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Authors: Christian Corey Melander, T. Vu Nguyen, Bradley M. Minrovic, and Roberta J. Melander

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Identification of novel anti-mycobacterial biofilm agents based upon the 2-aminoimidazole scaffold

T. Vu Nguyen,[a] Bradley M. Minrovic[b], Roberta J. Melander[b] and Christian Melander[b] *

[a] Department of Chemistry, North Carolina State University, Raleigh, NC 27695.
[b] Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556.
*to whom correspondence should be addressed: cmelande@nd.edu
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Abstract: Tuberculosis (TB) remains a significant global health problem for which new therapeutic options are sorely needed. The ability of the causative agent, *Mycobacterium tuberculosis*, to reside within host macrophages and form biofilm-like communities contributes to the persistent and drug tolerant nature of the disease. Compounds that can prevent or reverse the biofilm-like phenotype have the potential to serve alongside TB antibiotics to overcome this tolerance, and reduce treatment duration. Using *Mycobacterium smegmatis* as a surrogate organism, we report the identification of two new 2-aminoimidazole compounds that inhibit and disperse mycobacterial biofilms, work synergistically with isoniazid and rifampicin to eradicate preformed *M. smegmatis* biofilms *in vitro,* are non-toxic toward *Galleria mellonella*, and exhibit stability in mouse plasma.

Introduction

Tuberculosis (TB), caused by infection with *Mycobacterium tuberculosis* (*Mtb*), still reigns as one of deadliest diseases impacting global health in both developed and especially developing countries,[1] and is estimated to be responsible for more than 2 million deaths each year.[2] TB is highly contagious,[3] and manifestations of the disease include persistent coughing, extreme weakness or fatigue, weight loss, and constant chest pain.[4] Although tuberculosis is primarily an infection of the respiratory system, it also affects the central nervous system, kidney, brain, and other organs.[5] If left untreated, TB can lead to organ failure and mortality.[6] Currently, TB is the leading cause of death from a single infectious agent,[7] and is the biggest killer of people infected with HIV[8].

Current treatment regimens for drug susceptible TB consist of six to nine months of combination therapy with the first-line anti-TB drugs isoniazid, rifampicin, ethambutol, and pyrazinamide, while infections caused by multi-drug resistant (MDR) and extensively drug resistant (XDR) strains of *M. tuberculosis* require even more protracted regimens involving additional antibiotics.[9] The prolonged treatment times, and deleterious side effects of these regimens result in considerable patient noncompliance, and this is thought to be a significant underlying factor in the persistence of active TB.[10]

M. tuberculosis has a remarkable propensity to persist in stressful environments such as those experienced during prolonged antibiotic treatment.[11] This is thought to be a result of several factors including: the ability to reside within host macrophages,[12] and the ability to form multicellular surface attached communities, known as biofilms.[13] Many studies have proposed that biofilms play a key role in bacterial persistence and drug tolerant bacterial infections, and it is thought that *M. tuberculosis* uses biofilm formation as a defense strategy to evade host immune responses and drug therapy.[14] Targeting *M. tuberculosis* biofilm formation therefore represents a potential strategy to combat TB persistence, whereby compounds that inhibit and disperse *M. tuberculosis* biofilms could be administered alongside antibiotics to overcome tolerance. This approach has the potential to enable reduced duration of therapy, and subsequently improve patient compliance.

Our group has previously reported several small molecules that possess the ability to inhibit and disperse mycobacterial biofilms (Figure 1),[15] including aryl 2-aminoimidazole (2-AI) **1**, reverse amide 2-aminoimidazole **2**, and 2-aminobenzimidazole **3**. Compound **1**, which has also previously been shown to have anti-biofilm and antimicrobial activity in other pathogenic bacteria,[16] was observed to restore isoniazid susceptibility to *M. tuberculosis* expressing drug tolerance in an *in vitro* model system that utilizes lysed human leukocytes to mimic the *in vivo* microbial communities of virulent *M. tuberculosis*.[15b]

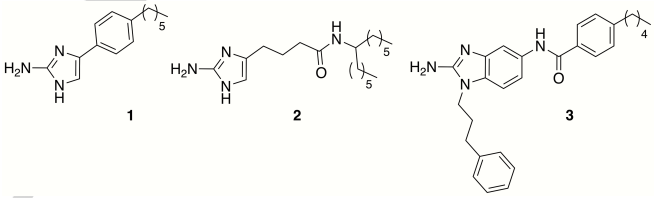


Figure 1. Structures of previously reported compounds with anti-mycobacterial biofilm activity.

As in our[15] and other group's[17] previous reports, we employed *M. smegmatis* as a surrogate organism for *M. tuberculosis* due to the slow growing nature of *M. tuberculosis,* as well as the fact that *M. tuberculosis* is a BSL3 organism. We have previously shown that anti-biofilm and antibiotic potentiation activity against *M. smegmatis* typically translates to activity against *M. tuberculosis*.[15a, 15b] Using this screening model, and by performing analogue synthesis upon the scaffold of compound **1**, we have identified two novel derivatives that possess potent anti-biofilm activity, work synergistically with isoniazid and rifampicin to eradicate preformed *M. smegmatis* biofilms *in vitro,* are non-toxic against *Galleria mellonella*, and exhibit stability in mouse plasma.

Results and Discussion

To delineate the structure activity relationship (SAR) of this class of compounds in the context of mycobacterial anti-biofilm activity, compound **1** along with members of a library of previously reported related 2-AI compounds and several newly synthesized

analogues, were probed for the ability to inhibit and disperse biofilms formed by *M. smegmatis* mc^{2}155 (ATCC 700084). Compound minimum inhibitory concentrations (MICs) were also determined to give an indication of the toxicity of each analogue toward planktonic bacteria.

In our initial studies with library stocks, an IC_{50} value (concentration required to inhibit 50% biofilm formation) of 8.0 μM was recorded for compound **1**, while the dispersion activity of this compound was found not to be dose responsive, thereby precluding the determination of an EC_{50} value (concentration required to disperse 50% of a pre-formed biofilm). Following resynthesis of compound **1** for this study, IC_{50} and EC_{50} values of 10.4 and 80.5 μM respectively were obtained (Table 1). The MIC of compound **1** is 100 μM. We next screened several previously reported substituted 2-aminoimidazoles,[18] for the ability to inhibit and disperse *M. smegmatis* biofilms. Of these, none of the compounds that contain substituted phenyl moieties at the 5-position of the 2-AI ring inhibited or dispersed biofilms to any significant degree (compounds S1-S4 Table S1). Compounds **4**, **5**, and **6** (Figure 2), which possess short aliphatic substituents (ethyl, isopropyl and butyl respectively) at the 31-position of the 2-AI ring exhibited IC_{50} values below 30 μM and/or EC_{50} values below 100 μM, however all exhibited reduced activity compared to the parent compound (Table 1). The most active compound of this series was the ethyl derivative **4**, which exhibited IC_{50} and EC_{50} values of 15.5 and 92.4 μM respectively. Larger substituents at this position, including heptyl, cyclohexyl and phenyl groups, abolished anti-biofilm activity (compounds S7-S9 Table S1).

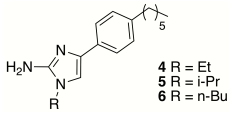
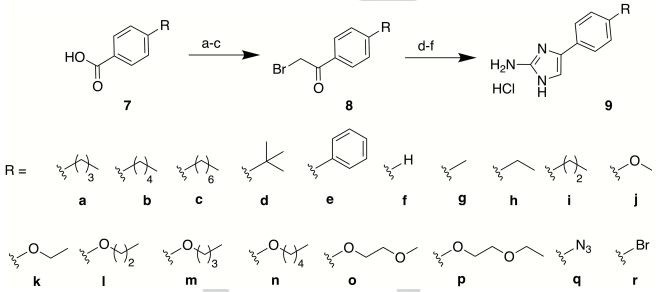


Figure 2. Structures of previously reported compounds **4**-**6**

A series of mono-substituted analogues of compound **1**, in which the *para*-hexyl substituent on the phenyl ring was varied, was next explored to probe the SAR of this portion of the molecule. Previously reported compounds **9a**-**e**,[16a] along with several novel derivatives (**9f**-**r**) containing a variety of *para*-substituted phenyl groups were synthesized as outlined in Scheme 1. Briefly, carboxylic acids **7a**-**r** were converted to the corresponding α-bromo ketones **8a**-**r** by treatment with oxalyl chloride followed by reaction with diazomethane and finally quenching with hydrobromic acid. The α-bromo ketones were then cyclized with Boc-guanidine and, following immediate Boc-deprotection with 30% TFA in dichloromethane, were purified and then treated with HCl/MeOH to afford compounds **9a-r** as HCl salts for biological testing.

Each compound was assayed for its inherent toxicity toward the bacterium, and no compound was found to have an MIC less than 100 μM. Compounds **9a-c and 9f-i**, which possess aliphatic chains of increasing length, displayed no improvement in anti-biofilm activity in comparison to the parent compound **1**, with the most active being pentyl derivative **9b**, which exhibited an IC_{50} of 13.6 μM. These analogues dispersed pre-formed biofilms with EC_{50} values ranging from 94.9 to >200 μM, with the pentyl derivative again exhibiting the greatest activity of the new compounds, returning an EC_{50} of 94.9 μM. The heptyl derivative **9c** exhibited IC_{50} and EC_{50} values of 23.5 and 130 μM respectively, indicating that for this series, the hexyl chain of compound **1** was optimal for anti-biofilm activity.



