DOI: 10.1002/cmdc.201100316

Evaluation of 4,5-Disubstituted-2-Aminoimidazole–Triazole Conjugates for Antibiofilm/Antibiotic Resensitization Activity Against MRSA and *Acinetobacter baumannii*

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A library of 4,5-disubstituted-2-aminoimidazole–triazole conjugates (2-AITs) was synthesized, and the antibiofilm activity was investigated. This class of small molecules was found to inhibit biofilm formation by methicillin-resistant *Staphylococcus aureus* (MRSA) at low-micromolar concentrations; 4,5-disubstituted-2-AITs were also able to inhibit and disperse *Acinetobacter baumannii* biofilms. The activities of the lead compounds were compared against the naturally occurring biofilm dispersant *cis*-2-decenoic acid and were revealed to be more potent. The ability of selected compounds to resensitize MRSA to traditional antibiotics (resensitization activity) was also determined. Lead compounds were observed to resensitize MRSA to oxacillin by 2–4-fold.

Introduction

Antibiotic-resistant pathogens have become an emerging crisis during the last decade.[1] Bacteria have demonstrated the ability to develop resistance to virtually every antibiotic introduced by the medical community.[2] Among the many defense mechanisms that bacteria have developed, biofilm formation plays an important role in establishing protection against antibacterial agents.[3] Biofilms are defined as sessile communities of microorganisms that exist as differentiated entities embedded in an extracellular matrix of biomolecules (typically exopolysaccharides).[4] Bacteria within a biofilm display up to 1000-fold increased resistance against traditional antibiotics relative to their planktonic counterparts.[5] The US National Institutes of Health (NIH) have estimated that three of every four cases of bacterial infection are mediated by biofilms. Biofilms are the preferred living mode for bacteria, as ~80% of the world's microbial mass exists as a biofilm.[6]

There are a number of naturally occurring molecular scaffolds that are capable of inhibiting biofilm formation[7] including brominated furanone **1**, *cis*-2-decenoic acid **2**, and oroidin **3** (Figure 1). Our research group has focused on studying the ability of oroidin derivatives to modulate biofilm formation and, through a focused synthesis and screening effort, we have developed a number of oroidin analogues that inhibit and disperse bacterial biofilms via non-microbicidal mechanisms (Figure 2).[8–16] Among these molecules, dihydrosventrin **4** (DHS)[8] and reverse amide **5** (RA-11)[9] were found to possess outstanding antibiofilm activity against *Pseudomonas aeruginosa* (PA), while the 2-aminoimidazole–triazole conjugate (2-AIT) **6**[10] was identified as the first small molecule to inhibit and dis-

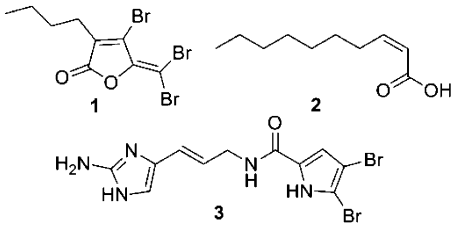

Figure 1. Antibiofilm natural products.

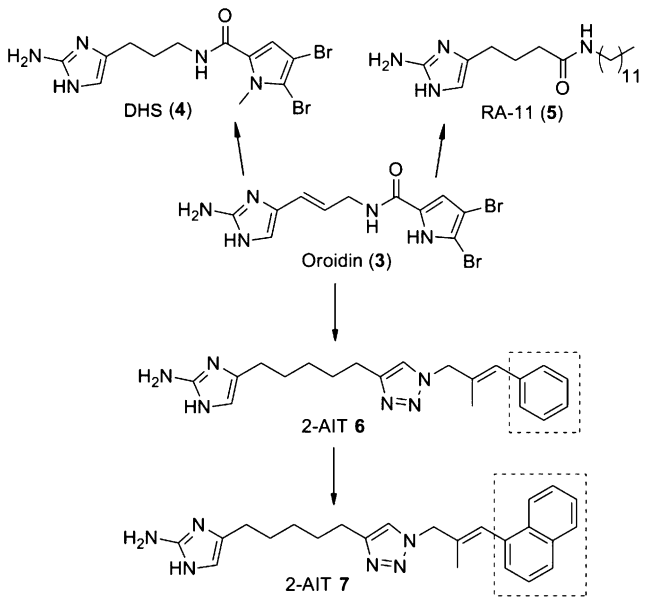

Figure 2. Small molecules inspired by oroidin that have antibiofilm activity.

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.201100316.

