.[5] Bacteria become resistant to colistin by modifying lipid A in a number of ways, including modification of the phosphate group with positively charged residues. Addition of aminoarabinose, galactosamine, and phosphoethanolamine residues to the terminal phosphate moieties of lipid A, the membrane anchor of lipopolysaccharide (LPS) has been documented.[6] These modifications all impart decreased electrostatic attraction toward the polycationic polymyxin, leading to drug resistance. This resistance mechanism is typically evolved under intense selection pressure, comes at a

fitness cost, and is controlled and encoded by endogenous genomic material.[7] The latter of the three modifications, however, can also be acquired from the *mobile colistin resistance – 1* (*mcr*-1) or related genes. These compact, plasmid-borne transcripts can be rapidly disseminated amongst pathogenic bacteria.[8] While *mcr-1* was first identified in China, several additional *mcr* gene variants (*mcr*-1-8) have since been found in bacteria across the globe.[9]

Antibiotic adjuvant therapy offers an alternative to novel antibiotic discovery by targeting non-essential bacterial systems and restoring susceptibility to a given antibiotic in a concurrent treatment strategy. Our research group has demonstrated diverse modulation of bacterial behavior using compounds derived from and inspired by marine natural products. Among these active compounds, a 2-aminoimidazole (2-AI) or 2-aminobenzimidazole (2-ABI) moiety has been a key component of the adjuvant scaffold in previous reports.[10, 11] Herein, we expand the breadth of active scaffolds and report that tryptamine, a well-established biogenic amine and component of the amino acid tryptophan, serves as a synthetic platform for novel, non-toxic small molecules that successfully disarm colistin resistance, and report a lead molecule active against multiple colistin-resistant (col^R) strains.

2. Results and Discussion

In a previous report from our group, a comprehensive screening of our in-house library identified that compound **1 (Figure 1)** disarms colistin resistance in Gram-negative strains carrying the *mcr-1* gene.[12] When dosed at 60 μM, **1** returned up to a 32-fold reduction in the colistin minimum inhibitory concentration (MIC) against five engineered *mcr-1* col^R Gram-negative strains.[13] This compound was one of six tryptamine derivatives (**1-6**, **Figure 1**) of the natural product oroidin first prepared in a previous report from our group aimed toward discovery of anti-biofilm agents against *P. aeruginosa.*[14] Interestingly, biofilm dispersion or

inhibition activity was not noted for any of these six molecules. When we screened the remaining five compounds from this original study (**2-6**) for synergy with colistin in four col^{R} Gram-negative strains, two that harbor chromosomally encoded resistance (*A. baumannii* 4106, *K. pneumoniae* B9), and two transfected with the *mcr-1* gene contained in a plasmid vector (*A. baumannii* ATCC 17978^{+mcr-1}, and *E. coli* ATCC 25922^{+mcr-1}) (**Table 1**), we found consensus activity in the molecule subset. Of these, compound **2** was consistently twice as active as **1** and returned MICs below the CLSI breakpoint (4 μg/mL) and thus became our lead molecule and the subject of a structure activity relationship (SAR) study.

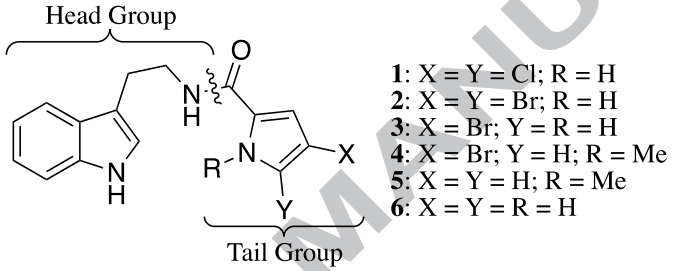


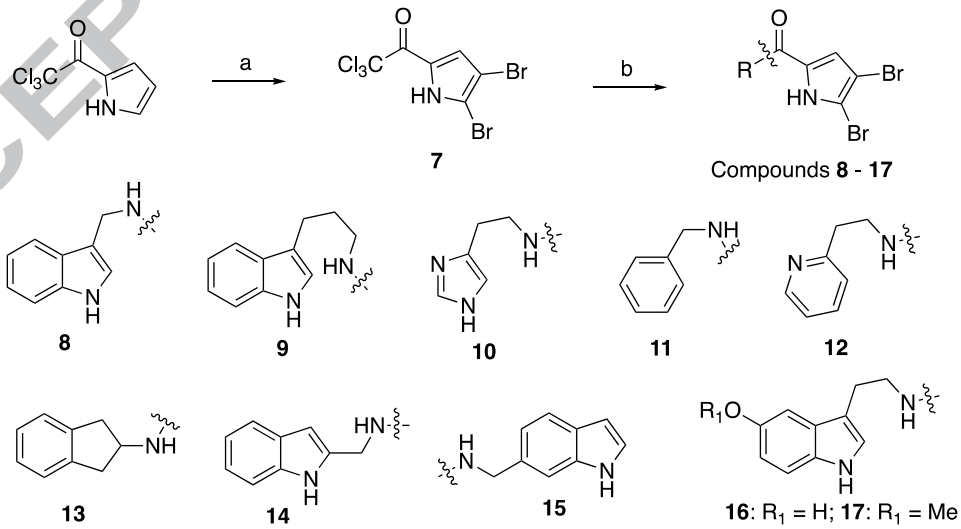
Figure 1. Structures of compounds **1-6** and structural segmentation.

Table 1. MIC (μg/mL (fold reduction)) of colistin with in-house library adjuvants dosed at 60 μM.

	A. baumannii 4106	*K. pneumoniae* B9	*A. baumannii* ATCC 17978^{+mcr-1}	*E. coli* ATCC 25922^{+mcr-1}
No compound	1024 μg/mL	1024 μg/mL	16 μg/mL	8 μg/mL
1 (19.3 μg/mL)	4 (256)	1 (1024)	0.5 (32)	1 (8)
2 (24.7 μg/mL)	2 (512)	0.5 (2048)	0.5 (32)	1 (8)
3 (19.9 μg/mL)	16 (64)	8 (128)	1 (16)	4 (2)
4 (20.8 μg/mL)	32 (32)	256 (4)	4 (4)	4 (2)
5 (16.1 μg/mL)	512 (2)	1024 (0)	16 (0)	8 (0)
6 (15.2 μg/mL)	512 (2)	1024 (0)	8 (2)	8 (0)

With lead compound **2** in hand, we envisioned an SAR strategy that focused on dividing the molecule along the amide bond and launching two separate diversification campaigns, one focused on the indole head group and carbon linker, and the other on the brominated carboxy-pyrrole tail (**Figure 1**). As each of the thirty-three analogs were synthesized, their activity with

colistin was determined in the same four Gram-negative col^R strains used previously (**Table 2**). Interestingly, all analogs synthesized in this study showed no antimicrobial activity in monotherapy (MIC = >200 μM) and were initially tested at 60 μM when co-dosed with colistin. We synthesized a panel of analogs with a variety of changes to the head group scaffold via condensing **7** with commercially available amines under basic conditions (**Scheme 1**).[14] The two-carbon linker of **2** was first both shortened and lengthened using 1*H*-indole-3-methanamine and homotryptamine as reactive amines to afford compounds **8** and **9** respectively, both of which showed increased activity over **2** in several strains. Reactions with histamine, benzylamine, 2-pyridineethanamine, and 2-aminoindane yielded compounds **10**, **11**, **12**, and **13**, respectively, all of which were inactive. We moved the tail group from the 3- position to the 2- position of the indole ring (**14**) via reaction with 1*H*-indole-2-methanamine,and moved it to the 6- position (**15**) using 1*H*-indole-6-methanamine as the amine source. Both **14** and **15** were roughly equipotent with **2**. Using this same methodology, we then employed both 5-hydroxytryptamine and 5-methoxytryptamine to afford the corresponding indole ring substitution in final products **16** and **17**, which were both upwards of eight-fold less active than **2**.



Scheme 1. Reaction scheme and structures of compounds **8** through **17**. (a): Br_2, $CHCl_3$, 2h, RT. (b): RCH_2NH_2, K_2CO_3, DMF, 18h, RT.

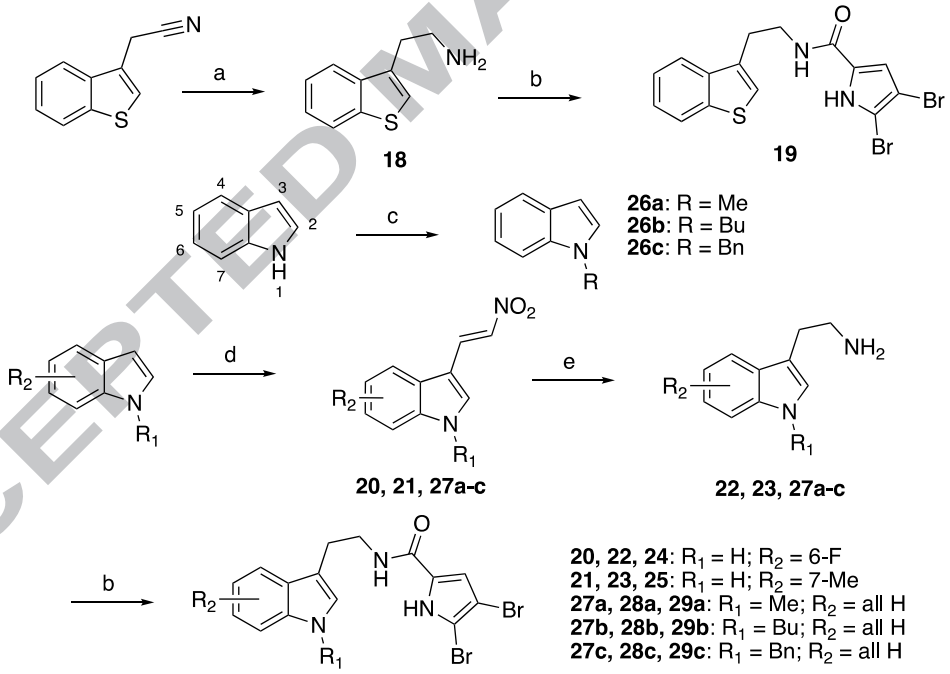
Further modifications to the head group required preparation of derivatized amines (**Scheme 2**). We first sought to substitute benzothiophene for indole. Starting from commercially available benzo[*b*]thiophene-3-acetonitrile, reduction with SmI_2 gave the corresponding ethylamine **18**,[15] which was then condensed with **7** to give **19**, a product with greatly reduced (32 fold) activity compared to **2**. To access additional substitutions of the indole ring, synthesis of substituted tryptamine precursors was necessary due to either lack of commercial availability or cost. Briefly, a nitrovinyl group was appended to the 3-position of the appropriately substituted indole via an addition-elimination reaction with 1-(dimethylamino)-2-nitroethylene (DMANE) in trifluoroacetic acid and dichloromethane. The nitroalkenes were then reduced with lithium aluminum hydride at room temperature to give the corresponding tryptamine derivatives.[16] Starting from 6-fluoroindole and 7-methylindole, this route gave both 6-fluorotryptamine (**22**) and 7-methyltryptamine (**23**), which were each condensed with **7** to afford **24** and **25** in three steps overall. This same route also proved useful to access *N*-alkylated analogs of **2**. *N*-methyl, *N*-butyl, and *N*-benzyl indole (**26a-c**) were each accessed by *N*-alkylating with the appropriate alkyl halide, and were then converted to the nitroalkene intermediate (**27a-c**) and finally reduced to the tryptamine derivative (**28a-c**). Each was then condensed with **7** to afford **29a-c**.

Table 2. Initial screen of head-group substituted analogs dosed at 60 μM with colistin given as MIC (μg/mL (fold reduction)).