Scheme 1. Synthesis of 2-AI analogues with varying substituents at the aryl tail group. Reagents and Conditions: a) $(COCl)_2$, DCM, DMF_{cat} 0 ˚C, 1 h; b) CH_2N_2, Et_2O, 0 ˚C, 1 h; c) HBr, 0 ˚C, 20 min: d) Boc-guanidine, DMF, r.t., 48 h; e) TFA/DCM 1:4, r.t. 2 h; f) HCl/MeOH

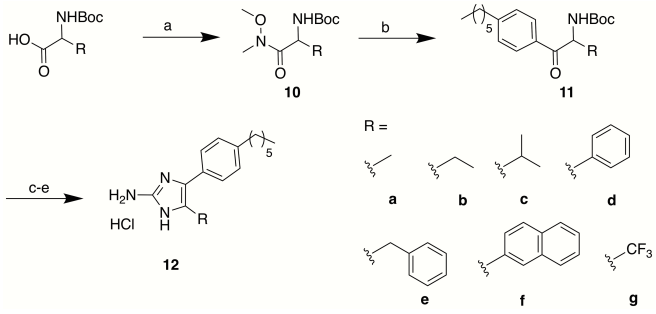
Table 1 Biofilm inhibition and dispersion activity of 2-aminoimidazole derivatives.

Compound	MIC (μM)	IC_{50} (μM)	EC_{50} (μM)
1	100	10.4 ± 0.6	80.6 ± 2.2
4	100	15.5 ± 2.3	92.4 ± 5.4
5	100	16.4 ± 1.1	90.1 ± 4.3
6	200	27.1 ± 4.5	>200a
9a	150	19.8 ± 2.8	98.1 ± 2.3
9b	200	13.6 ± 1.2	94.9 ± 4.2
9c	150	23.5 ± 0.9	130 ± 2.3
9d	150	>50a	>200
9e	>200	>50	>200
9f	100	>50	>200
9g	100	45.0 ± 3.3	>200
9h	100	29.0 ± 4.3	150 ± 6.2
9i	200	17.3 ± 1.2	152 ± 10
9j	200	30.1 ± 4.4	136 ± 5.6
9k	200	27.9 ± 1.8	170 ± 8.6
9l	100	20.9 ± 2.2	143.0 ± 4.8
9m	>200	18.7 ± 1.9	105 ± 6.3
9n	150	12.5 ± 1.3	88.2 ± 4.3
9o	>200	>50	180 ± 10
9p	>200	>50	>200
9q	200	>50	>200
9r	150	>50	>200

[a]Highest concentration tested was 50 μM for IC_{50} and 200 μM for EC_{50}.

We next investigated the effect that replacement of the alkyl chain on the phenyl ring of **1** with an alkoxy chain had upon anti-biofilm activity. Compounds **9j-p** exhibited similar activity to the corresponding alkyl derivatives, with pentoxy derivative **9n**,

which has the same chain length as compound **1**, exhibiting the greatest activity, returning IC_{50} and EC_{50} values of 12.5 and 88.2 μM respectively. The introduction of additional oxygen atoms into the alkyl chain in poly(ethylene glycol) (PEG) derivatives **9o** and **9p** resulted in a dramatic loss in activity, with IC_{50}/EC_{50} values greater than 50 μM and 200 μM respectively. The introduction of a *tert*-butyl, azido, phenyl, or bromo substituent (compounds **9d-e** and **9q-r**) was not well tolerated as evident by the inability of these analogues to inhibit and disperse biofilms below 50 μM and 200 μM respectively.



Scheme 2 Synthesis of 4,5-substituted 2-AI analogues. Reagents and Conditions: a) HN(OMe)MeHCl, BOP,Et_3N, DCM, 0 ˚C, 1 h; b) 4-hexyl phenyl lithium, THF, -78 ˚C –r.t. 16 h; c) TFA/DCM, 0 ˚C - r.t. 2 h; d) H_2NCN, H_2O/EtOH pH 4.3, 95 ˚C, 3 h; e) HCl/MeOH

To further this SAR study, we next investigated the impact of introducing various substituents at the 5-position of the 2-AI on the inhibition and dispersion of *M. smegmatis* biofilms by synthesizing a series of 4,5-substituted 2-aminoimidazole derivatives as previously reported[16]. Briefly, readily available Boc-protected amino acids were converted to the corresponding Weinreb amides **10a**-**g**, followed by reaction with 4-hexylphenyl lithium to form the α-amino ketones **11a**-**g**. Boc-deprotection and subsequent condensation with cyanamide yielded the 4,5-disubstituted 2-AIs **12a-g** (Scheme 2). After purification, each compound was converted to the corresponding HCl salt for biological testing.

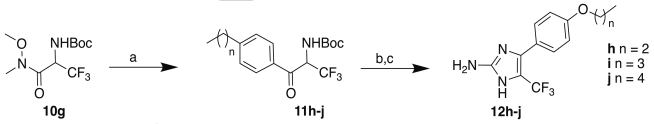
Table 2 Biofilm inhibition and dispersion activity of 4,5 substituted 2-AIs

Compound	MIC	IC_{50}	EC_{50}
12a	25	8.6 ± 0.5	86.5 ± 3.4
12b	25	13.5 ± 1.4	94.4 ± 3.8
12c	25	10.1 ± 0.8	90.1 ± 4.3
12d	100	>50[a]	>200[a]
12e	200	>50	185.6 ± 9.3
12f	200	>50	150.5 ± 10.3
12g	>200	6.8 ± 0.9	70.1 ± 3.6

[a]Highest concentration tested was 50 μM for IC_{50} and 200 μM for EC_{50}.

Many of the 4,5-substituted analogues exhibited both good inhibition and dispersion activity against *M. smegmatis* (Table 2), and this was especially so for the trifluoromethyl derivative **12g**, for which an IC_{50} value of 6.8 μM and an EC_{50} value of 70.1 μM was recorded. Compound **12g** was also inherently nontoxic toward the bacterium (MIC >200 μM). The corresponding 4-methyl analogue **12a** displayed greater toxicity toward the bacterium (MIC 25 μM), as did the 4-ethyl and 4-ispropyl analogues **12b** and **c** (MICs 25 μM). All three analogues were potent biofilm inhibitors with IC_{50} values in the range of 8.6-13.5 μM, but were less effective at dispersing biofilms in comparison to the parent compound **1**. Compounds possessing aromatic substituents at the 4-position (**12d-f**), displayed a reduced ability to inhibit or disperse *M. smegmatis* biofilms (IC_{50} and EC_{50} values >50 μM and 150 μM respectively).

As a result of the activity displayed by compound **12g**, we next synthesized three additional 4-trifluoromethyl analogues **12h-j** (Scheme 3). The goal was to elucidate if the trifluoromethyl group would improve biofilm activity for the well-tolerated alkoxy derivatives. Briefly, the 1-iodobenzene 4-alkoxy benzene analogues were reacted with n-butylithium and then subsequently added to the *N*-Boc trifluoroalanine Weinreb amide **10g** to form α-amino ketones **11h-j**. Boc deprotection and subsequent condensation with cyanamide yielded the 4,5-disubstituted 2-AIs **12h-j**. After purification, each compound was converted to the corresponding HCl salt for biological testing.



Scheme 3 Synthesis of additional trifluoromethyl 2-AI analogues. Reagents and Conditions: a) 4-alkoxyphenyl lithium, THF, -78 ˚C –r.t. i h; b) TFA/DCM, 0 ˚C - r.t. 2 h; c) H_2NCN, H_2O/EtOH pH 4.3, 95 ˚C, 3 h; d) HCl/MeOH

The presence of the 4-trifluoromethyl substituent in compounds **12h-j** resulted in increased anti-biofilm activity in comparison to the corresponding unsubstituted alkoxy derivatives **7l-n** (Table 3). This series followed the same SAR trend observed for the unsubstituted analogues, with pentoxy derivative **12j** being the most active of this series, and exhibiting comparable, although slightly reduced activity to **12g**, with IC_{50} and EC_{50} values of 7.4 μM and 74.2 μM respectively. Finally, colony count analysis at 48 hours was performed for each compound at its IC_{50} to verify a non-bactericidal mode of action.

Table 3 Biofilm inhibition and dispersion activity of trifluoromethyl 2-AIs

Compound	MIC	IC_{50}	EC_{50}
12h	200	17.1 ± 0.9	119.5 ± 3.0
12i	200	15.4 ± 1.0	95.2 ± 5.4
12j	200	7.4 ± 0.60	74.2 ± 2.5

We next asked the question whether lead compounds **12g** and **12j** would show synergistic effects with conventional anti-tuberculosis antibiotics upon biofilm dispersion. Studies by the Ojha Lab have shown that *M. tuberculosis* and *M. smegmatis* can form biofilms that tolerate more than 50 times the MIC of both isoniazid (INH) and rifampicin (RIF).[19-21] These two anti-tuberculosis drugs were chosen because they are the antibiotics most commonly administered in the first 6 to 9 months of TB chemotherapy.[22]

To study synergistic effects, biofilms were established in 96-well PVC plates for 48 hours. Media and planktonic bacteria were removed, and the wells were treated with either: media alone, media plus antibiotic, media plus compound **12g** or **12j**, or media plus a combination of compound and antibiotic. Dose response studies were performed to determine the EC_{50} of the

tested compound as a function of antibiotic concentration and this value was compared to the EC_{50} of the compound alone.