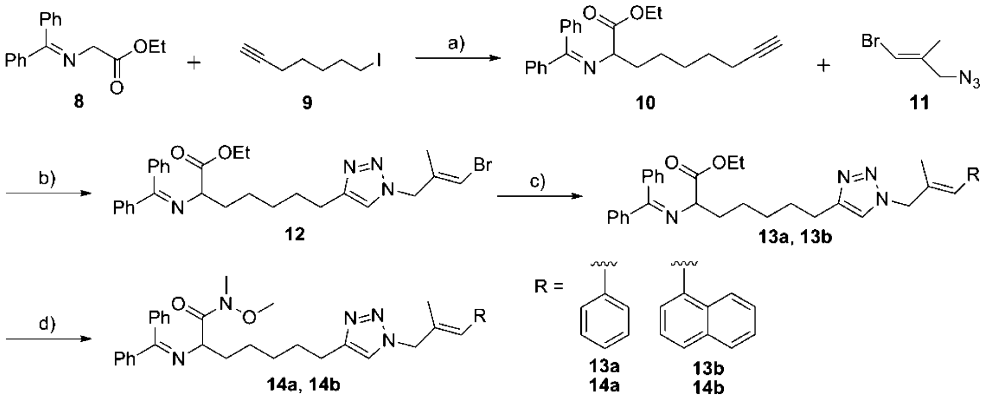
perse bacterial biofilms across order, class, and phylum. 2-AIT **6** was also reported to work synergistically with conventional antibiotics.[17] In an effort to augment the activity of lead 2-AIT **6**, we recently reported the synthesis of a second-generation 2-AIT library.[11] Subsequent screening of the library demonstrated that 2-AIT **7** is better able to inhibit methicillin-resistant *Staphylococcus aureus* (MRSA) biofilm formation and to disperse preformed *Acinetobacter baumannii* biofilms than compound **6**.

In a parallel structure–activity relationship (SAR) study, 4,5-disubstituted-2-AI analogues with simple substituent patterns were synthesized by a nitroenolate approach, and we identified a number of broad-spectrum antimicrobial reagents against a wide range of terrestrial and marine bacteria.[18] Given the success of our 2-AIT **6** toward both modulating biofilm formation and resensitizing multidrug-resistant bacteria to conventional antibiotics (resensitization activity), we elected to study the biological impact of imparting a 4,5-disubstitution pattern on 2-AITs **6** and **7** in the context of antibiofilm and antibiotic resensitization activity. Specifically, we were interested in investigating the outcome that augmenting the antibiofilm properties of 2-AITs **6** and **7** through analogue synthesis would have upon resensitization activity. Our original nitroenolate approach, however, was incompatible with accessing 2-AIT analogues based on **6** and **7**. To overcome this limitation, we report herein a general approach to 4,5-disubstituted-2-AIT derivatives based on the use of Weinreb amides. Furthermore, we show that imparting the 4,5-disubstitution pattern augments antibiofilm properties without compromising antibiotic resensitization activity. The activity of our lead compounds were also compared with the naturally occurring biofilm dispersant *cis*-2-decenoic acid **2**.