	A. baumannii 4106	*K. pneumoniae* B9	*A. baumannii* ATCC 17978$^{+mcr\text{-}1}$	*E. coli* ATCC 25922$^{+mcr\text{-}1}$
No compound	1024 μg/mL	1024 μg/mL	16 μg/mL	8 μg/mL
8 (23.8 μg/mL)	0.5 (2048)	0.5 (2048)	0.5 (32)	0.5 (16)
9 (25.5 μg/mL)	2 (512)	0.25 (4096)	0.5 (32)	0.5 (16)
10 (21.7 μg/mL)	128 (8)	1024 (0)	16 (0)	8 (0)
11 (21.5 μg/mL)	512 (2)	512 (2)	16 (0)	8 (0)
12 (22.4 μg/mL)	1024 (0)	512 (2)	16 (0)	8 (0)
13 (23.0 μg/mL)	128 (8)	1024 (0)	16 (0)	8 (0)
14 (23.8 μg/mL)	0.5 (2048)	0.25 (4096)	0.5 (32)	0.5 (16)
15 (23.8 μg/mL)	4 (256)	0.5 (2048)	0.5 (32)	1 (8)

16 (25.6 μg/mL)	256 (4)	128 (8)	4 (4)	2 (4)
17 (26.5 μg/mL)	8 (128)	8 (128)	2 (8)	4 (2)
19 (25.7 μg/mL)	32 (32)	64 (16)	2 (8)	2 (4)
24 (25.7 μg/mL)	2 (512)	16 (64)	0.5 (32)	1 (8)
25 (25.5 μg/mL)	4 (256)	2 (512)	0.5 (32)	1 (8)
29a (25.5 μg/mL)	256 (4)	1024 (0)	16 (0)	8 (0)
29b (28.0 μg/mL)	1024 (0)	1024 (0)	16 (0)	8 (0)
29c (30.1 μg/mL)	1024 (0)	1024 (0)	16 (0)	8 (0)

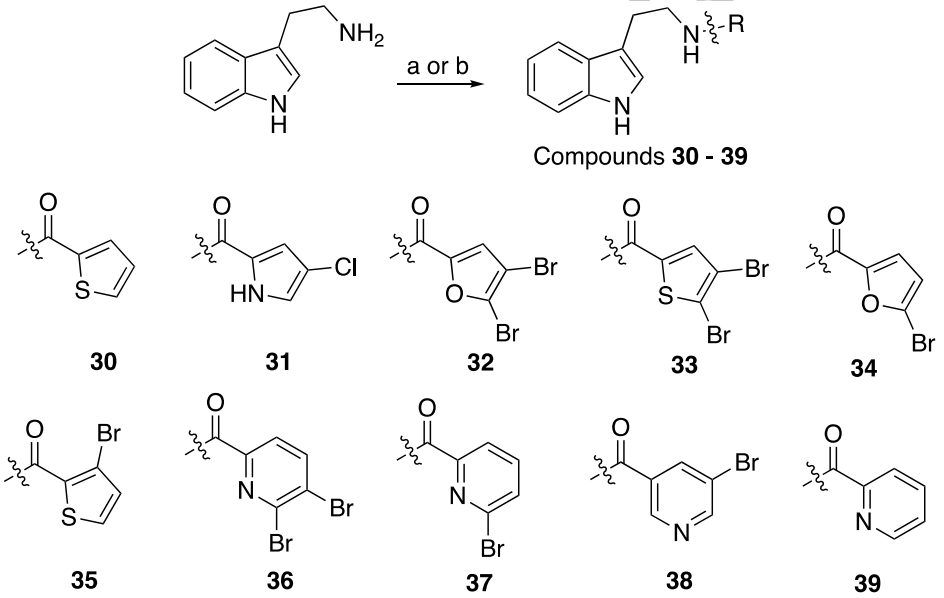
From the biological data, we see that in general, an indole ring is required for activity though its orientation with respect to the aliphatic linker is flexible, as is the case for the overall length of the aliphatic linker itself. Substitutions about the indole ring are well tolerated in some cases but do not return increases in activity over the original lead **2**, while indole *N*-alkylation renders a complete loss of activity.



Scheme 2. Reaction scheme and structures of compounds **18** through **29c**; (a): SmI_2, TEA, H_2O, THF; (b): K_2CO_3, DMF, 18h, RT (c): NaH, DMF, 0°C, 30 min, then R-X, 18h, RT; (d): DMANE, TFA, DCM; (e): LAH, THF.

Turning our attention to the pyrrole tail moiety, tryptamine was either directly reacted with commercially available acid chlorides, or coupled with commercially available carboxylic

acids through activation with PyBOP, to access compounds **30-39** (**Scheme 3,** biological data given in **Table 3**). These compounds offer diversification of the 4,5-dibromopyrrole with multiple five and six membered heterocycles with varying halide substituents, and revealed that alteration of the 4,5-dibromopyrrole moiety is deleterious for activity with few exceptions. Isolated activity was seen with **37** in *K. pneumoniae* B9, returning a colistin MIC of 1 µg/mL (1024 fold reduction) when dosed at 60 µM, while remaining inactive in other Gram-negative strains.



Scheme 3. Reaction scheme and structures of compounds **30** through **39**; (a): RCOCl, TEA, DMF; (b): RCOOH, TEA, PyBOP, DMF.

Mirroring efforts towards the indole *N*-alkylated analogs **29a-c**, we accessed *N*-alkylated derivatives of the 4,5-dibromopyrrole moiety, namely *N*-methyl, *N*-butyl, and *N*-benzyl analogs of **7**. Each of these derivatives was condensed with tryptamine to afford compounds **41a-c** (**Scheme 4**). We observed diminished biological activity with increased steric bulk. To further explore the impact that halogenation patterns of the pyrrole tail had upon activity, the 4,5-diiodopyrrole analog **44** was synthesized from **7** (**Scheme 4**). Iodination of the 2-trichloroacetyl

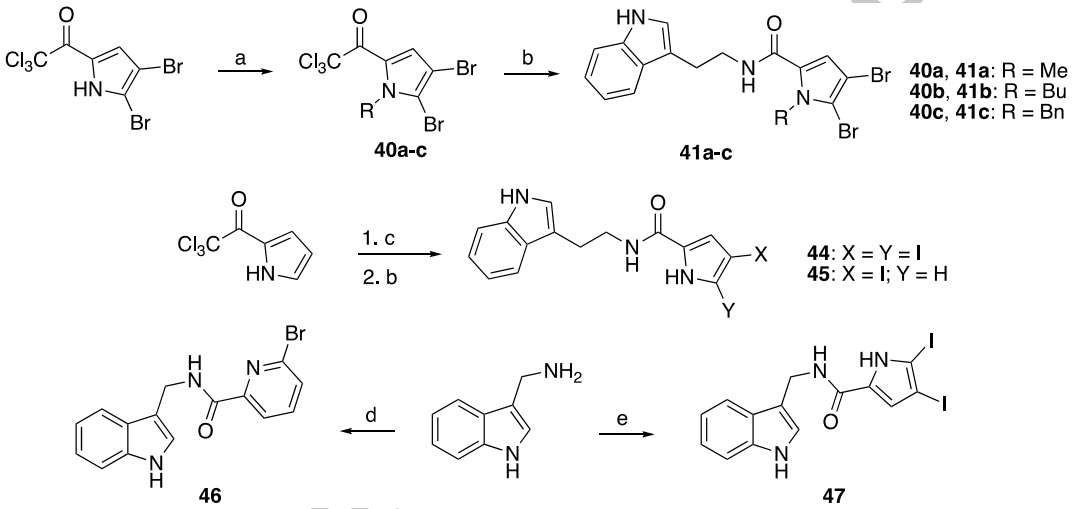
pyrrole using molecular iodine and silver trifluoroacetate generated the 4,5-diiodinated pyrrole along with the 4-iodopyrrole as a side product as an inseparable mixture.[17] Condensation of this mixture with tryptamine gave both the 4,5-diiodoinated product **44,** as well as the 4-iodinated **45**, which were readily isolated on silica gel. Both **44** and **45** were most effective in *K. pneumoniae* B9 and returned a 2048 fold reduction in colistin MIC when dosed at 60 μM.

Table 3. Colistin MIC (μg/mL (fold reduction)) of col^{R} strains co-challenged with tail-group substituted and shortened analogs of **2** dosed at 60 μM.

	A. baumannii 4106	*K. pneumoniae* B9	*A. baumannii* ATCC 17978^{+mcr-1}	*E. coli* ATCC 25922^{+mcr-1}
No compound	1024 μg/mL	1024 μg/mL	16 μg/mL	8 μg/mL
30 (16.2 μg/mL)	128 (8)	1024 (0)	16 (0)	8 (0)
31 (17.3 μg/mL)	64 (16)	128 (8)	8 (2)	4 (2)
32 (24.7 μg/mL)	16 (64)	2 (512)	1 (16)	4 (2)
33 (25.7 μg/mL)	32 (32)	2 (512)	1 (16)	4 (2)
34 (20.0 μg/mL)	64 (16)	16 (64)	8 (2)	8 (0)
35 (21.0 μg/mL)	16 (64)	8 (128)	4 (4)	4 (2)
36 (25.4 μg/mL)	128 (8)	8 (128)	16 (0)	8 (0)
37 (20.7 μg/mL)	64 (16)	1 (1024)	8 (2)	4 (2)
38 (20.7 μg/mL)	256 (4)	128 (8)	16 (0)	8 (0)
39 (15.9 μg/mL)	128 (8)	1024 (0)	16 (0)	8 (0)
41a (25.5 μg/mL)	32 (32)	4 (256)	1 (16)	4 (2)
41b (28.0 μg/mL)	64 (16)	64 (16)	8 (2)	8 (0)
41c (30.1 μg/mL)	128 (8)	64 (16)	8 (2)	8 (0)
44 (30.3 μg/mL)	8 (128)	0.5 (2048)	1 (16)	2 (4)
45 (22.8 μg/mL)	16 (64)	0.5 (2048)	1 (16)	4 (2)
46 (20.7 μg/mL)	32 (32)	32 (32)	16 (0)	8 (0)
47 (29.5 μg/mL)	1024 (0)	1024 (0)	16 (0)	8 (0)

Preliminary screening showed promising results for compounds **8**, **37**, and **44** (MIC fold-reduction of >512 in any tested strain, **Tables 2** and **3**) and thus we elected to combine successful alterations on both sides of the amide bond (**Scheme 4**). Coupling 1*H*-indole-3-methanamine with 6-bromopicolinic acid delivered **46**, a shortened analog of **37**. We also generated **47**, the analogous shortened derivative of **44** via condensation of 1*H*-indole-3-methanamine with the

corresponding 4,5-diiodopyrrole to afford **47.** Compound **46** had modest activity (32-fold MIC colistin reduction) against two strains (*A. baumannii* 4106, *K. pneumoniae* B9), while compound **47** was completely inactive (**Table 3**). All analogs having with equal or greater activity compared to **2** in any of the four test strains were then taken forward for further testing. These criteria eliminated twenty-three analogs and identified a cohort of nine molecules of interest (**8**, **9**, **14**, **15**, **24**, **25**, **37**, **44**, and **45**.)



Scheme 4. Reaction scheme and structures of compounds **41a** through **47**; (a): NaH, DMF, 30 mins, 0°C, then R-X, RT, 18h; (b): tryptamine, K_2CO_3, DMF, 18h; (c): I_2, $AgCOCF_3$, $CHCl_3$; (d): 6-bromopicolinic acid, PyBOP, TEA, DMF; (e): **42**, K_2CO_3, DMF.

These nine compounds were then subjected to further screening with nine additional bacterial strains: five primary clinical isolates that harbor chromosomally-encoded colistin resistance (*A. baumannii* 3941, *A. baumannii* 3942, *A. baumannii* 4112, *K. pneumoniae* A5, and *K. pneumoniae* C3) as well as the *mcr-1* parent strains without plasmid (*A. baumannii* ATCC 17978, and *E. coli* ATCC 25922) and two other colistin sensitive strains (*A. baumannii* 5075 and *K. pneumoniae* ATCC BAA 2146). These results are tabulated in **Table 4**.

Table 4. MIC (μg/mL (fold reduction)) of colistin with adjuvants dosed at 60 μM against nine Gram-negative strains.