Neither INH nor (RIF) affected biofilm mass at the concentrations tested (up to 1024 μg mL^{-1}) when compared to samples treated with media only. The addition of 256 μg/mL INH lowered the EC_{50} of **12g** from 70.1 to 25.4 μM, while a similar effect was seen for RIF (128 μg/mL), which lowered the EC_{50} to 30.3 μM. Concurrent addition of both antibiotics resulted in an EC_{50} value of 20.5 μM, a three-fold reduction (Table 4) See SI for a full list of concentrations tested). Likewise, the EC_{50} value of compound **12j** was reduced in the presence of the same concentrations of INH, RIF, or INH + RIF to 27.5, 26.6 and 17.7 μM respectively, the latter representing a four-fold reduction.

Table 4 Dispersal enhancement between compounds **12g** and **12j** and INH/RIF

Compound	Antibiotic	Antibiotic Concentration (μg mL^{-1})	EC_{50} (μM)
12g	INH	256	25.4 ± 1.2
	RIF	128	30.3 ± 2.6
	INH + RIF	256 + 128	20.5 ± 2.9
12j	INH	256	27.5 ± 1.3
	RIF	128	26.6 ± 1.5
	INH + RIF	256 + 128	17.7 ± 1.9

After establishing the synergistic effects of compounds **12g** and **12j**, we next asked whether the cells being dispersed displayed the same response to antibiotics as cells grown under planktonic conditions. Under planktonic growth, incubation with isoniazid or rifampicin at their MIC of 32 μg mL^{-1} and 8 μg mL^{-1}, respectively, resulted in cell death. We have also established that *M. smegmatis* biofilms were non-responsive to isoniazid and rifampicin up to 1024 μg mL^{-1}. To determine whether cells being dispersed by **12g** and **12j** showed phenotypic variation, we first established *M. smegmatis* biofilms over 48 hours. The biofilms were washed to remove planktonic bacteria then treated with either compound alone at the EC_{50} concentration or compound with isoniazid (256 μg mL^{-1}) plus rifampicin (128 μg mL^{-1}) at its lowest synergistic EC_{50} concentration. After 24 hours of treatment, an aliquot of the culture was serially diluted and plated to determine the number of colony forming units (CFUs). The log reduction was calculated by taking the log of the CFUs from treatment with just INH and RIF minus the log of the CFUs from compound treatment with INH and RIF. Compound **12g** in combination with isoniazid (256 μg mL^{-1}) plus rifampicin (128 μg $mL^{-}1$) gave a log reduction of 2.94 ± 0.28. Combinatorial treatment with compound **12j** similarly resulted in a log reduction of 2.66 ± 0.37. The log reductions indicate that the dispersed *M. smegmatis* cells are drug susceptible.

To assess the potential of lead compounds as candidates for *in vivo* studies, both the toxicity profiles, and stability were investigated. To assess eukaryotic toxicity, the hemolytic activity of **1**, **12g**, and **12j** was first assessed using difibrinated sheep blood.[19] Compounds **12g** and **12j** showed some hemolytic activity, effecting 50% lysis (compared to 1% Triton-X) at concentrations of 151 μM and 159 μM respectively. These concentrations are well above (7-8 fold) the EC_{50} concentrations observed for synergy with anti-tuberculosis drugs. The parent compound **1** elicited less than 50% red blood cell lysis at the highest concentration tested (500 μM). While the amphipathic nature of these compounds likely contributes to the observed moderate hemolytic activty, and may also play a role in the mechanism of action with regards to anti-biofilm activity, it is unlikely that the anti-biofilm activity is derived entirely from this characteristic given the similarly structured compounds in this library that do not possess antibiotfilm activity. Determination of the mechnism of action is currently being investigated.

We next evaluated whole organism toxicity of all three compounds in *Galleria melonella*, a model organism that has been employed to predict treatment efficacy and outcome for mycobacterial infections.[20] All three compounds were dosed at 400 mg/Kg with >93% survival observed for each dose after six days.

The stability of the lead compounds was assessed in both microsomes and plasma. Microsome stability was assessed using microsomes from liver pooled from (CD-1) male mice, Phenacetin was used as positive control and was rapidly degraded with a half-life ($t_{1/2}$) of 35.4 minutes. The half-life for compound **1** was calculated to be 66.0 min, while the trifluoromethyl derivatives **12g** and **12j** exhibited similar half-lives of 58.7 min. and 61.3 min respectively. The stability of the lead compounds in mouse plasma was next determined. Verapamil was used as a negative control and showed prolonged stability in plasma with a half-life >480 minutes. Propanolol was used as a positive control and was rapidly degraded with a half-life of 20.6 minutes. The parent compound **1** exhibited a $t_{1/2}$ of 230 min. in mouse plasma, while the trifluoromethyl compounds **12g** and **12j** showed improved stability in plasma compared to compound **1**, with half-lives of 266 min and 288 min respectively. The toxicity and stability profiles of the lead compounds indicate their suitability as viable leads for an *in vivo* proof-of-concept assessment of this therapeutic approach.

Conclusions

In summary, using the previously identified compound **1** as a lead, SAR studies identified trifluoromethyl derivatives **12g** and **12j** with increased activity, inhibiting the formation of *M. smegmatis* biofilms below 10 μM. The compounds exhibited synergistic biofilm dispersion activity with the frontline TB antibiotics isoniazid and rifampicin, with 3-4 fold reductions in EC_{50} value. The compounds were non-toxic against *G. mellonella*, showed plasma stability beyond 4 hours. Current efforts are focused on further tuning this class of 2-AI molecule to improve upon its anti-biofilm activity, stability and toxicity profile, and examining its activity against *M. tuberculosis.*

Experimental Section

General Chemistry Experimental All reagents used for chemical synthesis were purchased from commercially available sources and used without further purification. Chromatography was performed using 60 Å mesh standard grade silica gel from Sorbtech. NMR solvents were obtained from Cambridge Isotope Labs and used as is. ^{1}H NMR (300 MHz or 400 MHz) and ^{13}C NMR (75 MHz or 100 MHz) spectra were recorded at 25 °C on Varian Mercury spectrometers. Chemical Shifts (δ) are given in ppm relative to tetramethylsilane or respective NMR solvent; coupling constants (*J*) are in hertz (Hz). Abbreviations used are s = singlet, bs = broad singlet, d = doublet, dd = doublet of doublets, t =

triplet, dt = doublet of triplets, bt = broad triplet, qt = quartet, m = multiplet, bm = broad multiplet and br = broad. High and low resolution mass spectra were obtained at the NCSU Department of Chemistry Mass Spectroscopy Facility. Infrared Spectra were obtained on an FT/IR-4100 spectrophotometer (vmax in cm^{-1}). UV absorbance was recorded on a Genesys 10 scanning UV/visible spectrophotometer (λmax in nm). The purities of the tested compounds were all verified to be >95% by LC-MS analysis on a Shimadzu LC-MS 2020 with Kinetex, 2.6 mm, C18 50 x 2.10 mm.

Compounds **9f, 9c, 9d, 9q, 9e, 9r, 12d, 12e, 12f** were previously synthesized, and characterized.[16] Compounds were tested without further purification from 100 mM stock solutions in biological grade DMSO. Compounds **1**, and **12a-f** were synthesized as previously reported, ^{13}C NMR, and LRMS (ESI) aligned with previously reported spectral and MS data.[16]

General procedure for α-bromo ketone synthesis:10 mmol of the carboxylic acid was dissolved in dichloromethane and allowed to stir under an inert atmosphere. Three drops of DMF were added and the reaction was cooled to 0 °C. Oxalyl chloride (3 equivalents) was added dropwise and left to stir for one hour. In a diazomethane kit was added a stir bar, KOH (9 equivalents), 25 mL of ethanol, 25 mL of water was added to the top of the diazomethane apparatus and was heated to 65 °C and stirred. To a diazomethane addition funnel was added diazald (3 equivalents) and diethyl ether (100 mL). The diethyl ether/diazald mixture was added dropwise to the KOH/water/ethanol mixture so that the diazomethane was generated and distilled over to the collection flask that was cooled to 0 °C. Once the diazomethane was collected, the carboxylic acid, oxalyl chloride reaction had reacted for 1 hour and the mixture was concentrated in vacuo, dissolved in 5 mL of dichloromethane and added slowly to the flask containing the diazomethane while being cooled at 0 °C. The reaction mixture was allowed to stir for 1 hour at 0 °C, followed by addition of concentrated hydrobromic acid (10 equivalents) and allowed to stir for 20 minutes. Then 100 mL of saturated aqueous sodium bicarbonate was added to the reaction mixture and allowed to stir for 30 minutes. The resulting mixture was extracted with ethyl acetate, washed twice with brine, and concentrated in vacuo and then purified via column chromatography with 10% ethyl acetate/hexanes solution providing the α-bromo keto product**.**

General procedure for Boc-guanidine cyclization followed by Boc-deprotection: The α-bromo ketone (5 mmol) was dissolved in 10 mL of DMF. Boc guanidine (3 equivalents) was then added to the reaction mixture it was allowed to stir for 48 hours. Water was then added to the reaction mixture and it was placed in a seperatory funnel and washed twice with water and twice with brine, and concentrated in vacuo. The crude product was then dissolved in a 1:4 mixture of trifluoroacetic acid/dichloromethane and allowed to stir for 2 hours, then washed with saturated sodium bicarbonate twice, concentrated in vacuo and purified via column chromatography (5% methanol saturated ammonia/95% dichloromethane) to provide the 2-aminoimidazole product. The sample was then dissolved in dilute HCl/methanol (5 drops HCl/ 10 mL methanol) and then concentrated in vacuo to provide the HCl salt.