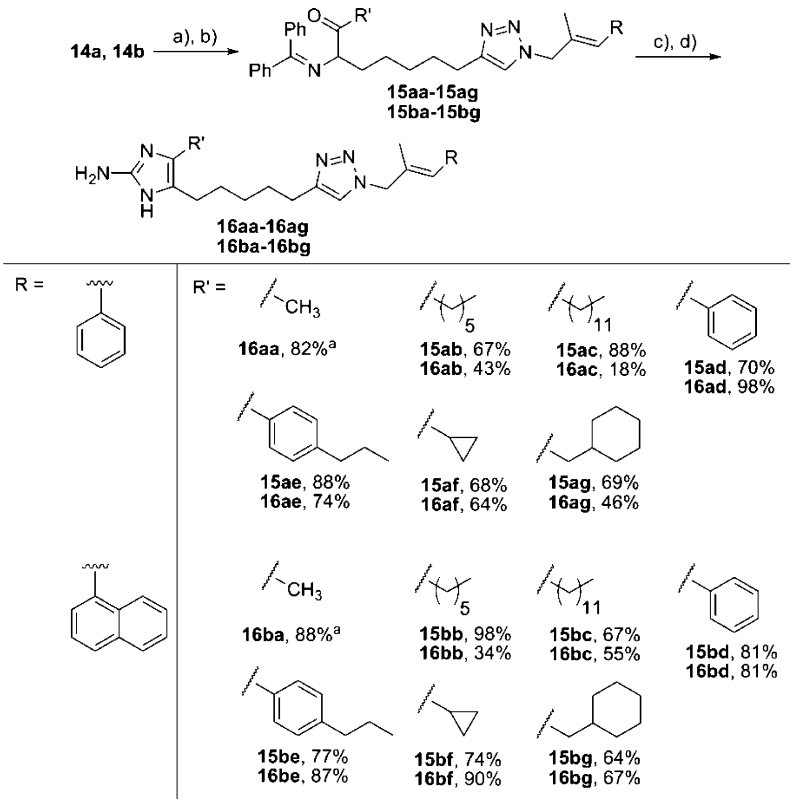
Results and Discussion

The synthesis of our Weinreb intermediate and subsequent generation of a 4,5-disubstituted-2-aminoimidazole–triazole pilot library is outlined in Scheme 1. Alkylation of the protected glycine ethyl ester **8** with iodoalkyne **9** delivered the protected α-amino ester **10** in 71% yield. A click reaction was then performed between **10** and vinyl bromide **11** to generate triazole **12**. Suzuki–Miyaura cross-coupling was then used to install both phenyl and naphthyl moieties (**13a** and **13b**).[11] Weinreb amides (**14a** and **14b**) were then accessed from **13** by treatment with *N*,*O*-dimethylhydroxyamine hydrochloride and isopropylmagnesium chloride.

With Weinreb amides **14** in hand, diversity was installed through reaction of each respective amide with a variety of commercially available Grignard reagents (Scheme 2). Each of the resulting ketones was then deprotected (2 N hydrochloric



Scheme 1. Synthetic route to Weinreb amides. *Reagents and conditions:* a) *t*BuOK, THF, −78 °C, 69 h (71%); b) sodium ascorbate, $CuSO_4$, *t*BuOH/H_2O/CH_2Cl_2, RT, 12 h (82%); c) $RB(OH)_2$, $PdCl_2(PPh_3)_2$, Na_2CO_3 (aq.), THF, 69 °C, 20 h (**13a** 96%, **13b** 98%); d) *O*,*N*-dimethylhydroxyamine·HCl, *i*PrMgCl, THF, −20 °C, 2 h (**14a** 69%, **14b** 56%)



Scheme 2. Pilot libraries of 4,5-disubstituted-2-AITs. *Reagents and conditions:* a) R′MgBr, THF; b) NH_4Cl (aq.); c) 2 N HCl, EtOH; d) NH_2CN, EtOH, pH 4.3. [a] Yield over three steps.

acid/ethanol) and cyclized with cyanamide to deliver the target 4,5-disubstituted-2-AIT. All derivatives, after purification, were prepared as their respective hydrochloride salts for biological screening.

The pilot library was first assessed for its ability to inhibit MRSA biofilm formation at 100 μM using the crystal violet reporter assay.[19] To our delight, all members in the library were capable of inhibiting biofilm formation by >95% at this concentration. A dose–response study was subsequently undertaken to determine the IC_{50} value of each compound for biofilm inhibition (Table 1). All compounds were subjected to growth-curve analysis (A_{600}) at their respective IC_{50} concentrations, and their antibiofilm activity was established to be non-microbicidal except for compounds **16bb**, **16bc**, **16be**, and **16bf**. We performed follow-up colony-count analysis with **16ae** and verified that biofilm modulation occurs via a non-microbicidal mechanism.

Table 1. Biofilm inhibition activity of pilot libraries against MRSA.

Compd	IC_{50} [μM][a]	Compd	IC_{50} [μM][a]
16aa	25.73 ± 0.28	**16ba**	2.95 ± 0.37
16ab	2.25 ± 0.04	**16bb**[b]	2.19 ± 0.03
16ac	1.91 ± 0.02	**16bc**[b]	10.08 ± 0.13
16ad	2.01 ± 0.01	**16bd**	2.23 ± 0.07
16ae	1.42 ± 0.18	**16be**[b]	2.15 ± 0.23
16af	2.10 ± 0.13	**16bf**[b]	8.20 ± 0.10
16ag	1.89 ± 0.05	**16bg**	1.97 ± 0.03

[a] Values represent the mean ± SD as determined by at least three independent parallel experiments. [b] Growth curve defects were noted at these concentrations.

According to the data outlined in Table 1, it was generally noted that compounds with the phenyl substituent (**16a** series) present an overall trend toward increased antibiofilm activity over the **16b** series, which contains the naphthyl subunit. Additionally, several of the compounds with the naphthyl moiety were found to be microbicidal during growth-curve analysis, whereas all compounds with the phenyl moiety were determined to inhibit biofilm formation via a non-microbicidal mechanism. We also noted, as previously observed, that activity is modulated by introduction of the 4,5-disubstitution pattern. Overall, compound **16ae** is the most potent compound of the pilot library, registering an IC_{50} value of 1.42 μM.