	Colistin MIC	**2** (24.7 µg/mL)	**8** (23.8 µg/mL)	**9** (25.5 µg/mL)	**14** (23.8 µg/mL)	**15** (23.8 µg/mL)	**24** (25.7 µg/mL)	**25** (25.5 µg/mL)	**37** (20.7 µg/mL)	**44** (30.3 µg/mL)	**45** (22.8 µg/mL)
A. baumannii 3941	512 µg/mL	2 (256)	0.5 (1024)	2 (256)	2 (256)	4 (128)	1 (512)	2 (256)	64 (8)	4 (128)	8 (64)
A. baumannii 3942	512 µg/mL	1 (512)	0.25 (2048)	2 (256)	2 (256)	4 (128)	1 (512)	2 (256)	64 (8)	4 (128)	16 (32)
A. baumannii 4112	1024 µg/mL	2 (512)	0.5 (2048)	4 (256)	2 (512)	4 (256)	2 (512)	4 (256)	128 (8)	8 (128)	32 (32)
K. pneumoniae A5	1024 µg/mL	4 (256)	2 (512)	4 (256)	2 (512)	4 (256)	4 (256)	4 (256)	1024 (0)	4 (256)	32 (32)
K. pneumoniae C3	512 µg/mL	0.5 (1024)	0.25 (2048)	0.5 (1024)	0.25 (2048)	1 (512)	1 (512)	1 (512)	1 (512)	1 (512)	4 (128)
A. baumannii 5075	1 µg/mL	0.5 (2)	0.25 (4)	0.25 (4)	0.5 (2)	0.5 (2)	0.5 (2)	0.5 (2)	1 (0)	0.5 (2)	1(0)
A. baumannii ATCC 17978^{parent}	1 µg/mL	0.25 (4)	0.25 (4)	0.25 (4)	0.5 (2)	0.5 (2)	0.5 (2)	0.5 (2)	0.5 (2)	0.5 (2)	0.5 (2)
E. coli ATCC 25922^{parent}	1 µg/mL	0.25 (4)	0.25 (4)	0.5 (2)	0.5 (2)	0.5 (2)	0.5 (2)	0.5 (2)	0.5 (2)	0.5 (2)	0.5 (2)
K. pneumoniae ATCC 2146^{NDM-1}	1 µg/mL	0.5 (2)	1 (0)	0.5 (2)	1 (0)	1 (0)	0.5 (2)	0.5 (2)	0.5 (2)	0.5 (2)	0.5 (2)

This second round of screening established that all molecules were efficacious in col^{R} primary clinical isolates of *A. baumannii* and *K. pneumoniae*, and identified **8** as the most active colistin modulator, in some cases returning a 2048-fold reduction in colistin MIC and a four-fold increase in activity compared to the original lead molecule **2**. A subsequent dose-response study with **8** (**Table 5**) showed concentration-dependent activity in two Gram-negative clinical isolates (*A. baumannii* 3942, *K. pneumoniae* C3). To explore potential aggregate effects, this same experiment was repeated supplementing Cation-Adjusted Mueller Hinton Broth (CAMHB) with 0.001% Triton X100. No significant changes in colistin MIC were observed in the presence of detergent (**Table S1**, Supporting Information). Additionally, growth curves were constructed for *A. baumannii* 3942 treated with 60 µM **8**, (**Figure S1)** which showed that **8** did not perturb bacterial proliferation. To identify changes in membrane permeability, the BacLight cell viability assay was used. This duplex colorimetric fluorescence screen offers quantized detection of potential increases in cell permeability caused by xenobiotics. The ratio of nucleic-acid bound

STYO-9 (green-fluorescent), which readily crosses healthy cellular membranes, to the red-fluorescence of propidium iodide, which only enters cells with compromised cellular envelopes, is determined by scanning the distinct emission wavelengths of the two dyes. In endotoxin-free water dosed with 60 μM **8**, membrane permeability was increased by a factor of 1.9 in *A. baumannii* 3942 and of 1.5 in *K. pneumoniae* C3 compared to untreated controls. Conversely, no significant change in cell permeability was detected in either strain treated with the inactive compound **29b** at the same concentration. In a separate assay, neither **8** nor **29b** lysed red blood cells (<1%) when dosed in phosphate-buffered saline (PBS) at 100 μM.

Table 5. Colistin MIC (μg/mL (fold-reduction) of *A. baumannii* 3942 and *K. pneumoniae* C3 treated with various concentrations of **8**.

	A. baumannii 3942	*K. pneumoniae* C3
60 μM **8** (23.8 μg/mL)	0.25 (2048)	0.25 (2048)
50 μM **8** (19.8 μg/mL)	1 (512)	1 (512)
40 μM **8** (15.9 μg/mL)	2 (256)	2 (256)
30 μM **8** (11.9 μg/mL)	4 (128)	4 (128)
20 μM **8** (7.9 μg/mL)	32 (16)	4 (128)
15 μM **8** (6.0 μg/mL)	64 (8)	8 (64)
10 μM **8** (4.0 μg/mL)	256 (2)	16 (32)
5 μM **8** (2.0 μg/mL)	512 (0)	128 (4)

In conclusion, we have presented the synthesis of thirty-three analogs of a tryptamine-based lead-molecule previously observed to suppress colistin resistance. This diverse panel allowed for preliminary relationships between structure and activity to be uncovered indicating that there are rigid constraints on the scaffold to retain suppression of colistin resistance. The indole head group is necessary for activity, and neither substitution about the ring nor *N*-alkylation enhance efficacy. We further saw that deviation from the 4,5-dibromopyrrole to other five and six membered heterocycles greatly reduced activity. These molecules are non-toxic to bacteria, each having an MIC >200 μM in all strains that were studied. Based on activity, this panel of thirty-three analogs was narrowed to nine compounds for further screening against an

additional nine bacterial strains. This second round of screening revealed that while they had unremarkable activity in colistin-sensitive strains, they were effective in both col^R primary clinical isolates of *A. baumannii* and *K. pneumoniae*, as well as strains transfected with the *mcr-1* gene, and returned sub-breakpoint colistin MICs, in some cases as low as 0.25 μg/mL.

In the end, shortening the aliphatic linker by one carbon accessed compound **8** with four-fold greater activity than the parent molecule. In subsequent studies we observed dose-dependent activity of **8**, and when given as monotherapy, it caused no abnormalities in bacterial growth and did not lyse red blood cells. Currently, the mechanism-of-action of this family of compounds is unknown. Previous reports from our research group have disclosed colistin adjuvants containing a 2-AI heterocycle reversed lipid A modification leading to re-sensitization to colisin. These tryptamine-based adjuvants, however, do not reverse lipid A modification, thus appear to act via a novel mechanism. In all, this study identified several scaffolds that can be tasked in future probes of colistin resistance pathways and potentially new leads for further SAR studies while concurrent *in vivo* investigations are pursued.

3. Experimental Methods

General Biological Assay Methods: All antibiotics, media, and other biological reagents were purchased from commercial sources and used without further purification. Compounds synthesized in this study were dosed from DMSO stocks.

Single Compound MIC Determination via Broth Microdilution: This procedure follows the guidelines set by the Clinical Laboratory Sciences Institute.[4] Bacteria were cultured for 4 to 6 hours in Cation-Adjusted Mueller-Hinton Broth (CAMHB) and subcultured to 5 $x10^5$ CFU/mL in fresh CAMHB. For each compound to be tested, a 1 mL aliquot of subculture was taken and dosed with compound to a final concentration of 200 μM. Samples were then dispensed (200 μL)

into the first row of a 96-well microtiter plate in which subsequent wells were prefilled with 100 µL of subculture. 100 µL of dosed subculture was then serially diluted a total of 6 times in each subsequent row of the plate save for the last row which served as a control. Plates were then sealed and incubated stationary at 37°C. After 18 hours, the plates were removed and MIC values were recorded. All compounds tested had a purity of >95%.

Antibiotic Resensitization MIC Determination via Broth Microdilution: This procedure was adapted from the guidelines set by the Clinical Laboratory Sciences Institute.[4] Bacteria were cultured for 4 to 6 hours in Cation-Adjusted Mueller-Hinton Broth (CAMHB) and subcultured to 5 x10^5 CFU/mL in fresh CAMHB. For each compound to be tested, a 5 mL aliquot of subculture was taken and dosed with adjuvant to a set concentration. A 1 mL aliquot of each dosed subculture was collected and dosed with antibiotic to a set concentration. Co-dosed aliquots were then dispensed (200 µL) into the first row of a 96-well microtiter plate in which subsequent wells were prefilled with 100 µL of the corresponding dosed subculture. 100 µL of co-dosed subculture was then serially diluted a total of 6 times in each subsequent row of the plate save the last row as a control to afford serial dilution of the antibiotic while holding a constant concentration of adjuvant. Plates were then sealed and incubated stationary at 37°C. After 18 hours, the plates were removed and MIC values were recorded. All compounds tested had a purity of >95%.

Bacterial Growth Curve: Bacteria were cultured overnight in CAMHB and subcultured to 5 × 10^5 CFU/mL in fresh CAMHB. The subculture was then transferred to culture tubes in 5 mL aliquots, which were dosed with adjuvant to a set concentration, save one aliquot as a control. All subcultures were then incubated at 37°C with shaking. At 2, 4, 6, 8, and 24 hours, 100 µL samples of each culture was serially diluted in 900 µL aliquots of CAMHB for a total of 5 to 7

times. 100 µL of each dilution point were plated on nutrient agar and incubated stationary overnight. The total number of bacterial colonies on each plate was recorded.

BacLight Cell Permeability Assay: Bacteria were cultured overnight in CAMHB at 37°C and diluted 1:10 in fresh CAMHB and grown for an additional 4 hours to an OD_{600} of ~0.3. Cultures were centrifuged at 10,000 g for 15 mins, supernatants were discarded, and cell pellets were washed once with sterile water and resuspended in $1/10^{th}$ the original volume in sterile water or water dosed with compounds. Suspensions were incubated for 1 hour at 37°C with shaking and centrifuged at 10,000 g for 15 mins, washed with sterile water and resuspended in sterile water supplemented with 1:1 SYTO-9 and propidium iodide (3 µL/mL, from Invitrogen BacLight Kit). 100 µL of each test condition were added to a 96 well plate which was covered and allowed to stand at room temperature. After 15 mins, the plate was read at 530 nm and 645 nm (excitation 485 nm). The ratio of green to red fluorescence was calculated as a percentage of the control.

Ovine Erythrocyte Lysis Assay: Hemolysis assays were performed on mechanically difibrinated sheep blood (Hemostat Labs: DSB100). Difibrinated blood (1.5 mL) was placed into a microcentrifuge tube and centrifuged for 10 min at 10,000 rpm. The supernatant was then removed and then the cells were resuspended in 1 mL of phosphate-buffered saline (PBS). The suspension was centrifuged, the supernatant was removed and cells were resuspended two additional times in PBS. The final cell suspension was then diluted 10-fold in PBS dosed with test compounds from DMSO stock solutions. DMSO was used as a negative control and a zero hemolysis marker. Triton X100 (1%) was used as a positive control serving as the 100% lysis marker. Samples were incubated at 37°C with shaking. After one hour, the samples were centrifuged for 10 min at 10,000 rpm. The supernatant was diluted 1:40 in distilled water. The absorbance of the supernatant at 540 nm was then measured with a UV spectrometer.

General Synthetic Methods: All reagents used for chemical synthesis were purchased from commercial sources and used without further purification. Flash chromatography was performed using 60Å mesh silica gel (Sorbtech, Norcross, GA). Both ^{1}H (300 and 400 MHz) and ^{13}C NMR (75 MHz and 100 Mhz) spectra were collected on Varian Mercury spectrometers at 25°C. Ultraviolet spectra were obtained from a Genesys 10 Scanning UV/Vis spectrophotometer (λ_{max} in nm). Infrared spectra were obtained on a solid-phase FT-IR-4100 spectrophotometer (ν_{max} in cm^{-1}). High-resolution mass spectra were obtained by the NCSU Molecular Education, Technology, and Research Innovation Center (METRIC). All NMR spectra are available in this article's supplemental information document. The purity of all reported compounds was determined to be greater than or equal to 95% both by NMR spectra and subsequent liquid chromatography UV/Vis trace analysis.

General Procedure for Condensing Primary Amines with 2,2,2-trichloroacetylpyrroles: This method was adapted from methods described by Richards et al.[14] One equivalent of the appropriate 2,2,2-trichloroacetylpyrrole was combined with 2 equivalents of the appropriate primary amine along with 3 equivalents of anhydrous potassium carbonate and dissolved in 4 mLs of anhydrous dimethylformamide (DMF) under argon and stirred for 18 hours. The reaction was poured into 75 mLs EtOAc and 25 mLs of deionized water, the aqueous layer was discarded and the organic was further washed thrice with water, twice with 25 mLs 1N HCl, and once with 25 mLs brine. The organic layer was then dried with anhydrous magnesium sulfate and evaporated under reduced pressure. The crude solid was purified by flash chromatography (5-20% EtOAc/Hexanes) to yield the final products.