2-Bromo-1-(*p*-tolyl)ethan-1-one 8g was synthesized as described in the general procedure for α-bromo ketone synthesis. Following purification via flash column chromatography the product was obtained as a white solid (73 % yield). ^1H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, *J* = 8.2 Hz, 1H), 7.31 – 7.24 (m, 1H), 4.42 (d, *J* = 0.6 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.0, 145.1, 131.6, 129.7, 129.6, 129.1, 128.7, 46.0, 31.1, 29.8, 21.8. IR vmax (cm^{-1}) 3250, 1976, 1608 1514; UV (λmax nm) 280; HRMS (ESI) calcd for C_9H_9BrO (M+) 214.0740, found 214.0738.

2-Bromo-1-(4-ethylphenyl)ethan-1-one 8h was synthesized as described in the general procedure for α-bromo ketone synthesis. Following purification via flash column chromatography the product was obtained as a clear oil (64 % yield). ^1H NMR (400 MHz, cdcl3) δ 8.08 – 7.79 (m, 2H), 7.31 (d, *J* = 7.9 Hz, 2H), 4.44 (d, *J* = 0.9 Hz, 1H), 2.72 (q, *J* = 7.6 Hz, 2H), 1.26 (td, *J* = 7.6, 2.2 Hz, 4H). 13C NMR (101 MHz, cdcl3) δ 191.1, 151.3, 131.8, 129.3, 128.9, 128.5, 128.5, 46.1, 31.1, 29.8, 29.1, 15.2. IR vmax (cm^{-1}) 3087, 2065, 1524, 1510, 1309; UV (λmax nm) 282; HRMS (ESI) calcd for $C_{10}H_{11}BrO$ (M+) 228.1010, found 228.1029.

2-Bromo-1-(4-propylphenyl)ethan-1-one 8i was synthesized as described in the general procedure for α-bromo ketone synthesis. Following purification via flash column chromatography the product was obtained as a clear oil (75 % yield) ^1H NMR (400 MHz, $cdcl_3$) δ 8.13 – 7.72 (m, 2H), 7.50 – 7.23 (m, 2H), 4.44 (s, 2H), 2.65 (dd, *J* = 8.5, 6.8 Hz, 2H), 1.86 – 1.47 (m, 2H), 0.95 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $cdcl_3$) δ 191.1, 149.8, 131.8, 129.2, 129.1, 129.1, 128.8, 46.1, 38.2, 31.1, 24.3, 13.9. IR vmax (cm^{-1}) 3208, 1927, 1604, 1590; UV (λmax nm) 274; HRMS (ESI) calcd for $C_{11}H_{13}BrO$ (M+) 242.1280, found 242.1283.

2-Bromo-1-(4-methoxyphenyl)ethan-1-one 8j was synthesized as described in the general procedure for α-bromo ketone synthesis. Following purification via flash column chromatography the product was obtained as a brown oil (55 % yield) ^1H NMR (400 MHz, $cdcl_3$) δ 8.12 – 7.80 (m, 2H), 6.95 (dd, *J* = 9.1, 0.7 Hz, 2H), 4.40 (d, *J* = 0.7 Hz, 2H), 3.88 (d, *J* = 0.7 Hz, 3H). ^{13}C NMR (101 MHz, $cdcl_3$) δ 190.1, 166.5, 164.3, 131.5, 131.1, 127.1, 114.2, 55.7, 30.8. IR vmax (cm^{-1}) 3059, 1804, 1467; UV (λmax nm) 286; HRMS (ESI) calcd for C_9H_9BrO2 (M+) 230.0754, found 230.0753.

2-Bromo-1-(4-ethoxyphenyl)ethan-1-one 8k was synthesized as described in the general procedure for α-bromo ketone synthesis. Following purification via flash column chromatography the product was obtained as a brown oil (55 % yield) ^1H NMR (400 MHz, $cdcl_3$) δ 7.92 (d, *J* = 8.9 Hz, 2H), 6.91 (d, *J* = 8.8 Hz, 2H), 4.38 (s, 2H), 4.21 – 3.98 (m, 2H), 1.42 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, $cdcl_3$) δ 190.0, 166.5, 163.7, 131.5, 131.0, 126.8, 114.6, 64.0, 45.9, 31.0, 29.8, 14.8. IR vmax (cm^{-1}) 2945, 2807, 1623, 1580; UV (λmax nm) 270; HRMS (ESI) calcd for $C_{10}H_{11}BrO_2$ (M+) 244.2793, found 244.2790.

2-Bromo-1-(4-propoxyphenyl)ethan-1-one 8l was synthesized as described in the general procedure for α-bromo ketone synthesis. Following purification via flash column chromatography the product was obtained as a clear oil (72 % yield). ^1H NMR (400 MHz, $cdcl_3$) δ 8.06 – 7.80 (m, 2H), 6.94 (d, *J* = 9.1 Hz, 2H), 4.39 (s, 2H), 3.99 (t, *J* = 6.5 Hz, 2H), 1.83 (dt, *J* = 7.5, 6.6 Hz, 3H), 1.04 (t, *J* = 7.4 Hz, 4H). ^{13}C NMR (101 MHz, $cdcl_3$) δ 190.0, 189.7, 163.9, 163.9, 131.4, 131.0, 127.0, 126.7, 114.6, 114.6, 69.9, 45.8, 30.9, 22.5, 10.5. IR vmax (cm^{-1}) 3280, 2986, 1542; UV (λmax nm) 292; HRMS (ESI) calcd for $C_{11}H_{12}BrO_2$ (M+) 258.0078, found 258.0080.

2-Bromo-1-(4-butoxyphenyl)ethan-1-one 8m was synthesized as described in the general procedure for α-bromo ketone synthesis. Following purification via flash column chromatography the product was obtained as a pale yellow oil (56% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 7.94 (dt, *J* = 9.0, 1.1 Hz, 2H), 6.93 (dt, *J* = 7.8, 0.9 Hz, 2H), 4.38 (d, *J* = 0.8 Hz, 2H), 4.03 (t, *J* = 6.5 Hz, 2H), 1.90 – 1.67 (m, 2H), 1.64 – 1.39 (m, 2H), 0.98 (td, *J* = 7.4, 0.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.0, 163.9, 163.9, 131.4, 131.0, 126.8, 114.6, 114.6, 68.2, 45.8, 31.2, 30.8, 19.3, 13.9. IR vmax (cm^{-1}) 3168, 1645; UV (λmax nm) 282; HRMS (ESI) calcd for $C_{12}H_{14}BrO_2$ (M+) 272.0236, found 272.0234.

2-Bromo-1-(4-(pentyloxy)phenyl)ethan-1-one 8n was synthesized as described in the general procedure for α-bromo ketone synthesis. Following purification via flash column chromatography the product was obtained as a light brown solid (76% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 7.94 (dd, *J* = 10.5, 9.0 Hz, 2H), 6.94 (dd, *J* = 9.0, 1.2 Hz, 2H), 4.40 (s, 2H), 4.02 (td, *J* = 6.5, 0.9 Hz, 3H), 1.81 (td, *J* = 6.6, 1.6 Hz, 3H), 1.57 – 1.26 (m, 4H), 0.93 (t, *J* = 7.1 Hz, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 189.9, 189.6, 163.8, 163.8, 131.3, 130.9, 126.9, 126.7, 114.5, 114.5, 68.4, 45.6, 30.7, 28.7, 28.1, 22.4, 14.0. IR vmax (cm^{-1}) 2930, 1853, 1632,

1309; UV (λmax nm) 270; HRMS (ESI) calcd for $C_{13}H_{15}BrO_2$ (M+) 286.0392, found 286.0395

2-Bromo-1-(4-(2-methoxyethoxy)phenyl)ethan-1-one 8o was synthesized as described in the general procedure for α-bromo ketone synthesis. Following purification via flash column chromatography the product was obtained as white solid (80% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, *J* = 8.9 Hz, 2H), 6.97 (d, *J* = 8.9 Hz, 2H), 4.39 (s, 2H), 4.25 – 4.13 (m, 2H), 3.83 – 3.68 (m, 2H), 3.44 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.0, 163.5, 163.5, 131.4, 131.0, 127.5, 127.2, 114.8, 114.7, 70.8, 67.7, 59.4, 45.8, 30.9. IR vmax (cm^{-1}) 3143, 2954, 13,45, 1288; UV (λmax nm) 280; HRMS (ESI) calcd for $C_{11}H_{13}BrO_3$ (M+) 274.0031, found 274.0026.

2-Bromo-1-(4-(2-ethoxyethoxy)phenyl)ethan-1-one 8p was synthesized as described in the general procedure for α-bromo ketone synthesis. Following purification via flash column chromatography the product was obtained as a white solid (78% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, *J* = 8.9 Hz, 2H), 6.96 (dd, *J* = 9.0, 1.4 Hz, 2H), 4.38 (s, 2H), 4.25 – 4.05 (m, 2H), 3.93 – 3.73 (m, 2H), 3.58 (d, *J* = 7.0 Hz, 2H), 1.22 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.0, 189.7, 163.6, 163.5, 131.4, 131.0, 127.4, 127.1, 114.8, 114.8, 68.7, 67.9, 67.0, 45.8, 30.9, 15.2. IR vmax (cm^{-1}) 3095, 1873, 1652, 1273; UV (λmax nm) 286; HRMS (ESI) calcd for $C_{12}H_{15}BrO_3$ (M+) 288.0184, found 288.0185.

4-(*p*-Tolyl)-1H-imidazol-2-amine hydrochloride 9g was synthesized as described in the general procedure for Boc-guanidine cyclization followed by Boc-deprotection. Following purification via flash column chromatography the product was obtained as a light red solid (0.83 g, 52%) ^{1}H NMR (400 MHz, CD_3OD) δ 7.42 (d, *J* = 8.2 Hz, 2H), 7.11 (d, *J* = 8.1 Hz, 3H), 6.82 (s, 1H), 2.30 (s, 3H) ppm; ^{13}C NMR (101 MHz, CD_3OD) δ 136.8, 130.2, 124.9, 21.2 ppm; IR vmax (cm^{-1}) 3323, 2123, 1578; UV (λmax nm) 286; HRMS (ESI) calcd for $C_{10}H_{11}N_3$ (M+) 173.2187, found 173.2186.

4-(4-Ethylphenyl)-1H-imidazol-2-amine hydrochloride 9h was synthesized as described in the general procedure for Boc-guanidine cyclization followed by Boc-deprotection. Following purification via flash column chromatography the product was obtained as a brown solid (45%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.46 (d, *J* = 8.2 Hz, 2H), 7.28 – 7.06 (m, 2H), 2.60 (q, *J* = 7.6 Hz, 2H), 1.18 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 152.6, 148.9, 147.2, 146.0, 129.5, 128.9, 126.1, 125.5, 109.1, 15.9, 15.5. IR vmax (cm^{-1}) 3086, 1423, 1367, 1232; UV (λmax nm) 250; HRMS (ESI) calcd for $C_{11}H_{13}N_3$ (M+) 188.1132, found 188.1139.

4-(4-Propylphenyl)-1H-imidazol-2-amine hydrochloride 9i was synthesized as described in the general procedure for Boc-guanidine cyclization followed by Boc-deprotection. Following purification via flash column chromatography the product was obtained as a light solid (52%). ^{1}H NMR (300 MHz, CD_3OD) δ 7.48 – 7.39 (m, 2H), 7.10 (d, *J* = 8.2 Hz, 1H), 6.82 (s, 1H), 2.53 (t, *J* = 7.6 Hz, 2H), 1.61 (h, *J* = 7.3 Hz, 2H), 0.92 (t, *J* = 7.3 Hz, 2H). ^{13}C NMR (75 MHz, CD_3OD) δ 151.6, 141.5, 134.5, 131.9, 129.6, 129.5, 124.8, 112.4, 38.7, 25.7, 14.1. IR vmax (cm^{-1}) 2845, 1723, 1532, 1190; UV (λmax nm) 256; HRMS (ESI) calcd for $C_{12}H_{15}N_3$ (M+) 202.1356, found 202.1350.

4-(4-Butylphenyl)-1H-imidazol-2-amine hydrochloride 9a was synthesized as described in the general procedure for Boc-guanidine cyclization followed by Boc-deprotection. Following purification via flash column chromatography the product was obtained as a brown oil (60%). ^{1}H NMR, ^{13}C NMR, and LRMS (ESI) aligned with previously reported spectral and MS data.[16]

4-(4-Pentylphenyl)-1H-imidazol-2-amine hydrochloride 9b was synthesized as described in the general procedure for Boc-guanidine cyclization followed by Boc-deprotection. Following purification via flash column chromatography the product was obtained as a light yellow solid (43%). ^{1}H NMR, ^{13}C NMR, and LRMS (ESI) aligned with previously reported spectral and MS data.[16]

4-(4-Methoxyphenyl)-1H-imidazol-2-amine hydrochloride 9j was synthesized as described in the general procedure for Boc-guanidine cyclization followed by Boc-deprotection. Following purification via flash column chromatography the product was obtained as a dark brown solid (35%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.51 – 7.41 (m, 2H), 6.94 – 6.83 (m, 2H), 6.76 (s, 1H), 3.78 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 126.3, 115.0, 111.2, 55.7. IR vmax (cm^{-1}) 3345, 2980; UV (λmax nm) 296; HRMS (ESI) calcd for $C_{10}H_{11}N_3O$ (M+) 190.0923, found 190.0921.

4-(4-Ethoxyphenyl)-1H-imidazol-2-amine hydrochloride 9k was synthesized as described in the general procedure for Boc-guanidine cyclization followed by Boc-deprotection. Following purification via flash column chromatography the product was obtained as a light red solid (49%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.48 (d, *J* = 8.9 Hz, 2H), 7.02 – 6.93 (m, 3H), 4.06 (q, *J* = 7.0 Hz, 2H), 1.39 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 160.7, 149.0, 129.1, 127.1, 121.2, 116.1, 108.4, 64.6, 15.1. IR vmax (cm^{-1}) 3433, 3006, 2807; UV (λmax nm) 248; HRMS (ESI) calcd for $C_{11}H_{13}N_3O$ (M+) 204.1088, found 204.1079

4-(4-Propoxyphenyl)-1H-imidazol-2-amine hydrochloride 9l was synthesized as described in the general procedure for Boc-guanidine cyclization followed by Boc-deprotection. Following purification via flash column chromatography the product was obtained as a light red solid (54%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.47 (d, *J* = 8.8 Hz, 2H), 7.02 – 6.90 (m, 3H), 3.93 (t, *J* = 6.4 Hz, 2H), 1.90 – 1.70 (m, 2H), 1.03 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 160.9, 148.9, 129.0, 127.1, 121.2, 116.1, 108.3, 70.7, 23.6, 10.8. IR vmax (cm^{-1}) 3392, 2854, 1673, 1345; UV (λmax nm) 280; HRMS (ESI) calcd for $C_{12}H_{15}N_3O$ (M+) 218.1276, found 218.1268.

4-(4-Butoxyphenyl)-1H-imidazol-2-amine hydrochloride 9m was synthesized as described in the general procedure for Boc-guanidine cyclization followed by Boc-deprotection. Following purification via flash column chromatography the product was obtained as a light red solid (68%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.48 (d, *J* = 8.9 Hz, 2H), 7.03 – 6.92 (m, 3H), 4.00 (d, *J* = 6.4 Hz, 2H), 1.75 (m, 2H), 1.58 – 1.45 (m, 2H), 0.98 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 160.9, 148.9, 129.0, 127.1, 121.2, 116.1, 108.3, 68.8, 32.4, 20.3, 14.2. IR vmax (cm^{-1}) 3124, 3076, 2974, 1569; UV (λmax nm) 254; HRMS (ESI) calcd for $C_{13}H_{17}N_3O$ (M+) 232.1444, found 232.1442.

4-(4-(Pentyloxy)phenyl)-1H-imidazol-2-amine hydrochloride 9n was synthesized as described in the general procedure for Boc-guanidine cyclization followed by Boc-deprotection. Following purification via flash column chromatography the product was obtained as a light brown sticky oil (57%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.45 (d, *J* = 8.8 Hz, 2H), 6.85 (d, *J* = 8.8 Hz, 2H), 6.74 (s, 1H), 3.94 (t, *J* = 6.4 Hz, 2H), 1.76 (dd, *J* = 8.0, 6.3 Hz, 2H), 1.52 – 1.34 (m, 4H), 0.95 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 159.1, 151.6, 127.4, 126.2, 115.6, 69.0, 30.2, 29.4, 23.6, 14.4. IR vmax (cm^{-1}) 3473, 3193, 1543, 1123; UV (λmax nm) 270; HRMS (ESI) calcd for $C_{14}H_{19}N_3O$ (M+) 246.1573, found 246.1569.

4-(4-(2-Methoxyethoxy)phenyl)-1H-imidazol-2-amine hydrochloride 9o was synthesized as described in the general procedure for Boc-guanidine cyclization followed by Boc-deprotection. Following purification via flash column chromatography the product was obtained as a white solid (64%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.53 – 7.46 (m, 2H), 7.05 – 6.96 (m, 3H), 4.18 – 4.09 (m, 2H), 3.78 – 3.71 (m, 2H), 3.42 (s, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 160.5, 149.0, 128.9, 127.1, 121.6, 116.2, 108.5, 72.1, 68.5, 59.2. IR vmax (cm^{-1}) 3213, 2921, 1734; UV (λmax nm) 250; HRMS (ESI) calcd for $C_{12}H_{15}N_3O_2$ (M+) 234.1153, found 234.1152.

4-(4-(2-Ethoxyethoxy)phenyl)-1H-imidazol-2-amine hydrochloride 9p was synthesized as described in the general procedure for Boc-guanidine cyclization followed by Boc-deprotection. Following purification

via flash column chromatography the product was obtained as a white solid (54%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.49 (d, *J* = 8.8 Hz, 2H), 7.04 – 6.99 (m, 3H), 4.18 – 4.09 (m, 2H), 3.83 – 3.74 (m, 2H), 3.60 (q, *J* = 7.0 Hz, 2H), 1.22 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 160.6, 127.1, 121.6, 116.2, 108.5, 70.0, 68.7, 67.7, 15.4. IR vmax (cm^{-1}) 3419, 1734, 1245, 1167; UV (λmax nm) 264; HRMS (ESI) calcd for $C_{13}H_{17}N_3O_2$ (M+) 248.1365, found 248.1263.