General Procedure for Synthesis of 3-(2-nitrovinyl) indoles: This procedure was adapted from the methods described by Holloway.[16] The corresponding indole (0.9 equiv) was dissolved

in 4 mLs anhydrous dichloromethane (DCM) under argon and stirred for 15 mins at RT. In a separate flask, *N*,*N*-dimethyl-2-nitroethen-1-amine (DMANE) (1 equiv) was dissolved in 3 mLs trifluoroacetic acid (TFA) and stirred for 15 mins at RT. The solution of indole in DCM was added dropwise to the stirring solution of TFA and stirred for an additional 30 mins at RT. The reaction was quenched with the addition of water (10 mLs) and was poured into 25 mLs water and extracted three times with 25 mLs DCM. The organic layers were combined and dried with anhydrous magnesium sulfate and evaporated under reduced pressure. The resulted crude solid was recrystallized from chloroform to give the products with no further purification necessary.

General Procedure for N-alkylation of Indole: Indole (1 g, 8.54 mmols) was dissolved in 15mLs DMF and cooled to 0°C under argon. Sodium hydride (410 mg, 10.2 mmols) was added in portions to the solution which was then stirred at 0°C for 30 mins. Two equivalents of the appropriate alkyl halide were then added to the solution which was allowed to warm to RT for 18 hours. The reaction was poured into 75 mLs EtOAc and 25 mLs of deionized water, the aqueous layer was discarded and the organic was further washed thrice with water, twice with 25mLs 1N HCl, and once with 25 mLs brine. The organic layer was then dried with anhydrous magnesium sulfate and evaporated under reduced pressure. The crude solid was purified by flash chromatography (5-20% EtOAc/Hexanes) to yield the final products.

General Procedure for the Reduction of N-alkylated 3-nitrovinyl Indoles: This procedure was adapted from the methods described by Muratore.[18] The corresponding N-alkylated 3-nitrovinyl indole was dissolved in 15 mLs anhydrous tetrahydrofuran (THF) and cooled to -78°C for 20 mins. A 2.4M solution (5 mLs) of lithium aluminum hydride (LAH) in THF) was added dropwise to the stirring solution which was then allowed to come to room temperature for 18 hours. The stirring solution was then cooled to 0°C and the following solutions were added

dropwise in sequence: 0.86 mL of water, 0.86 mL of 15% aq. NaOH, and finally, 2.2 mL of deionized water. After 30 mins, magnesium sulfate was added to the slurry which was subsequently filtered through a pad of celite and washed twice with 20 mLs DCM. The organics were evaporated under reduced pressure and the crude amine was taken to further transformations with no further purification.

General Procedure for the Coupling of Carboxylic Acids to Primary Amines using PyBOP: The corresponding carboxylic acid (1 equiv), benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) (1 equiv), and triethylamine (TEA) (3 equiv) were dissolved in anhydrous DMF under argon and stirred for 15 mins at RT. The appropriate primary amine (2 equiv) was then added in one portion and the reaction was allowed to stir for 18 hours at RT and then poured into 75 mLs EtOAc and 25 mLs deionized water. The aqueous layer was discarded and the organic layer was washed twice with water (25 mLs) and once with brine (25 mLs). The organic layer was dried with magnesium sulfate and evaporated under reduced pressure. The crude solids were purified by flash chromatography (5-20% EtOAc/Hexanes) to afford pure products.

N-(2-(1H-indol-3-yl)ethyl)-4,5-dichloro-1H-pyrrole-2-carboxamide (1): Compound was synthesized using the methods previously reported by Richards *et al*. Spectral data were consistent with previous reports.[14]

***N*-(2-(1*H*-indol-3-yl)ethyl)-4,5-dibromo-1*H*-pyrrole-2-carboxamide (2):** Compound was synthesized using the methods previously reported by Richards *et al*. Spectral data were consistent with previous reports.[14]

***N*-(2-(1*H*-indol-3-yl)ethyl)-4-bromo-1*H*-pyrrole-2-carboxamide (3):** Compound was synthesized using the methods previously reported by Richards *et al*. Spectral data were consistent with previous reports.[14]

***N*-(2-(1*H*-indol-3-yl)ethyl)-4-bromo-1-methyl-1*H*-pyrrole-2-carboxamide (4):** Compound was synthesized using the methods previously reported by Richards *et al*. Spectral data were consistent with previous reports.[14]

***N*-(2-(1*H*-indol-3-yl)ethyl)-1-methyl-1*H*-pyrrole-2-carboxamide (5):** Compound was synthesized using the methods previously reported by Richards *et al*. Spectral data were consistent with previous reports.[14]

***N*-(2-(1*H*-indol-3-yl)ethyl)-1*H*-pyrrole-2-carboxamide (6):** Compound was synthesized using the methods previously reported by Richards *et al*. Spectral data were consistent with previous reports.[14]

2,2,2-trichloro-1-(4,5-dibromo-1*H*-pyrrol-2-yl)ethan-1-one (7): Compound was synthesized using the methods previously reported by Richards *et al*. Spectral data were consistent with previous reports.[14]

***N*-((1*H*-indol-3-yl)methyl)-4,5-dibromo-1*H*-pyrrole-2-carboxamide (8):** Compound was synthesized using the general procedure for condensing primary amines with 2,2,2-trichloroacetyl pyrroles with (1*H*-indol-3-yl)methanamine (323 mg, 2.14 mmols) and **7** (396 mg, 1.07 mmols) as condensation partners to afford **8** as a tan solid (372 mg, 77%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.69 (s, 1H), 10.94 (s, 1H), 8.41 (s, 1H), 7.60 (d, J = 7.9 Hz, 1H), 7.36 (d, J = 8.1 Hz, 1H), 7.28 (s, 1H), 7.07 (t, J = 7.5 Hz, 1H), 7.01 – 6.88 (m, 2H), 4.57 (d, J = 5.5 Hz, 2H); ^{13}C NMR (101 MHz, dmso) δ 158.54, 136.20, 128.26, 126.46, 123.82, 121.20, 118.81, 118.56, 112.71, 112.25, 111.44, 104.21, 97.86, 33.91 ppm; UV (λ_{max} nm) 288; IR ν_{max} (cm^{-1})

3392, 1504, 804, 749, 492; HRMS (ESI) calcd for $C_{14}H_{11}Br_2N_3O$ $[M+H]^-$ 393.9196, found 393.9192.

4,5-dibromo-*N*-(2-(1-butyl-1*H*-indol-3-yl)ethyl)-1*H*-pyrrole-2-carboxamide (9): Compound was synthesized using the general procedure for condensing primary amines with 2,2,2-trichloroacetyl pyrroles with (1*H*-indol-3-yl)propylamine (150 mg, 0.86 mmols) and **7** (287 mg, 0.78 mmols) as condensation partners to afford **9** as a tan solid (186 mg, 38%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.70 (s, 1H), 8.26 (s, 1H), 7.58 (d, J = 7.8 Hz, 1H), 7.39 (d, J = 8.1 Hz, 1H), 7.16 (s, 1H), 7.14 – 7.07 (m, 1H), 7.05 – 6.96 (m, 1H), 6.92 (s, 1H), 4.07 (t, J = 6.5 Hz, 2H), 3.62 – 3.39 (m, 2H), 2.91 (t, J = 6.9 Hz, 2H), 1.82 – 1.49 (m, 2H), 1.19 (dd, J = 14.7, 7.3 Hz, 2H), 0.84 (t, J = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, acetone) δ 160.09, 137.48, 129.43, 129.00, 126.97, 122.00, 119.62, 119.26, 113.08, 113.05, 112.35, 110.34, 99.44, 46.24, 40.79, 33.20, 26.21, 20.74, 14.01 ppm; UV (λ_{max} nm) 296; IR ν_{max} (cm^{-1}) 3117, 1611, 1557, 742, 492; HRMS (ESI) calcd for $C_{19}H_{21}Br_2N_3O$ $[M+H]^-$ 463.9978, found 463.9976.

***N*-(2-(1*H*-imidazol-4-yl)ethyl)-4,5-dibromo-1*H*-pyrrole-2-carboxamide (10):** Compound was synthesized using the methods previously reported by Richards *et al*. Spectral data were consistent with previous reports.[14]

***N*-benzyl-4,5-dibromo-1*H*-pyrrole-2-carboxamide (11):** Compound was synthesized using the general procedure for condensing primary amines with 2,2,2-trichloroacetyl pyrroles with benzylamine (89 µL, 0.81 mmols) and **7** (300 mg, 0.81 mmols) as condensation partners to afford **11** as a white solid (162 mg, 56%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.73 (s, 1H), 8.68 (s, 1H), 7.40 – 7.17 (m, 5H), 6.98 (d, J = 2.8 Hz, 1H), 4.42 (d, J = 5.8 Hz, 2H); ^{13}C NMR (75 MHz, dmso) δ 158.93, 139.51, 128.36, 128.06, 127.21, 126.86, 112.73, 104.78, 97.92, 41.99

ppm; UV (λ_{max} nm) 286; IR ν_{max} (cm^{-1}) 3187, 1602, 1558, 1232, 711; HRMS (ESI) calcd for $C_{12}H_{10}Br_2N_2O$ $[M+H]^-$ 354.9087, found 354.9088.

4,5-dibromo-*N*-(2-(pyridin-2-yl)ethyl)-1*H*-pyrrole-2-carboxamide (12): Compound was synthesized using the general procedure for condensing primary amines with 2,2,2-trichloroacetyl pyrroles with 2-(pyridin-2-yl)ethan-1-amine (97 µL, 0.81 mmols) and **7** (300 mg, 0.81 mmols) as condensation partners to afford **12** as a white solid (97 mg, 32%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.67 (s, 1H), 8.50 (d, *J* = 6.4 Hz, 1H), 8.23 (t, *J* = 5.4 Hz, 1H), 7.69 (td, *J* = 7.6, 1.9 Hz, 1H), 7.23 (dd, *J* = 14.0, 7.2 Hz, 2H), 6.87 (s, 1H), 3.56 (q, *J* = 6.7 Hz, 2H), 2.95 (t, *J* = 7.3 Hz, 2H); ^{13}C NMR (101 MHz, dmso) δ 159.05, 158.91, 149.11, 136.55, 128.25, 123.23, 121.60, 112.49, 104.52, 97.84, 38.65, 37.50 ppm; UV (λ_{max} nm) 288; IR ν_{max} (cm^{-1}) 3108, 1626, 1566, 1327, 765; HRMS (ESI) calcd for $C_{12}H_{11}Br_2N_3O$ $[M+H]^-$ 369.9196, found 369.9197.

4,5-dibromo-*N*-(2,3-dihydro-1*H*-inden-2-yl)-1*H*-pyrrole-2-carboxamide (13): Compound was synthesized using the general procedure for condensing primary amines with 2,2,2-trichloroacetyl pyrroles with 2-aminoindane (211 µL, 1.62 mmols) and **7** (300 mg, 0.81 mmols) as condensation partners to afford **13** as a gray solid (102 mg, 33%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.68 (s, 1H), 8.29 (d, *J* = 6.2 Hz, 1H), 7.20 (d, *J* = 20.0 Hz, 4H), 6.99 (s, 1H), 4.85 – 4.38 (m, 1H), 3.22 (dd, *J* = 16.3, 7.3 Hz, 2H), 2.88 (dd, *J* = 16.0, 5.5 Hz, 2H); ^{13}C NMR (101 MHz, dmso) δ 158.80, 141.22, 128.21, 126.46, 124.52, 112.97, 104.50, 97.84, 50.12, 39.07 ppm; UV (λ_{max} nm) 288; IR ν_{max} (cm^{-1}) 3133, 1635, 1503, 811, 740; HRMS (ESI) calcd for $C_{14}H_{12}Br_2N_2O$ $[M+H]^-$ 380.9243, found 380.9245.

***N*-((1*H*-indol-2-yl)methyl)-4,5-dibromo-1*H*-pyrrole-2-carboxamide (14):** Compound was synthesized using the general procedure for condensing primary amines with 2,2,2-trichloroacetyl pyrroles with (1*H*-indol-2-yl)methanamine (237 mg, 1.62 mmols) and **7** (300

mg, 0.81 mmols) as condensation partners to afford **14** as a yellow solid (239 mg, 74%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.76 (s, 1H), 10.96 (s, 1H), 8.64 (s, 1H), 7.45 (d, J = 7.7 Hz, 1H), 7.33 (d, J = 8.2 Hz, 1H), 7.07 – 6.99 (m, 2H), 6.98 – 6.89 (m, 1H), 6.28 (s, 1H), 4.57 (s, 2H); ^{13}C NMR (101 MHz, dmso) δ 158.98, 137.01, 136.18, 128.08, 127.98, 120.72, 119.60, 118.92, 113.08, 111.14, 104.82, 99.20, 98.01, 36.25 ppm; UV (λ_{max} nm) 294; IR ν_{max} (cm^{-1}) 3314, 1685, 1521, 1229, 684; HRMS (ESI) calcd for $C_{15}H_{14}ClN_3O$ $[M+H]^-$ 393.9196, found 393.9198.