***tert*-Butyl (1,1,1-trifluoro-3-(methoxy(methyl)amino)-3-oxopropan-2-yl)carbamate 10g** 1.50 g (6.16 mmol) of 2-((tert-butoxycarbonyl)amino)-3,3,3-trifluoropropanoic acid, a stir bar, and 12 mL of dichloromethane were added to a 100-mL round bottom flask and allowed to stir. To the mixture was added 2.36 g (12.33 mmol) of 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride and 1.89g (12.33 mmol) of hydroxybenzotriazole hydrate and 2.58 mL (18.51 mmol) of trimethylamine and the mixture was allowed to stir for 2 minutes. Following was added 0.90g (9.25 mmol) of N,O-dimethylhydroxylamine hydrochloride. The reaction was allowed to stir overnight and the solvent was then removed in vacuo. The mixture was dissolved in 20 mL of ethyl acetate and washed with saturated sodium bicarbonate twice and saturated brine twice. The organic layer was dried over magnesium sulfate and purified via silica gel column chromatography using 0 to 25% ethyl acetate in hexanes to give tert-butyl (1,1,1-trifluoro-3-(methoxy(methyl)amino)-3-oxopropan-2-yl)carbamate as a white solid (1.3 g, 76% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.75 – 5.43 (m, 1H), 3.75 (s, 3H), 3.23 (s, 3H), 1.42 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$ δ 154.6, 124.3, 81.0, 61.6, 51.1, 50.8, 32.1, 28.1, 28.1. IR vmax (cm^{-1}) 1823, 1139, 1032; melting point 84 – 88 °C; HRMS (ESI) calcd for $C_{10}H_{17}F_3N_2O_4$ (M+) 287.1164, found 287.1160.

***tert*-Butyl (1,1,1-trifluoro-3-(4-hexylphenyl)-3-oxopropan-2-yl)carbamate 11g** To a flame dried 100-mL round bottom flask under a nitrogen atmosphere was added 1-bromo-4-hexylbenzene (0.14 mL, 0.70 mmol), a stir bar and 8 mL of dry tetrahydrofuran. The mixture was stirred at -78 °C and n-butyllitium (0.29 mL, 2.5 M solution in hexanes) was added dropwise over a two minute period. After another five minutes, tert-butyl (1,1,1-trifluoro-3-(methoxy(methyl)amino)-3-oxopropan-2-yl)carbamate (0.1 g, 0.35 mmol) was dissolved in 2 mL of dry tetrahydrofuran and added dropwise to the reaction mixture over a two minutes. After a three minute period, the reaction was removed from the -78 °C bath and allowed to warm to room temperature over an hour period. Then, the reaction was placed at 0 °C and saturated ammonium chloride was added dropwise. After stirring for ten minutes, the mixture was diluted with 20 mL of ethyl acetate and washed twice with water and twice with brine. The organic layer was dried over sodium sulfate and then concentrated in vacuo. The resulting residue was then purified via silica gel column chromatography 0-25% ethyl acetate in hexanes to give *tert*-butyl (1,1,1-trifluoro-3-(4-hexylphenyl)-3-oxopropan-2-yl)carbamate as a light yellow solid (0.80g, 59% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, *J* = 7.9 Hz, 2H), 7.27 (d, *J* = 8.1 Hz, 2H), 5.32 (d, *J* = 8.7 Hz, 2H), 2.65 (t, *J* = 7.8 Hz, 2H), 1.61 (d, *J* = 8.5 Hz, 2H), 1.43 (s, 9H), 1.30 (tt, *J* = 4.5, 2.6 Hz, 4H), 1.06 (m, 2H), 0.88 (t, *J* = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.6, 155.7, 149.4, 128.8, 79.5, 53.4, 43.0, 36.0, 31.6, 31.0, 28.9, 28.3, 25.0, 23.4, 22.5, 21.8, 14.0. IR vmax (cm^{-1}) 3304, 1823, 1256; UV (λmax nm) 204; HRMS (ESI) calcd for $C_{20}H_{28}F_3NO_3$ (M+) 388.2042, found 388.2039.

4-(4-Hexylphenyl)-5-(trifluoromethyl)-1H-imidazol-2-amine 12g To a 6 dram vial open to air was added tert-butyl (1,1,1-trifluoro-3-(4-hexylphenyl)-3-oxopropan-2-yl)carbamate (0.24g, 0.62 mmol) in 3.5 mL of dichloromethane. The mixture was placed in a 0 °C ice bath and 1.5 mL of trifluoroacetic acid was added dropwise. After five minutes, the reaction was removed from the ice bath and allowed to stir at room temperature over two hours and monitored by TLC. Then, the solvent was removed in vacuo and the residue was dissolved in 10 mL of ethyl acetate and washed three times with saturated sodium bicarbonate. The ethyl acetate layer was then dried over sodium sulfate and concentrated in vacuo in a 100 mL round bottom flask. The sticky residue was placed on a high vacuum pump for one hour. Then, to the round bottom was added 5 mL of ethanol and 5 mL of water and cyanamide (0.26 g, 6.19 mmol). The pH of the reaction was adjusted to pH 4.3 using 1N HCl. The reaction was heated at 95 °C for 3 hours. The reaction was allowed to cool to room temperature and poured into ethyl acetate and washed two times with sodium bicarbonate and two times with brine. The ethyl acetate layer was dried over magnesium sulfate and then concentrated in vacuo. The residue was purified via silica gel column chromatography (0-5% methanol saturated ammonia in dicholomethane). The purified product was then dissolved in five drops of concentrated HCl in methanol to give 4-(4-hexylphenyl)-5-(trifluoromethyl)-1H-imidazol-2-amine as a pale yellow solid (0.10 g, 53% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 7.45 (d, *J* = 8.2 Hz, 2H), 7.37 – 7.33 (m, 2H), 2.69 (t, *J* = 7.7 Hz, 2H), 1.68 – 1.60 (m, 2H), 1.32 (d, *J* = 4.3 Hz, 6H), 0.94 – 0.87 (m, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 154.4, 149.3, 147.2, 130.1, 129.8 (q *J* = 1.6 Hz), 124.0, 36.7, 32.8, 32.4, 30.0, 23.6, 14.4. IR vmax (cm^{-1}) 3287, 2987, 1653, 1522, 1197; UV (λmax nm) 258; HRMS (ESI) calcd for $C_{16}H_{22}F_3N_3$ (M+) 312.1634, found 312.1630.

***tert*-Butyl (1,1,1-trifluoro-3-oxo-3-(4-propoxyphenyl)propan-2-yl)carbamate 11h** was synthesized as described in the general procedure for **11g**. Following purification via flash column chromatography the product was obtained as a light yellow solid in 47% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.7 Hz, 2H), 6.96 (d, *J* = 8.9 Hz, 2H), 5.92 – 5.79 (m, 1H), 4.00 (t, *J* = 6.5 Hz, 2H), 1.83 (h, *J* = 7.0 Hz, 2H), 1.45 (s, 9H), 1.04 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 189.0, 166.5, 164.7, 154.8, 141.1, 132.0, 127.1, 124.5, 121.7, 114.7, 81.2, 70.0, 55.2, 54.9, 54.6, 54.3, 28.3, 22.5, 10.5. IR vmax (cm^{-1}) 3520, 3147, 1429, 1278; UV (λmax nm) 228; HRMS (ESI) calcd for $C_{17}H_{22}F_3NO_4$ (M+) 362.1548, found 362.1550.

***tert*-Butyl (3-(4-butoxyphenyl)-1,1,1-trifluoro-3-oxopropan-2-yl)carbamate 11i** was synthesized as described in the general procedure for **11g**. Following purification via flash column chromatography the product was obtained as a light yellow solid in 57% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (dd, *J* = 8.9, 2.8 Hz, 2H), 6.94 (dd, *J* = 6.8, 2.4 Hz, 2H), 6.01 – 5.81 (m, 1H), 4.03 (q, *J* = 6.7 Hz, 2H), 1.78 (q, *J* = 6.9 Hz, 2H), 1.44 (s, 9H), 0.96 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 189.0, 164.7, 154.8, 132.0, 127.1, 114.7, 81.2, 68.3, 55.2, 54.6, 54.3, 31.1, 28.2, 19.2, 13.8. IR vmax (cm^{-1}) 3377, 2743, 1604; UV (λmax nm) 234; HRMS (ESI) calcd for $C_{18}H_{24}F_3NO_4$ (M+) 376.1675, found 376.1676.

***tert*-Butyl (1,1,1-trifluoro-3-oxo-3-(4-(pentyloxy)phenyl)propan-2-yl)carbamate 11j** was synthesized as described in the general procedure for **11g**. Following purification via flash column chromatography the product was obtained as a white solid in 53% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, *J* = 9.1 Hz, 2H), 6.96 (d, *J* = 8.9 Hz, 2H), 5.90 – 5.79 (m, 1H), 4.04 (t, *J* = 6.6 Hz, 2H), 1.80 (dt, *J* = 8.0, 6.5 Hz, 2H), 1.46 (s, 11H), 0.93 (t, *J* = 7.1 Hz, 3H). 13C NMR (101 MHz, $CDCl_3$) δ 166.5, 164.8, 154.9, 132.1, 127.2, 114.8, 81.3, 68.7, 55.0, 54.7, 28.3, 14.1. IR vmax (cm^{-1}) 3428, 3046, 1420, 1265; UV (λmax nm) 260; HRMS (ESI) calcd for $C_{19}H_{26}F_3NO_4$ (M+) 390.1847, found 390.1849.

4-(4-Propoxyphenyl)-5-(trifluoromethyl)-1H-imidazol-2-amine 12h was synthesized as described in the general procedure for **12g**. Following purification via flash column chromatography the product was obtained as a white solid in 41% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18 (d, *J* = 7.3 Hz, 2H), 7.13 (d, *J* = 7.3 Hz, 2H), 6.41 (q, *J* = 7.3 Hz, 1H), 4.09 (t, 2H), 1.83 – 1.68 (m, 2H), 0.98 (t, *J* = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.0, 161.6, 132.8, 126.8, 115.3, 70.2, 22.3, 10.7. IR vmax (cm^{-1}) 3066, 2875, 1567, 1123; UV (λmax nm) 246; HRMS (ESI) calcd for $C_{13}H_{14}F_3N_3O$ (M+) 286.1138, found 286.1139.

4-(4-Butoxyphenyl)-5-(trifluoromethyl)-1H-imidazol-2-amine 12i was synthesized as described in the general procedure for **12g**. Following purification via flash column chromatography the product was obtained as a white solid in 35% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 8.12 (d, *J* =

8.9 Hz, 2H), 7.11 (d, *J* = 8.9 Hz, 2H), 6.24 (q, *J* = 7.4 Hz, 1H), 4.13 (t, *J* = 6.4 Hz, 2H), 1.87 – 1.75 (m, 2H), 1.59 – 1.46 (m, 2H), 1.00 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 166.9, 133.5, 127.5, 124.6, 121.8, 116.1, 69.5, 56.3, 56.0, 32.2, 20.2, 14.1. IR vmax (cm^{-1}) 3366, 3048, 1765, 1527; UV (λmax nm) 228; HRMS (ESI) calcd for $C_{14}H_{16}F_3N_3O$ (M+) 300.1264, found 300.1258.

4-(4-(Pentyloxy)phenyl)-5-(trifluoromethyl)-1H-imidazol-2-amine 12j was synthesized as described in the general procedure for **12g**. Following purification via flash column chromatography the product was obtained as a pale yellow solid in 57% yield. ^{1}H NMR (400 MHz, CD_3OD) δ 7.45 (d, *J* = 8.7 Hz, 2H), 7.04 (d, *J* = 8.8 Hz, 2H), 4.03 (t, *J* = 6.4 Hz, 2H), 1.87 – 1.72 (m, 2H), 1.54 – 1.34 (m, 4H), 0.95 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.4, 131.3, 131.3, 131.3, 118.5, 115.9, 69.2, 30.0, 29.3, 23.5, 14.4. IR vmax (cm^{-1}) 3143, 2854, 1697; UV (λmax nm) 264; HRMS (ESI) calcd for $C_{15}H_{18}F_3N_3O$ (M+) 314.1455, found 314.1457.

Biological Methods

Bacterial strains, media, and antibiotics The *M. smegmatis* strain (ATCC 700084, mc^{2}155) was obtained from ATCC (Manassas, VA). Stock cultures were stored in glycerol stock media (50% v/v glycerol and 7H9, ADC, Tween 80) and maintained at -80 °C. Prior to use, colonies were grown on 7H10 agar (OADC, glyercol) for 2 days, and single colonies were subcultured in 7H9 (ADC, Tween 80) for 2 days. 7H10, 7H9, OADC, and ADC were purchased from BD Diagnostics. Glycerol and Tween 80 were purchased from Sigma Aldrich. Ampicillin, carbenicillin, cefaclor, cefadroxil, cefmetazole, cefotaxime, cefoxitin, ceftazidime, cephalothin, methicillin, naficillin, and penicillin were purchased from Sigma Aldrich. Oxacillin was purchased from TCI America. All assays were run in duplicate and repeated at least two separate times. All compounds were dissolved as their HCl salts in molecular biology grade DMSO as 100 mM stock solutions and stored at -20 °C.

Broth microdilution method for determination of minimum inhibitory concentrations *M. smegmatis* was grown in 7H9 (ADC, 0.5% Tween 80) for 48 h, and this culture was used to inoculate fresh 7H9 (5×10^5 CFU mL^{-1}, OD_{600} = 0.006). Aliquots (1 mL) were placed in culture tubes, and compound was added from 100 mM stock solutions in DMSO, such that the compound concentration equaled the highest concentration tested. Antibiotics, from a water stock, were added at the highest concentration tested to 1 mL aliquots of cultures. Inoculated media not treated with compound served as the control. Samples were then aliquoted (200 µL) into the first wells of a 96-well plate, with all remaining wells being filled with 100 µL of initial bacterial subculture. Row 1 wells were mixed 8 times, before 100 µL was transferred to row 2. Row 2 was then mixed 8 times, and 100 µL was transferred to row 3. This process was repeated to serially dilute the rest of the rows. One row with bacteria subculture served as the control. Plates were then covered with GLAD Press n' Seal and incubated under stationary conditions at 37 °C. After 48 h, 10 µL of alamarBlue was added to each well, and the plate was resealed and incubated under stationary conditions at 37 °C. After 6 h, the minimum inhibitory concentration (MIC) values were recorded as the lowest concentration of compound or antibiotic at which no visible growth of bacteria was observed.

General biofilm inhibition assay protocol for *M. smegmatis* Cultures were incubated for 48 hr and then subcultured to OD_{600} = 0.01 in Difco M9 minimal salts media. 100 µL of subculture containing the appropriate concentration of compound was added to all of the wells in columns 2–4 and 9–11 of 96- well PVC microtiter plate. Columns 1 and 12 were filled with 100 µL of sterile M9 minimal salts media to serve as controls. Plates were covered with Glad Press n' Seal and were incubated under stationary conditions at 37°C for 48 hr. After 48 hr, the media were discarded, and the plates were washed thoroughly with water. 110 µL of a 0.1% aqueous solution of crystal violet was added to every well, and the plates were left at ambient temperature for 30 min. After 30 min, the crystal violet was disposed, and the plates were washed thoroughly with water. 200 µL of 95% ethanol was added to each well, and the plates were left at ambient temperature for 10 min. 125 µL of the ethanol solution was transferred to a fresh polystyrene microtiter plate, and the plate was quantified by measuring the OD_{540}. The percent inhibition was calculated by comparing the OD540 of the treated wells with the OD_{540} of the untreated wells. The first and last column, which had only sterile media, were used as blanks and those values were subtracted from the OD_{540} obtained in the other columns.

General biofilm dispersion assay protocol for *M. smegmatis* Cultures were incubated for 48 hr and then subcultured to 0.01 in Difco M9 minimal salts media. 100 µL of the subculture was aliquoted into every well in columns 2–11 of a 96- well PVC microtiter plate. Columns 1 and 12 were left empty to serve as control wells. Plates were covered with Glad Press n' Seal and were incubated under stationary conditions at 37°C for 48 hr. After 48 hr, the media were discarded, and the plates were washed thoroughly with water. 100 µL of fresh media containing the appropriate concentration of compound was added to all of the wells in columns 2–4 and 9–11. 100 µL of sterile media was added to all of the wells in columns 1 and 12 and columns 5–8. Plates were covered with Glad Press n' Seal and were incubated under stationary conditions at 37°C for 24 hr. After 24 hr, media were dis-carded, and the plates were washed thoroughly with water. 110 µL of a 0.1% aqueous solution of crystal violet was added to every well, and the plates were left at ambient temperature for 30 min. After 30 min, the crystal violet was disposed, and the plates were washed thoroughly with water. 200 µL of 95% ethanol was added to each well, and the plates were left at ambient temperature for 10 min. 125 µL of the ethanol solution was transferred to a fresh polystyrene microtiter plate, and the plate was quantified by measuring the OD_{540}. The percent dispersion was calculated by comparing the OD_{540} of the treated wells with the OD_{540} of the untreated wells, which contained only media after biofilm growth. The first and last column, which had only sterile media, were used as blanks and those values were subtracted from the OD_{540} obtained in the other columns.

General synergy dispersion assay protocol for *M. smegmatis* Cultures were incubated for 48 hr and then subcultured to 0.01 in Difco M9 minimal salts media. 100 µL of the subculture was aliquoted into every well in columns 2–11 of a 96- well PVC microtiter plate. Columns 1 and 12 were left empty to serve as control wells. Plates were covered with Glad Press n' Seal and were incubated under stationary conditions at 37°C for 48 hr. After 48 hr, the media were discarded, and the plates were washed thoroughly with water. 100 µL of fresh media containing the appropriate concentration of compound and antibiotic was added to all of the wells in columns 2–4 and 9–11. 100 µL of sterile media was added to all of the wells in columns 1 and 12 and columns 5–8. Plates were covered with Glad Press n' Seal and were incubated under stationary conditions at 37°C for 24 hr. After 24 hr, media were discarded, and the plates were washed thoroughly with water. 110 µL of a 0.1% aqueous solution of crystal violet was added to every well, and the plates were left at ambient temperature for 30 min. After 30 min, the crystal violet was disposed, and the plates were washed thoroughly with water. 200 µL of 95% ethanol was added to each well, and the plates were left at ambient temperature for 10 min. 125 µL of the ethanol solution was transferred to a fresh polystyrene microtiter plate, and the plate was quantified by measuring the OD_{540}. The percent dispersion was calculated by comparing the OD540 of the treated wells with the OD_{540} of the untreated wells, which contained only media after biofilm growth. The first and last column, which had only sterile media, were used as blanks and those values were subtracted from the OD_{540} obtained in the other columns.