***N*-((1*H*-indol-6-yl)methyl)-4,5-dibromo-1*H*-pyrrole-2-carboxamide (15):** Compound was synthesized using the general procedure for condensing primary amines with 2,2,2-trichloroacetyl pyrroles with (1*H*-indol-6-yl)methanamine (236 mg, 1.62 mmols) and **7** (300 mg, 0.81 mmols) as condensation partners to afford **14** as a white solid (268 mg, 83%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.73 (s, 1H), 11.01 (s, 1H), 8.66 (s, 1H), 7.47 (d, J = 8.1 Hz, 1H), 7.30 (s, 2H), 7.00 (s, 1H), 6.94 (d, J = 9.0 Hz, 1H), 6.38 (s, 1H), 4.51 (d, J = 5.9 Hz, 2H); ^{13}C NMR (101 MHz, dmso) δ 158.85, 136.06, 132.19, 128.30, 126.64, 125.30, 119.90, 118.85, 112.73, 110.00, 104.68, 100.94, 97.97, 42.54 ppm; UV (λ_{max} nm) 292; IR ν_{max} (cm^{-1}) 3403, 1637, 1512, 867, 769; HRMS (ESI) calcd for $C_{15}H_{14}ClN_3O$ $[M+H]^-$ 393.9196, found 393.9198.

4,5-dibromo-*N*-(2-(5-hydroxy-1*H*-indol-3-yl)ethyl)-1*H*-pyrrole-2-carboxamide (16): Compound was synthesized using the general procedure for condensing primary amines with 2,2,2-trichloroacetyl pyrroles with one exception: final product was purified by flash chromatograpy (0.5 – 5% MeOH-NH3/DCM) in lieu of EtOAc/Hexanes. 5-hydroxytryptamine (117 mg, 0.54 mmols) and **7** (200 mg, 0.54 mmols) were condensed to afford **16** as a tan solid (48 mg, 21%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.68 (s, 1H), 10.50 (s, 1H), 8.61 (s, 1H), 8.25 (s, 1H), 7.13 (d, J = 8.5 Hz, 1H), 7.06 (s, 1H), 6.93 (s, 1H), 6.87 (s, 1H), 6.61 (d, J = 8.6 Hz, 1H),

3.53 – 3.42 (m, 2H), 2.82 (t, *J* = 7.0 Hz, 2H); ^{13}C NMR (101 MHz, dmso) δ 158.86, 150.24, 130.85, 128.44, 127.93, 123.16, 112.46, 111.73, 111.35, 110.79, 104.40, 102.29, 97.83, 39.44, 25.46 ppm; UV (λ_{max} nm) 296; IR ν_{max} (cm^{-1}) 3161, 1569, 1178, 829, 614; HRMS (ESI) calcd for $C_{15}H_{13}Br_2N_3O_2$ $[M+H]^-$ 423.9301, found 423.9304.

4,5-dibromo-*N*-(2-(5-methoxy-1*H*-indol-3-yl)ethyl)-1*H*-pyrrole-2-carboxamide (17): Compound was synthesized using the general procedure for condensing primary amines with 2,2,2-trichloroacetyl pyrroles with 5-methoxytryptamine (318 mg, 1.62 mmols) and **7** (300 mg, 0.81 mmols) as condensation partners to afford **14** as a pink solid (207 mg, 58%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.69 (s, 1H), 10.66 (s, 1H), 8.26 (s, 1H), 7.22 (d, *J* = 8.6 Hz, 1H), 7.12 (s, 1H), 7.04 (s, 1H), 6.91 (s, 1H), 6.71 (d, *J* = 8.7 Hz, 1H), 3.53 – 3.39 (m, 2H), 2.87 (t, *J* = 7.1 Hz, 2H); ^{13}C NMR (101 MHz, dmso) δ 158.94, 153.04, 131.43, 128.46, 127.65, 123.43, 112.50, 112.09, 111.69, 111.18, 104.42, 100.16, 97.85, 55.28, 39.72, 25.35 ppm; UV (λ_{max} nm) 296; IR ν_{max} (cm^{-1}) 3340, 1606, 1467, 1167, 923; HRMS (ESI) calcd for $C_{16}H_{15}Br_2N_3O_2$ $[M+H]^-$ 437.9458, found 437.9460.

2-(benzo[*b*]thiophen-3-yl)ethan-1-amine (18): Compound was synthesized using the methods previously reported by Szostak. Spectral data were consistent with previous reports.[15]

***N*-(2-(benzo[*b*]thiophen-3-yl)ethyl)-4,5-dibromo-1*H*-pyrrole-2-carboxamide (19):** Compound was synthesized using the general procedure for condensing primary amines with 2,2,2-trichloroacetyl pyrroles with **18** (100 mg, 0.56 mmols) and **7** (209 mg, 0.56 mmols) as condensation partners to afford **19** as a yellow solid (105 mg, 44%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.71 (s, 1H), 8.35 (s, 1H), 7.95 (dd, *J* = 16.0, 7.2 Hz, 2H), 7.48 (s, 1H), 7.45 – 7.33 (m, 2H), 6.89 (s, 1H), 3.54 (q, *J* = 6.8 Hz, 2H), 3.05 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR (101 MHz, dmso) δ 159.03, 139.73, 138.81, 133.73, 128.24, 124.35, 124.11, 122.98, 122.86, 121.77,

112.52, 104.59, 97.85, 38.50, 28.34 ppm; UV (λ_{max} nm) 286; IR ν_{max} (cm^{-1}) 3410, 3117, 2921, 1629, 739; HRMS (ESI) calcd for $C_{15}H_{12}Br_2N_2OS$ $[M+H]^-$ 424.8964, found 424.8969.

(*E*)-6-fluoro-3-(2-nitrovinyl)-1*H*-indole (20): Compound was synthesized using the methods previously reported by Muratore. Spectral data were consistent with previous reports.[18]

(*E*)-7-methyl-3-(2-nitrovinyl)-1*H*-indole (21): Compound was synthesized using the general procedure for synthesis of 3-(2-nitrovinyl) indoles with 7-methyl indole (800 mg, 6.09 mmols) as the starting material to afford **21** as an orange solid (730 mg, 59%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.22 (s, 1H), 8.35 (d, *J* = 13.4 Hz, 1H), 8.19 (d, *J* = 2.7 Hz, 1H), 7.93 (d, *J* = 13.4 Hz, 1H), 7.68 (d, *J* = 7.8 Hz, 1H), 7.07 (t, *J* = 7.5 Hz, 1H), 7.00 (d, *J* = 7.1 Hz, 1H), 2.44 (s, 3H); ^{13}C NMR (101 MHz, dmso) δ 137.17, 135.99, 134.82, 131.02, 124.53, 123.99, 122.21, 122.09, 117.98, 108.68, 16.62 ppm; UV (λ_{max} nm) 396; IR ν_{max} (cm^{-1}) 3104, 1609, 1521, 1201, 793; HRMS (ESI) calcd for $C_{11}H_{10}N_2O_2$ $[M+H]^-$ 201.0669, found 201.0669.

2-(6-fluoro-1*H*-indol-3-yl)ethan-1-amine (22): Compound was synthesized using the methods previously reported by Muratore and taken to the next reaction without further purification.[18]

2-(7-methyl-1*H*-indol-3-yl)ethan-1-amine (23): Compound was synthesized using the methods previously reported by Blough and taken on to the next reaction without further purification.[19]

4,5-dibromo-*N*-(2-(6-fluoro-1*H*-indol-3-yl)ethyl)-1*H*-pyrrole-2-carboxamide (24): Compound was synthesized using the general procedure for condensing primary amines with 2,2,2-trichloroacetyl pyrroles with **22** (204 mg, 1.14 mmols) and **7** (300 mg, 0.81 mmols) as condensation partners to afford **24** as a brown solid (129 mg, 37%). ^{1}H NMR (300 MHz, Chloroform-*d*) δ 11.25 (s, 1H), 8.11 (s, 1H), 7.50 (dd, *J* = 8.6, 5.3 Hz, 1H), 7.09 – 7.00 (m, 2H), 6.90 (td, *J* = 9.1, 2.1 Hz, 1H), 6.37 (s, 1H), 5.97 (s, 1H), 3.76 (q, *J* = 6.4 Hz, 2H), 3.03 (t, *J* = 6.5 Hz, 2H); ^{13}C NMR (101 MHz, cd_3od) δ 161.64, 159.89, 137.95, 128.85, 125.40, 123.79, 120.13,

114.21, 113.48, 108.09, 105.88, 99.94, 98.19, 41.34, 26.33 ppm; UV (λ_{max} nm) 288; IR ν_{max} (cm^{-1}) 2924, 1613, 1556, 1454, 799; HRMS (ESI) calcd for $C_{15}H_{12}Br_2FN_3O$ $[M+H]^-$ 425.9258, found 425.9262.

4,5-dibromo-*N*-(2-(7-methyl-1*H*-indol-3-yl)ethyl)-1*H*-pyrrole-2-carboxamide (25): Compound was synthesized using the general procedure for condensing primary amines with 2,2,2-trichloroacetyl pyrroles with **23** (201 mg, 1.14 mmols) and **7** (300 mg, 0.81 mmols) as condensation partners to afford **25** as a tan solid (141 mg, 41%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.70 (s, 1H), 10.79 (s, 1H), 8.27 (s, 1H), 7.40 (d, J = 7.4 Hz, 1H), 7.15 (s, 1H), 6.93 (s, 1H), 6.88 (t, J = 9.1 Hz, 2H), 3.50 (q, J = 6.3 Hz, 2H), 2.91 (t, J = 7.2 Hz, 2H), 2.44 (s, 3H); ^{13}C NMR (101 MHz, dmso) δ 158.85, 135.79, 128.42, 126.92, 122.39, 121.45, 120.46, 118.49, 115.90, 112.43, 112.19, 104.34, 97.79, 39.57, 25.42, 16.79 ppm; UV (λ_{max} nm) 286; IR ν_{max} (cm^{-1}) 3392, 3085, 1559, 777, 448; HRMS (ESI) calcd for $C_{16}H_{15}Br_2N_3O$ $[M+H]^-$ 421.9509, found 421.9514.

1-methyl-1*H*-indole (26a): Compound was synthesized via the general procedure for N-alkylation of indole with methyl iodide as the electrophile. Spectral data were consistent with previous reports.[20]

1-butyl-1*H*-indole (26b): Compound was synthesized via the general procedure for N-alkylation of indole with butyl iodide (4.84 mLs, 42.7 mmols) as the electrophile to afford **26b** as a yellow oil (1.36 g, 92%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.90 (d, J = 8.5 Hz, 1H), 7.57 (d, J = 8.2 Hz, 1H), 7.46 (t, J = 7.6 Hz, 1H), 7.37 (t, J = 7.4 Hz, 1H), 7.28 (d, J = 3.1 Hz, 1H), 6.74 (d, J = 3.1 Hz, 1H), 4.27 (t, J = 7.1 Hz, 2H), 2.01 (p, J = 7.2 Hz, 2H), 1.59 – 1.51 (m, 2H), 1.17 (t, J = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $cdcl_3$) δ 136.08, 128.71, 127.79, 121.34, 120.99, 119.22, 109.44, 100.92, 46.06, 32.37, 20.22, 13.75 ppm; UV (λ_{max} nm) 298; IR ν_{max} (cm^{-1}) 2928, 1510, 1313, 734, 425; HRMS (ESI) calcd for $C_{12}H_{15}N$ $[M+H]^+$ 174.1277, found 174.1277.

1-benzyl-1*H*-indole (26c): Compound was synthesized using the methods previously reported by Yu. Spectral data were consistent with previous reports.[21]

(*E*)-1-methyl-3-(2-nitrovinyl)-1*H*-indole (27a): Compound was synthesized using the general procedure for synthesis of 3-(2-nitrovinyl) indoles with **26a** (536 mg, 4.08 mmols) as the starting material to afford **27a** as an orange solid (115 mg, 14%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (d, J = 13.4 Hz, 1H), 8.16 (s, 1H), 8.03 – 7.89 (m, 2H), 7.56 (d, J = 8.0 Hz, 1H), 7.33 (t, J = 7.6 Hz, 1H), 7.27 (t, J = 7.5 Hz, 1H), 3.84 (s, 3H); ^{13}C NMR (101 MHz, dmso) δ 139.48, 138.27, 134.20, 131.00, 125.06, 123.42, 122.24, 120.69, 111.26, 107.25, 33.33 ppm; UV (λ_{max} nm) 394; IR ν_{max} (cm^{-1}) 2918, 1614, 1296, 948, 740; HRMS (ESI) calcd for $C_{11}H_{10}N_2O_2$ $[M+H]^-$ 201.0669, found 201.0672

(*E*)-1-butyl-3-(2-nitrovinyl)-1*H*-indole (27b): Compound was synthesized using the general procedure for synthesis of 3-(2-nitrovinyl) indoles with **26b** (1.21 g, 6.98 mmols) as the starting material to afford **27b** as a brown oil (545 mg, 32%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.26 (d, J = 14.6 Hz, 1H), 7.78 – 7.71 (m, 2H), 7.55 (s, 1H), 7.44 (d, J = 7.9 Hz, 1H), 7.40 – 7.35 (m, 1H), 7.35 – 7.29 (m, 1H), 4.14 (t, J = 7.1 Hz, 2H), 1.84 (dq, J = 12.5, 5.7, 5.0 Hz, 2H), 1.37 (h, J = 7.3 Hz, 2H), 0.98 (t, J = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, cdcl$_3$) δ 137.74, 136.29, 133.63, 131.51, 125.51, 123.67, 122.32, 120.58, 110.83, 107.91, 46.75, 31.67, 19.96, 13.54 ppm; UV (λ_{max} nm) 394; IR ν_{max} (cm^{-1}) 2929, 1615, 1294, 1174, 740; HRMS (ESI) calcd for $C_{14}H_{16}N_2O_2$ $[M+H]^+$ 245.1284, found 245.1285.

(*E*)-1-benzyl-3-(2-nitrovinyl)-1*H*-indole (27c): Compound was synthesized using the general procedure for synthesis of 3-(2-nitrovinyl) indoles with **26c** (770 mg, 3.71 mmols) as the starting material to afford **27c** as an orange solid (390 mg, 38%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.23 (d, J = 13.4 Hz, 1H), 7.75 (d, J = 13.3 Hz, 2H), 7.53 (s, 1H), 7.37 (td, J = 14.0, 12.6, 9.3 Hz,

5H), 7.19 (d, *J* = 6.0 Hz, 2H), 5.33 (s, 2H); ^{13}C NMR (101 MHz, cdcl$_3$) δ 137.99, 136.27, 135.38, 133.43, 132.14, 129.11, 128.39, 127.17, 125.68, 124.04, 122.63, 120.72, 111.19, 108.55, 50.73 ppm; UV (λ_{max} nm) 394; IR ν_{max} (cm^{-1}) 3105, 1616, 1488, 1247, 701; HRMS (ESI) calcd for $C_{17}H_{14}N_2O_2$ $[M+H]^-$ 277.0982, found 277.0982.

(*E*)-7-methyl-3-(2-nitrovinyl)-1*H*-indole (28a): Compound was synthesized using the general procedure for the reduction of N-alkylated 3-nitrovinyl indoles and taken to the next reaction without further purification.

2-(1-butyl-1*H*-indol-3-yl)ethan-1-amine (28b): Compound was synthesized using the general procedure for the reduction of N-alkylated 3-nitrovinyl indoles and taken to the next reaction without further purification.

2-(1-benzyl-1*H*-indol-3-yl)ethan-1-amine (28c): Compound was synthesized using the general procedure for the reduction of N-alkylated 3-nitrovinyl indoles and taken to the next reaction without further purification.

4,5-dibromo-*N*-(2-(1-methyl-1*H*-indol-3-yl)ethyl)-1*H*-pyrrole-2-carboxamide (29a): Compound was synthesized using the general procedure for condensing primary amines with 2,2,2-trichloroacetyl pyrroles with **28a** (330 mg, 1.89 mmols) and **7** (701 mg, 1.89 mmols) as condensation partners to afford **29a** as a white solid (476 mg, 59%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.75 (s, 1H), 8.33 (s, 1H), 7.60 (d, *J* = 7.8 Hz, 1H), 7.35 (d, *J* = 8.1 Hz, 1H), 7.21 – 7.07 (m, 2H), 7.08 – 6.91 (m, 2H), 3.70 (s, 3H), 3.58 – 3.44 (m, 2H), 2.94 (t, *J* = 6.4 Hz, 2H); ^{13}C NMR (101 MHz, dmso) δ 158.99, 136.70, 128.45, 127.62, 127.13, 121.17, 118.59, 118.43, 112.57, 111.22, 109.60, 104.48, 97.92, 39.77, 32.26, 25.28 ppm; UV (λ_{max} nm) 298; IR ν_{max} (cm^{-1}) 3113, 1639, 1516, 824, 736; HRMS (ESI) calcd for $C_{16}H_{15}Br_2N_3O$ $[M+H]^-$ 421.9509, found 421.9521.

4,5-dibromo-*N*-(2-(1-butyl-1*H*-indol-3-yl)ethyl)-1*H*-pyrrole-2-carboxamide (29b): Compound was synthesized using the general procedure for condensing primary amines with 2,2,2-trichloroacetyl pyrroles with **28b** (204 mg, 0.95 mmols) and **7** (350 mg, 0.95 mmols) as condensation partners to afford **29b** as a tan solid (168 mg, 38%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.70 (s, 1H), 8.26 (s, 1H), 7.58 (d, *J* = 7.8 Hz, 1H), 7.39 (d, *J* = 8.1 Hz, 1H), 7.16 (s, 1H), 7.14 – 7.07 (m, 1H), 7.05 – 6.96 (m, 1H), 6.92 (s, 1H), 4.07 (t, *J* = 6.5 Hz, 2H), 3.62 – 3.39 (m, 2H), 2.91 (t, *J* = 6.9 Hz, 2H), 1.82 – 1.49 (m, 2H), 1.19 (dd, *J* = 14.7, 7.3 Hz, 2H), 0.84 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, acetone) δ 160.09, 137.48, 129.43, 129.00, 126.97, 122.00, 119.62, 119.26, 113.08, 113.05, 112.35, 110.34, 99.44, 46.24, 40.79, 33.20, 26.21, 20.74, 14.01 ppm; UV (λ_{max} nm) 296; IR ν_{max} (cm^{-1}) 3111, 1638, 1506, 745, 494; HRMS (ESI) calcd for $C_{19}H_{21}Br_2N_3O$ $[M+H]^-$ 463.9978, found 463.9976.

***N*-(2-(1-benzyl-1*H*-indol-3-yl)ethyl)-4,5-dibromo-1*H*-pyrrole-2-carboxamide (29c):** Compound was synthesized using the general procedure for condensing primary amines with 2,2,2-trichloroacetyl pyrroles with **28c** (400 mg, 1.59 mmols) and **7** (300 mg, 0.81 mmols) as condensation partners to afford **29c** as a tan solid (117 mg, 15%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.69 (s, 1H), 8.25 (s, 1H), 7.60 (d, *J* = 7.8 Hz, 1H), 7.40 (d, *J* = 8.1 Hz, 1H), 7.32 (s, 1H), 7.24 (t, *J* = 8.0 Hz, 3H), 7.16 (d, *J* = 7.7 Hz, 2H), 7.12 – 7.05 (m, 1H), 7.03 – 6.97 (m, 1H), 6.90 (s, 1H), 5.35 (s, 2H), 3.54 – 3.46 (m, 2H), 2.92 (t, *J* = 7.3 Hz, 2H); ^{13}C NMR (101 MHz, dmso) δ 158.91, 138.40, 136.10, 128.51, 128.40, 127.86, 127.26, 127.04, 126.75, 121.28, 118.74, 118.63, 112.44, 111.71, 110.11, 104.43, 97.84, 48.94, 39.43, 25.16 ppm; UV (λ_{max} nm) 290; IR ν_{max} (cm^{-1}) 3109, 1638, 824, 746, 490; HRMS (ESI) calcd for $C_{22}H_{19}Br_2N_3O$ $[M+H]^-$ 497.9822, found 497.9821.

***N*-(2-(1*H*-indol-3-yl)ethyl)thiophene-2-carboxamide (30):** Tryptamine (152 mg, 0.984 mmols) and TEA (0.13 mLs, 0.984 mmols) were dissolved in 3 mLs anhydrous DCM and stirred for 10 mins at RT. To the stirring solution was added thiophene-2-carbonyl chloride (100 µL, 0.984 mmols) dropwise over five minutes. The reaction was stirred for an additional hour at RT and then poured into 75 mLs EtOAc and 25 mLs of deionized water, the aqueous layer was discarded and the organic was further washed thrice with water, twice with 25mLs 1N HCl, and once with 25 mLs brine. The organic layer was then dried with anhydrous magnesium sulfate and evaporated under reduced pressure. The crude solid was purified by flash chromatography (5-20% EtOAc/Hexanes) to afford **30** as a brown solid (192 mg, 80%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.83 (s, 1H), 8.65 (s, 1H), 7.74 (d, *J* = 4.0 Hz, 2H), 7.58 (d, *J* = 7.8 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.18 (s, 1H), 7.16 – 7.11 (m, 1H), 7.07 (t, *J* = 7.4 Hz, 1H), 6.98 (t, *J* = 7.3 Hz, 1H), 3.57 – 3.47 (q, 2H), 2.94 (t, *J* = 7.5 Hz, 2H); ^{13}C NMR (101 MHz, cd_3od) δ 164.44, 140.40, 138.12, 131.36, 129.42, 128.78, 128.68, 123.41, 122.31, 119.60, 119.34, 113.30, 112.21, 42.03, 26.38 ppm; UV (λ_{max} nm) 292; IR ν_{max} (cm^{-1}) 3401, 1551, 741, 717, 504; HRMS (ESI) calcd for $C_{15}H_{14}N_2OS$ $[M+H]^+$ 271.0899, found 271.0899.

***N*-(2-(1*H*-indol-3-yl)ethyl)-4-chloro-1*H*-pyrrole-2-carboxamide (31):** Compound was synthesized using the general procedure for condensing primary amines with 2,2,2-trichloroacetyl pyrroles with tryptamine (81 mg, 0.41 mmols) and 2,2,2-trichloro-1-(4-chloro-1*H*-pyrrol-2-yl)ethan-1-one (50 mg, 0.20 mmols) as condensation partners to afford **31** as a tan solid (46 mg, 79%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.76 (s, 1H), 10.82 (s, 1H), 8.24 (s, 1H), 7.58 (d, *J* = 7.8 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.17 (s, 1H), 7.07 (t, *J* = 7.5 Hz, 1H), 7.03 – 6.92 (m, 2H), 6.77 (s, 1H), 3.49 (q, *J* = 6.8 Hz, 2H), 2.91 (t, *J* = 7.3 Hz, 2H); ^{13}C NMR (101

MHz, cd_3od) δ 162.67, 138.08, 128.72, 126.77, 123.39, 122.31, 120.07, 119.58, 119.31, 113.71, 113.31, 112.19, 110.69, 41.45, 26.50 ppm; UV (λ_{max} nm) 288; R ν_{max} (cm^{-1}) 2922, 1610, 1516, 1454, 740; HRMS (ESI) calcd for $C_{15}H_{14}ClN_3O$ $[M+H]^-$ 286.0752, found 286.0752.

***N*-(2-(1*H*-indol-3-yl)ethyl)-4,5-dibromofuran-2-carboxamide (32):** Compound was synthesized using the general procedure for coupling carboxylic acids to primary amines using PyBOP. Tryptamine (712 mg, 4.44 mmols) and 4,5-dibromofuran-2-carboxylic acid (400 mg, 1.48 mmols) were coupled to afford **32** as a white solid (290 mg, 48%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.51 (s, 1H), 7.62 (d, *J* = 7.8 Hz, 1H), 7.38 (d, *J* = 8.1 Hz, 1H), 7.22 (t, *J* = 7.2 Hz, 1H), 7.14 (t, *J* = 7.4 Hz, 1H), 7.10 (s, 1H), 7.02 (d, *J* = 1.8 Hz, 1H), 6.59 (s, 1H), 3.74 (q, *J* = 6.7 Hz, 2H), 3.06 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (101 MHz, $cdcl_3$) δ 156.64, 149.34, 136.51, 127.24, 125.21, 122.28, 122.22, 119.52, 118.61, 118.53, 112.40, 111.45, 104.32, 39.85, 25.37 ppm; UV (λ_{max} nm) 286; IR ν_{max} (cm^{-1}) 3286, 1643, 1479, 978, 738; HRMS (ESI) calcd for $C_{15}H_{12}Br_2N_2O_2$ $[M+H]^+$ 410.9338, found 410.9329.