Colony counts for dispersion assay protocol for *M. smegmatis* Cultures were incubated for 48 hr and then subcultured to 0.01 in Difco M9 minimal salts media. 100 µL of the subculture was aliquoted into every well in columns 2–11 of a 96- well PVC microtiter plate. Columns 1 and 12 were left empty to serve as control wells. Plates were covered

with Glad Press n' Seal and were incubated under stationary conditions at 37°C for 48 hr. After 48 hr, a 50 µL aliquot of the media from selected wells was serially diluted 10-fold. Then, a 10 µL aliquot was removed from each aliquot and plated out on a 7H10 + OADC + 0.5% glycerol round petri dish followed by 48 hours of incubation at 37 °C under stationary conditions. Viable colonies were quantified through the track-dilution method. Controls were employed in which only compound was added and sterile media was added. The number of colonies counted from the treatment with sterile media was subtracted from the counts of the other test samples.

Hemolysis 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. The final cell suspension was then diluted 10-fold. Test compounds from DMSO stock solutions were added to aliquots of the 10-fold suspension dilution of blood. PBS was used as a negative control and a zero hemolysis marker. Triton X (a 1% sample) was used as a positive control serving as the 100% lysis marker. Samples were then placed in an incubator at 37 ˚C while being shaken at 200 rpm for one hour. After one hour, the samples were centrifuged for 10 min at 10,000 rpm. The resulting supernatant was diluted by a factor of 40 in distilled water. The absorbance of the supernatant at 540 nm was then measured with a UV spectrometer.

Microsome stability assay A stock solution of compound was added to a solution of 0.1M phosphate-buffer saline (PBS, pH = 7.4) containing 1mM NADPH to make a final concentration of 100 µM. This solution was incubated at 37^{O}C for 5 minutes at which time mouse microsomes (pooled CD1 males from Sigma M9441) was added at a final concentration of 1.0 mg/mL and vortexed gently. A positive control solution without the addition of microsomes was also included to monitor compound stability over the course of the experiment. An aliquot was removed and immediately quenched with the addition of 10x volume of acetonitrile (HPLC grade). The remaining solution was incubated at 37 ˚C while being shaken at 200 rpm. An aliquot was removed at 0, 15, 30, 60, 90, 120, 240, and 480 minutes time points and quenched with acetonitrile. After quenching, samples were kept on ice and centrifuged at 10,000 rpm for 5 minutes at 4^{O}C, and the supernatant was transferred to appropriate LC-MS vials for analysis. Samples were analyzed using reverse phase liquid chromatography coupled with low resolution tandem mass spectrometry (LC-MS/MS) in the laboratory of Dr. Melander at North Carolina State University Department of Chemistry. A Shimadzu Scientific Instruments LC system (comprising a solvent degasser, two LC-20A pumps and a SCL-20A system controller) was coupled to and introduced into the ESI source of an Expression L CMS Advion mass spectrometer (Advion, Ithica, NY). Separation was achieved using a Restek Ultra C18 reversed-phase column (3 *µ*m, 50 x 4.6 mm). For analysis 20µL of sample was injected. The method used a solvent flow of 0.5 ml/min. Initial gradient conditions (90% water:10% acetonitrile with 0.1% formic acid) were held for 2 minutes. From 2 to 5.5 minutes, the mobile phase composition increased linearly to 10% water:90% acetonitrile with 0.1% formic acid. From 5.5 to 8 minutes, the mobile phase composition was held at 10% water:90% acetonitrile with 0.1% formic acid. At 8.01 to 10 min, the column was re-equilibrated with 90% water:10% acetonitrile with 0.1% formic acid. HPLC data acquisition and analysis were performed using LabSolutions software. The settings for the Advion ESI/MS in dual ion mode are as follows: capillary temperature = 250 °C; capillary voltage = 180 V; ESI voltage = 3500 V; source gas temperature = 200 °C; pirani pressure = 2.73 e^{-3} mbar; hexapole bias = 8 V; extraction electrode = 9 V. Data was acquired using an acquisition method: mass range m/z = 100 to 800; scan time = 632 ms, scan speed (m/z/sec) = 1107. Analysis performed using Advion Mass Express and Advion Data Express. The percentage of compound remaining is calculated as the ratio of [(compound peak area of solution with microsomes)/(compound peak without microsomes)] x 100. The *in* vitro microsome half-life ($t_{1/2}$) was calculated using the expression $t_{1/2}$=0.693/*b*, where *b* is the slope found in the linear fit of the natural logarithm of the fraction remaining of the parent compound vs. incubation time.

Plasma stability assay The *in* vitro stability of the test compounds was studied in mouse plasma (Sigma 9275). The plasma was diluted to 80% with 0.05 M PBS (pH 7.4) at 37°C. The reactions were initiated by the addition of the test compounds to 1 mL of preheated plasma solution to yield a final concentration of 100 µM. A positive control solution without the addition of plasma was also included to monitor compound stability over the course of the experiment. The assays were incubated at 37°C and shaken at 200 rpm. Samples (50 µl) were taken at 0, 15, 30, 45, 60, 120, 240, 480 min and added to 200 µl acetonitrile in order to deproteinize the plasma. The samples were subjected to vortex mixing for 1 min and then centrifugation at 4°C for 15 min at 12,000 rpm. The clear supernatants were transferred to appropriate LC-MS vials for analysis. Samples were analyzed using reverse phase liquid chromatography coupled with low resolution tandem mass spectrometry (LC-MS/MS) in the laboratory of Dr. Melander at North Carolina State University Department of Chemistry. A Shimadzu Scientific Instruments LC system (comprising a solvent degasser, two LC-20A pumps and a SCL-20A system controller) was coupled to and introduced into the ESI source of an Expression L CMS Advion mass spectrometer (Advion, Ithica, NY). Separation was achieved using a Restek Ultra C18 reversed-phase column (3 *µ*m, 50 x 4.6 mm). For analysis 20µL of sample was injected. The method used a solvent flow of 0.5 ml/min. Initial gradient conditions (90% water:10% acetonitrile with 0.1% formic acid) were held for 2 minutes. From 2 to 5.5 minutes, the mobile phase composition increased linearly to 10% water:90% acetonitrile with 0.1% formic acid. From 5.5 to 8 minutes, the mobile phase composition was held at 10% water:90% acetonitrile with 0.1% formic acid. At 8.01 to 10 min, the column was re-equilibrated with 90% water:10% acetonitrile with 0.1% formic acid. HPLC data acquisition and analysis were performed using LabSolutions software. The settings for the Advion ESI/MS in dual ion mode are as follows: capillary temperature = 250 °C; capillary voltage = 180 V; ESI voltage = 3500 V; source gas temperature = 200 °C; pirani pressure = 2.73 e^{-3} mbar; hexapole bias = 8 V; extraction electrode = 9 V. Data was acquired using an acquisition method: mass range m/z = 100 to 800; scan time = 632 ms, scan speed (m/z/sec) = 1107. Analysis performed using Advion Mass Express and Advion Data Express. The percentage of compound remaining is calculated as the ratio of [(compound peak area of solution with plasma)/(compound peak without plasma)] x 100. The *in* vitro plasma half-life ($t_{1/2}$) was calculated using the expression $t_{1/2}$=0.693/*b*, where *b* is the slope found in the linear fit of the natural logarithm of the fraction remaining of the parent compound vs. incubation time.

***G. mellonella* toxicity assay** *G. mellonella larvae* (Speedy Worm, Alexandria, MN) were used within 10 days of shipment from the vendor. After reception of worms, larvae were kept in the dark at room temperature for at least 24 h before infection. Larvae weighing between 200 to 300 mg were used in the survival assay. Using a 10- µL glass syringe (Hamilton, Reno, NV) fitted with a 30 G needle (Exel International, St. Petersburg, Fl), a 5 µL solution of the desired compound and concentration were injected into the last left proleg. Injected worms were left at room temperature in the dark while being assessed at 24 h intervals over 5 days. Larvae were considered dead if they did not respond to physical stimuli. Experiment was repeated three times using 10 larvae per experimental group.

Colony count procedure at 48 hour time point for *M. smegmatis* Cultures were incubated for 48 hr and then subcultured to an OD_{600} of 0.01 in 7H9 + ADC + 0.5% Tween 80 media. The resulting bacterial suspension was aliquoted into 3.0 mL culture tubes. Test compound was then added to the media at predetermined concentrations. Controls were employed in which no compound was added to the cultures. Samples were then placed in a 37 °C incubator with shaking at 200 rpm for 48

hours. At this time point, 100 µL was taken from each culture tube and diluted serially in 7H9 media. Then 10 µL of was removed from each serial dilution and plated out on a 7H10 + OADC + glycerol round petri dish followed by 48 hr incubation at 37 °C and quantification.

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Keywords: Biofilm • 2-Aminoimidazole • Tuberculosis • Mycobacteria • Synergy

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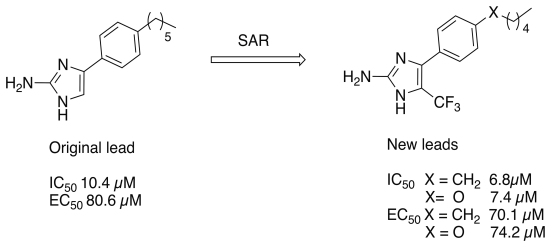

Compounds that can inhibit and disperse mycobacterial biofilms have the potential to serve alongside TB antibiotics to overcome drug tolerance and reduce treatment duration. Using *Mycobacterium smegmatis* as a surrogate organism, we have identified two new 2-aminoimidazole compounds that inhibit and disperse mycobacterial biofilms, and work synergistically with isoniazid and rifampicin to eradicate preformed *M. smegmatis* biofilms *in vitro.*