***N*-(2-(1*H*-indol-3-yl)ethyl)-4,5-dibromothiophene-2-carboxamide (33):** Compound was synthesized using the general procedure for coupling carboxylic acids to primary amines using PyBOP. Tryptamine (672 mg, 4.19 mmols) and 4,5-dibromothiophene-2-carboxylic acid (400 mg, 1.39 mmols) were coupled to afford **33** as a white solid (387 mg, 65%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (s, 1H), 8.80 (s, 1H), 7.76 (s, 1H), 7.55 (d, *J* = 7.8 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 1H), 7.17 (s, 1H), 7.07 (t, *J* = 7.5 Hz, 1H), 6.98 (t, *J* = 7.4 Hz, 1H), 3.51 (q, *J* = 6.7 Hz, 2H), 2.94 (t, *J* = 7.3 Hz, 2H) ; ^{13}C NMR (101 MHz, dmso) δ 159.17, 141.61, 136.26, 129.95, 127.20, 122.74, 120.95, 118.27, 118.20, 116.21, 113.72, 111.54, 111.41, 40.22, 25.00 ppm; UV (λ_{max}

nm) 294; IR ν_{max} (cm^{-1}) 3285, 2921, 1620, 1540, 738; HRMS (ESI) calcd for $C_{15}H_{12}Br_2N_2OS$ $[M+H]^-$ 424.8964, found 424.8966.

***N*-(2-(1*H*-indol-3-yl)ethyl)-5-bromofuran-2-carboxamide (34):** Compound was synthesized using the general procedure for coupling carboxylic acids to primary amines using PyBOP with modifications to the purification procedure. Tryptamine (671 mg, 4.19 mmols) and 5-bromofuran-2-carboxylic acid (400 mg, 2.09 mmols) were coupled under standard conditions. In lieu of flash chromatography, the crude product was recrystallized from chloroform to afford **34** as a white solid (202 mg, 29%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.82 (s, 1H), 8.57 (s, 1H), 7.57 (d, *J* = 7.9 Hz, 1H), 7.34 (d, *J* = 7.6 Hz, 1H), 7.20 – 7.16 (m, 1H), 7.12 (d, *J* = 3.5 Hz, 1H), 7.06 (t, *J* = 7.5 Hz, 1H), 6.98 (t, *J* = 7.4 Hz, 1H), 6.74 (dd, *J* = 3.5, 1.1 Hz, 1H), 3.49 (q, *J* = 6.7 Hz, 2H), 2.92 (t, *J* = 7.5 Hz, 2H); ^{13}C NMR (101 MHz, dmso) δ 156.60, 149.95, 136.23, 127.22, 124.10, 122.60, 120.92, 118.26, 118.21, 115.57, 113.91, 111.66, 111.36, 39.45, 25.16 ppm; UV (λ_{max} nm) 286; IR ν_{max} (cm^{-1}) 3385, 1596, 1120, 797, 582; HRMS (ESI) calcd for $C_{15}H_{13}BrN_2O_2$ $[M+H]^-$ 331.0087, found 331.0090.

***N*-(2-(1*H*-indol-3-yl)ethyl)-3-bromothiophene-2-carboxamide (35):** Compound was synthesized using the general procedure for coupling carboxylic acids to primary amines using PyBOP with modifications to the purification procedure. Tryptamine (619 mg, 3.86 mmols) and 3-bromothiophene-2-carboxylic acid (400 mg, 1.93 mmols) were coupled under standard conditions. Flash chromatography (1% MeOH-NH3/DCM) followed by trituration of the solid in diethyl ether gave **35** as a white solid (249 mg, 36%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84 (s, 1H), 8.20 (s, 1H), 7.79 (d, *J* = 5.2 Hz, 1H), 7.59 (d, *J* = 7.8 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.21 (d, *J* = 2.3 Hz, 1H), 7.17 (d, *J* = 5.2 Hz, 1H), 7.11 – 7.03 (m, 1H), 6.99 (td, *J* = 7.5, 7.1, 1.0 Hz, 1H), 3.60 – 3.46 (m, 2H), 2.95 (t, *J* = 7.4 Hz, 2H); ^{13}C NMR (101 MHz, dmso) δ 160.12,

136.29, 133.67, 131.61, 129.74, 127.20, 122.87, 120.98, 118.33, 118.28, 111.48, 111.40, 109.48, 40.31, 24.94 ppm; UV (λ_{max} nm) 290; IR ν_{max} (cm^{-1}) 3275, 1605, 1536, 729, 544; HRMS (ESI) calcd for $C_{15}H_{13}BrN_2OS$ $[M+H]^-$ 346.9859, found 346.9863.

***N*-(2-(1*H*-indol-3-yl)ethyl)-5,6-dibromopicolinamide (36):** Compound was synthesized using the general procedure for coupling carboxylic acids to primary amines using PyBOP. Tryptamine (684 mg, 4.27 mmols) and 5,6-dibromopicolinic acid (400 mg, 1.42 mmols) were coupled to afford **36** as a white solid (274 mg, 46%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84 (s, 1H), 8.86 (s, 1H), 8.36 (d, *J* = 8.1 Hz, 1H), 7.93 (d, *J* = 8.1 Hz, 1H), 7.62 (d, *J* = 7.7 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.18 (s, 1H), 7.06 (t, *J* = 7.5 Hz, 1H), 6.98 (t, *J* = 7.4 Hz, 1H), 3.57 (q, *J* = 6.7 Hz, 2H), 3.02 – 2.89 (m, 2H); ^{13}C NMR (101 MHz, dmso) δ 161.89, 149.71, 143.70, 141.51, 136.26, 127.22, 126.05, 122.69, 122.60, 120.95, 118.39, 118.23, 111.65, 111.36, 39.99, 25.14 ppm; UV (λ_{max} nm) 288; IR ν_{max} (cm^{-1}) 3256, 1672, 1524, 1339, 744; HRMS (ESI) calcd for $C_{16}H_{13}Br_2N_3O$ $[M+H]^+$ 421.9498, found 421.9493.

***N*-(2-(1*H*-indol-3-yl)ethyl)-6-bromopicolinamide (37):** Compound was synthesized using the general procedure for coupling carboxylic acids to primary amines using PyBOP. Tryptamine (476 mg, 2.97 mmols) and 6-bromopicolinic acid (300 mg, 1.49 mmols) were coupled to afford **37** as a white solid (251 mg, 49%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (s, 1H), 8.81 (s, 1H), 8.06 (d, *J* = 7.5 Hz, 1H), 7.94 (t, *J* = 7.7 Hz, 1H), 7.85 (d, *J* = 7.9 Hz, 1H), 7.63 (d, *J* = 7.8 Hz, 1H), 7.34 (d, *J* = 8.6 Hz, 1H), 7.19 (s, 1H), 7.07 (t, *J* = 7.5 Hz, 1H), 6.98 (t, *J* = 7.4 Hz, 1H), 3.59 (q, *J* = 6.7 Hz, 2H), 2.96 (t, *J* = 7.5 Hz, 2H); ^{13}C NMR (75 MHz, acetone) δ 163.21, 152.64, 141.30, 141.03, 137.58, 131.43, 128.50, 123.17, 122.14, 122.04, 119.45, 119.40, 113.20, 112.10, 40.96, 29.84, 26.28 ppm; UV (λ_{max} nm) 290; IR ν_{max} (cm^{-1}) 3372, 1666, 1552, 1408, 746; HRMS (ESI) calcd for $C_{16}H_{14}BrN_3O$ $[M+H]^+$ 344.0393, found 344.0391.

***N*-(2-(1*H*-indol-3-yl)ethyl)-5-bromonicotinamide (38):** Tryptamine (581 mg, 3.63 mmols) and trimethylamine (762 µL, 5.44 mmols) were dissolved in anhydrous DMF under argon and stirred for 15 mins and 5-bromonicotinoyl chloride (400 mg, 1.81 mmols) was added in a single portion. The reaction was allowed to stir at RT for 2 hours and was then poured into 75 mLs EtOAc / 25 mLs deionized water. The aqueous layer was discarded and the organic layer was washed twice with water (25 mLs) and once with brine (25 mLs). The organic layer was dried with anhydrous magnesium sulfate and evaporated under reduced pressure. The crude solid was triturated in diethyl ether to afford pure **38** as a tan solid (372 mg, 60%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.85 (s, 1H), 8.96 (s, 1H), 8.92 (s, 1H), 8.85 (s, 1H), 8.40 (s, 1H), 7.57 (d, *J* = 7.8 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.20 (s, 1H), 7.07 (t, *J* = 7.5 Hz, 1H), 6.98 (t, *J* = 7.4 Hz, 1H), 3.55 (q, *J* = 6.7 Hz, 2H), 2.96 (t, *J* = 7.3 Hz, 2H) ; ^{13}C NMR (101 MHz, dmso) δ 163.70, 152.81, 147.44, 137.69, 136.69, 132.14, 127.69, 123.21, 121.38, 120.48, 118.69, 118.66, 112.10, 111.86, 40.84, 25.37 ppm; UV (λ_{max} nm) 286; IR ν_{max} (cm^{-1}) 3392, 1628, 1545, 739, 692; HRMS (ESI) calcd for $C_{16}H_{14}BrN_3O$ $[M+H]^-$ 342.0247, found 342.0248.

***N*-(2-(1*H*-indol-3-yl)ethyl)picolinamide (39):** Compound was synthesized using the general procedure for coupling carboxylic acids to primary amines using PyBOP. Tryptamine (1.56 g, 9.74 mmols) and picolinic acid (400 mg, 3.25 mmols) were coupled to afford **39** as a tan solid (475 mg, 55%). ^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.74 (s, 1H), 8.49 (d, *J* = 4.7 Hz, 1H), 8.36 – 8.27 (m, 1H), 8.24 (d, *J* = 7.8 Hz, 1H), 7.85 – 7.74 (m, 1H), 7.68 (d, *J* = 7.7 Hz, 1H), 7.36 (dd, *J* = 7.5, 4.9 Hz, 2H), 7.24 – 7.17 (m, 1H), 7.17 – 7.08 (m, 1H), 7.03 (s, 1H), 3.85 (q, *J* = 7.0 Hz, 2H), 3.12 (t, *J* = 7.0 Hz, 2H) ; ^{13}C NMR (75 MHz, cdcl$_3$) δ 164.51, 149.87, 148.08, 137.35, 136.49, 127.32, 126.13, 122.27, 122.11, 121.93, 119.22, 118.72, 112.67, 111.37, 39.86, 25.55

ppm; UV (λ_{max} nm) 286; IR ν_{max} (cm^{-1}) 3244, 1662, 1522, 1430, 747; HRMS (ESI) calcd for $C_{16}H_{15}N_3O$ $[M+H]^+$ 266.1287, found 266.1282.

2,2,2-trichloro-1-(4,5-dibromo-1-methyl-1*H*-pyrrol-2-yl)ethan-1-one (40a): Compound was synthesized using the methods previously reported by Richards. Spectral data were consistent with previous reports.[14]

2,2,2-trichloro-1-(4,5-dibromo-1-butyl-1*H*-pyrrol-2-yl)ethan-1-one (40b): **7** (500 mg, 1.35 mmols) was dissolved in 15 mLs DMF and cooled to 0°C under argon. Sodium hydride (59 mg, 1.48 mmols) was added in portions to the solution which was then stirred at 0°C for 30 mins. Butyl iodide (460 µL, 4.05 mmols) was then added to the solution and stirred at RT for 18 hours. The reaction was poured into 75 mLs EtOAc and 25 mLs of deionized water, the aqueous layer was discarded and the organic was further washed thrice with water, twice with 25 mLs 1N HCl, and once with 25 mLs brine. The organic layer was then dried with anhydrous magnesium sulfate and evaporated under reduced pressure. The crude solid was purified by flash chromatography (5-20% EtOAc/Hexanes) to afford **40b** as a brown oil (299 mg, 52%). ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.58 (s, 1H), 4.60 – 4.30 (m, 2H), 1.68 (p, *J* = 7.6 Hz, 2H), 1.39 (dt, *J* = 14.9, 7.4 Hz, 2H), 1.02 – 0.93 (m, 3H); ^{13}C NMR (101 MHz, $cdcl_3$) δ 171.25, 125.09, 122.64, 119.02, 100.45, 95.57, 50.00, 32.33, 19.87, 13.83 ppm; UV (λ_{max} nm) 324; IR ν_{max} (cm^{-1}) 2959, 1675, 1360, 830, 683; HRMS (ESI) calcd for $C_{10}H_{10}Br_2Cl_3NO$ $[M+H]^-$ 421.8122, found 421.8130.

2,2,2-trichloro-1-(4,5-dibromo-1-butyl-1*H*-pyrrol-2-yl)ethan-1-one (40c): Compound was synthesized using the methods previously reported by Richards. Spectral data were consistent with previous reports.[14]

***N*-(2-(1*H*-indol-3-yl)ethyl)-4,5-dibromo-1-methyl-1*H*-pyrrole-2-carboxamide (41a):** Compound was synthesized using the methods previously reported by Richards. Spectral data were consistent with previous reports.[14]

***N*-(2-(1*H*-indol-3-yl)ethyl)-4,5-dibromo-1-butyl-1*H*-pyrrole-2-carboxamide (41b):** Compound was synthesized using the general procedure for condensing primary amines with 2,2,2-trichloroacetyl pyrroles with tryptamine (179 mg, 1.11 mmols) and **40b** (238 mg, 0.56 mmols) as condensation partners to afford **41b** as a tan solid (176 mg, 68%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (s, 1H), 8.34 (s, 1H), 7.56 (d, J = 7.8 Hz, 1H), 7.36 (d, J = 8.0 Hz, 1H), 7.18 (s, 1H), 7.08 (t, J = 7.5 Hz, 1H), 7.03 – 6.92 (m, 2H), 4.42 (t, J = 7.3 Hz, 2H), 3.49 (q, J = 6.8 Hz, 2H), 2.93 (t, J = 7.3 Hz, 2H), 1.59 (p, J = 7.6 Hz, 2H), 1.25 (dq, J = 14.3, 7.1 Hz, 2H), 0.86 (s, 3H); ^{13}C NMR (75 MHz, cdcl$_3$) δ 160.45, 136.43, 127.25, 127.18, 122.21, 122.16, 119.43, 118.58, 113.74, 112.50, 111.41, 110.53, 98.01, 48.03, 39.83, 33.00, 25.30, 19.73, 13.77 ppm; UV (λ_{max} nm) 286; IR ν_{max} (cm^{-1}) 3281, 1630, 1421, 807, 736; HRMS (ESI) calcd for $C_{19}H_{21}Br_2N_3O$ $[M+H]^-$ 463.9978, found 463.9980.

***N*-(2-(1*H*-indol-3-yl)ethyl)-1-benzyl-4,5-dibromo-1*H*-pyrrole-2-carboxamide (41c):** Compound was synthesized using the general procedure for condensing primary amines with 2,2,2-trichloroacetyl pyrroles with tryptamine (170 mg, 1.06 mmols) and **40c** (245 mg, 0.53 mmols) as condensation partners to afford **41c** as a tan solid (143 mg, 53%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.80 (s, 1H), 8.42 (s, 1H), 7.52 (d, J = 7.9 Hz, 1H), 7.29 (dt, J = 14.8, 8.4 Hz, 4H), 7.04 (dq, J = 21.3, 7.3 Hz, 6H), 5.76 (s, 2H), 3.46 – 3.39 (m, 2H), 2.86 (t, J = 7.2 Hz, 2H); ^{13}C NMR (101 MHz, dmso) δ 159.65, 137.66, 136.25, 128.53, 128.53, 128.07, 127.24, 127.18, 126.35, 122.70, 120.95, 118.27, 114.55, 111.72, 111.41, 110.35, 97.90, 50.07, 39.73, 25.10 ppm;

UV (λ_{max} nm) 290; IR ν_{max} (cm^{-1}) 3398, 1626, 1327, 772, 692; HRMS (ESI) calcd for $C_{22}H_{19}Br_2N_3O$ $[M+H]^-$ 497.9822, found 497.9827.

2,2,2-trichloro-1-(4,5-diiodo-1*H*-pyrrol-2-yl)ethan-1-one (42): Compound was synthesized using the methods previously reported by Essa. Spectral data were consistent with previous reports.[17]

2,2,2-trichloro-1-(4-iodo-1*H*-pyrrol-2-yl)ethan-1-one (43): Compound was synthesized using the methods previously reported by Essa and was isolated as a side product in this procedure along with the major product **42**. Spectral data were consistent with previous reports.[17]

***N*-(2-(1*H*-indol-3-yl)ethyl)-4,5-diiodo-1*H*-pyrrole-2-carboxamide (44):** Compound was synthesized using the general procedure for condensing primary amines with 2,2,2-trichloroacetyl pyrroles with tryptamine (376 mg, 2.34 mmols) and **42** (545 mg, 1.17 mmols) as condensation partners to afford **44** as a white solid (121 mg, 21%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.32 (s, 1H), 10.81 (s, 1H), 8.19 (s, 1H), 7.57 (d, *J* = 7.8 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 7.15 (s, 1H), 7.06 (t, *J* = 7.5 Hz, 1H), 6.97 (t, *J* = 7.3 Hz, 1H), 6.90 (s, 1H), 3.48 (q, *J* = 6.5 Hz, 2H), 2.90 (t, *J* = 7.2 Hz, 2H); ^{13}C NMR (101 MHz, cd_3od) δ 161.37, 138.12, 133.82, 128.74, 123.44, 122.30, 119.85, 119.58, 119.31, 113.29, 112.20, 82.15, 76.24, 41.46, 26.46 ppm; UV (λ_{max} nm) 288; IR ν_{max} (cm^{-1}) 3556, 2396, 1597, 1451, 747; HRMS (ESI) calcd for $C_{15}H_{13}I_2N_3O$ $[M+H]^-$ 503.9075, found 503.9075.

***N*-(2-(1*H*-indol-3-yl)ethyl)-4-iodo-1*H*-pyrrole-2-carboxamide (45):** Compound was synthesized using the general procedure for condensing primary amines with 2,2,2-trichloroacetyl pyrroles with tryptamine (516 mg, 3.22 mmols) and **43** (545 mg, 1.61 mmols**)** as condensation partners to afford **45** as a tan solid (172 mg, 28%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.80 (s, 1H), 10.81 (s, 1H), 8.22 (s, 1H), 7.58 (d, *J* = 7.8 Hz, 1H), 7.35 (d, *J* = 8.8 Hz, 1H),

7.17 (s, 1H), 7.07 (t, *J* = 7.5 Hz, 1H), 7.03 – 6.94 (m, 2H), 6.91 (s, 1H), 3.50 (q, *J* = 6.6 Hz, 2H), 2.92 (t, *J* = 7.4 Hz, 2H); ^{13}C NMR (101 MHz, cd_3od) δ 162.28, 138.06, 129.08, 128.71, 127.78, 123.39, 122.29, 119.58, 119.30, 118.13, 113.30, 112.18, 60.99, 41.44, 26.49 ppm; UV (λ_{max} nm) 284; IR ν_{max} (cm^{-1}) 3341, 1633, 1548, 752, 592; HRMS (ESI) calcd for $C_{15}H_{14}IN_3O$ $[M+H]^-$ 378.0108, found 378.0108.

***N*-((1*H*-indol-3-yl)methyl)-6-bromopicolinamide (46):** Compound was synthesized using the general procedure for coupling carboxylic acids to primary amines using PyBOP. (1*H*-indol-3-yl)methanamine (362 mg, 2.47 mmols) and 6-bromopicolinic acid (250 mg, 1.24 mmols) were coupled to afford **46** as a red solid (22 mg, 6.9%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.95 (s, 1H), 8.82 (s, 1H), 8.08 (d, *J* = 7.5 Hz, 1H), 7.93 (t, *J* = 7.9 Hz, 1H), 7.82 (d, *J* = 7.9 Hz, 1H), 7.67 (d, *J* = 7.9 Hz, 1H), 7.35 (d, *J* = 8.2 Hz, 1H), 7.31 (s, 1H), 7.07 (t, *J* = 7.5 Hz, 1H), 6.97 (t, *J* = 7.5 Hz, 1H), 4.64 (d, *J* = 6.0 Hz, 2H) ; ^{13}C NMR (101 MHz, $cdcl_3$) δ 162.75, 151.07, 140.61, 139.68, 136.48, 130.72, 126.64, 123.64, 122.39, 121.38, 119.88, 118.93, 112.26, 111.48, 35.16 ppm; UV (λ_{max} nm) 286; IR ν_{max} (cm^{-1}) 3434, 1665, 1515, 759, 492; HRMS (ESI) calcd for $C_{15}H_{12}BrN_3O$ $[M+Na]^+$ 352.0056, found 352.0049.

***N*-((1*H*-indol-3-yl)methyl)-4,5-diiodo-1*H*-pyrrole-2-carboxamide (47):** Compound was synthesized using the general procedure for condensing primary amines with 2,2,2-trichloroacetyl pyrroles with (1*H*-indol-3-yl)methanamine (182 mg, 1.25 mmols) and **42** (290 mg, 0.62 mmols) as condensation partners to afford **47** as a pink solid (146 mg, 48%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.31 (s, 1H), 10.92 (s, 1H), 8.31 (s, 1H), 7.59 (d, *J* = 7.8 Hz, 1H), 7.35 (d, *J* = 8.1 Hz, 1H), 7.27 (s, 1H), 7.07 (t, *J* = 7.5 Hz, 1H), 6.96 (t, *J* = 7.5 Hz, 1H), 6.92 (s, 1H), 4.55 (d, *J* = 5.3 Hz, 2H) ; ^{13}C NMR (101 MHz, dmso) δ 158.25, 136.31, 132.93, 126.47, 123.89, 121.13, 118.74, 118.50, 118.24, 112.31, 111.41, 83.00, 76.03, 33.94 ppm; UV (λ_{max} nm) 290; IR

ν_{max} (cm^{-1}) 3386, 3145, 1643, 1543, 809; HRMS (ESI) calcd for $C_{14}H_{11}I_2N_3O$ $[M+H]^-$ 489.8918, found 489.8915.

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5. Competing Interests

Dr. Melander is a co-founder and board of directors member of Agile Sciences, a biotechnology company seeking to commercialize antibiotic adjuvants.

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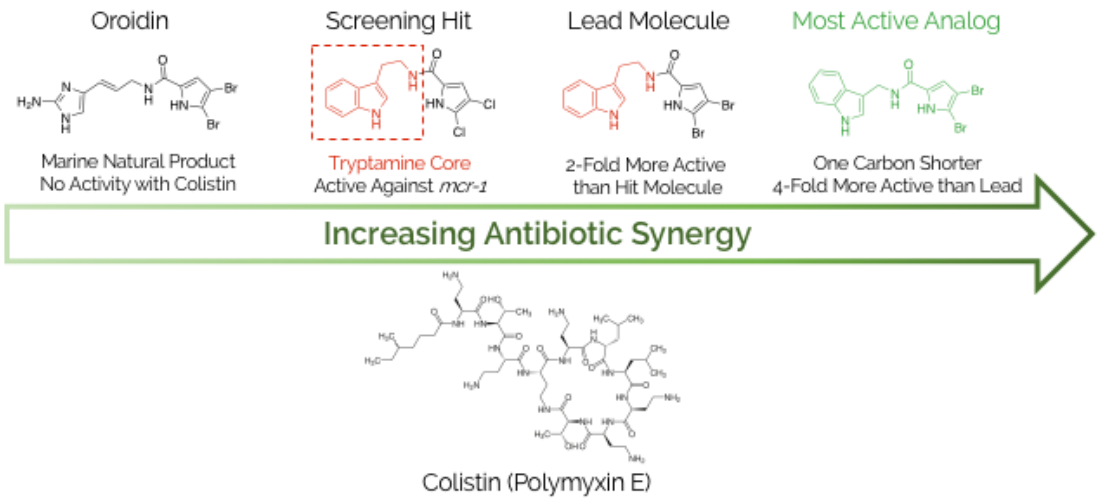

Colistin (Polymyxin E)