One of the most striking observations is that a propyl group on the newly introduced phenyl ring correlates with increased activity. Based on this observation, a second-generation library of eight compounds was synthesized (Figure 3), in which we further varied functionalities on the phenyl ring, including: methoxy, isopropyl, fluoro/difluoro, pyrrolidinyl, phenyl, and 1,3-benzodioxolyl. Antibiofilm activities of second-generation compounds were evaluated, and the IC_{50} values of biofilm inhibition against MRSA are outlined in Table 2. To our delight, two compounds displayed improved activity, with **16am** being the most active (1.34 μM). Growth-curve and colony-count analyses demonstrated that none of the second-generation compounds show toxicity against bacteria at their IC_{50} concentrations.

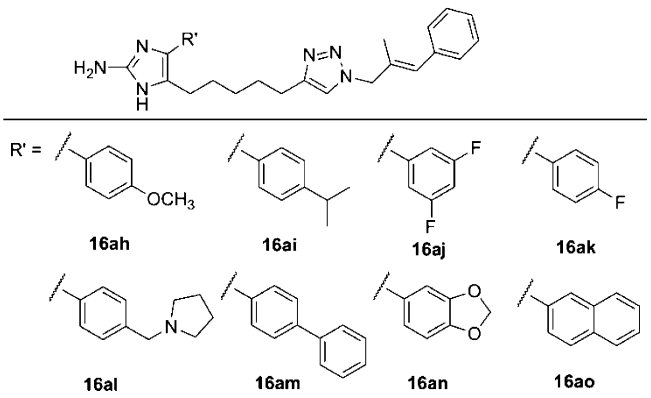


Figure 3. Second-generation library.

Table 2. Biofilm inhibition activity of second-generation compounds against MRSA.

Compd	IC_{50} [μM][a]	Compd	IC_{50} [μM][a]
16ah	1.50 ± 0.21	**16al**	1.52 ± 0.30
16ai	1.45 ± 0.05	**16am**	1.34 ± 0.10
16aj	1.89 ± 0.11	**16an**	1.96 ± 0.06
16ak	1.39 ± 0.08	**16ao**	1.95 ± 0.05

[a] Values represent the mean ± SD as determined by at least three independent parallel experiments.

Once the effect of these 4,5-disubstituted-2-AITs had been evaluated on a representative Gram-positive strain (MRSA), we assessed the antibiofilm activity of these compounds against *A. baumannii*, a representative Gram-negative bacterial strain. All compounds were initially screened for the ability to inhibit biofilm formation against *A. baumannii* at 100 μM. Each compound that showed >75% antibiofilm activity was subjected to a dose–response study to determine the IC_{50} value for biofilm inhibition. Several compounds displayed a precipitous decrease in their biofilm inhibition properties (**16ad**, **16ag**, **16ah**, **16ai**, **16aj**, **16ak**, **16an**, **16ba**, **16bf**, and **16bg**) over a narrow concentration range, which typically indicates that biofilm inhibition occurs through a traditional microbicidal mechanism (IC_{50} not determined). Other compounds with applicable IC_{50} values are summarized in Table 3. Growth-curve analysis was

Table 3. Biofilm inhibition and dispersion activity of representative compounds against *A. baumannii*.

Compd	IC_{50} [μM][a]	EC_{50} [μM][a]	Compd	IC_{50} [μM][a]	EC_{50} [μM][a]
16ab[b]	15.65 ± 0.27	–	**16am**	32.03 ± 2.88	64.55 ± 17.34
16ac	15.57 ± 0.31	50.03 ± 8.54	**16ao**	20.35 ± 0.07	60.80 ± 9.52
16ae	11.28 ± 0.68	44.61 ± 1.96	**16bb**[b]	19.73 ± 0.64	–
16af[b]	61.74 ± 2.54	–	**16bd**[b]	30.46 ± 0.57	–
16al	60.67 ± 3.18	–	**16be**[b]	22.92 ± 2.14	–

[a] Values represent the mean ± SD as determined by at least three independent parallel experiments. [b] Growth curve defects were noted at these concentrations.

conducted on each of the compounds outlined in Table 3 at their respective IC_{50} concentrations. 2-AIT conjugates **16ac**, **16ae**, **16al**, **16am**, and **16ao** were found to inhibit biofilm formation through a non-microbicidal mechanism, as no defect in the growth curve was noted over 24 h. Colony-count analysis on lead compounds **16ae** and **16ao** verified these results.

Table 4. Resensitization of MRSA to oxacillin.

Compd	MIC_{Oxa} [$\mu g\,mL^{-1}$]	Compd	MIC_{Oxa} [$\mu g\,mL^{-1}$]	Compd	MIC_{Oxa} [$\mu g\,mL^{-1}$]
16aa (20.0 μM)	32	**16ah** (4.1 μM)	64	**16ba** (8.9 μM)	32
16ab (2.1 μM)	32	**16ai** (2.0 μM)	32	**16bb** (1.0 μM)	32
16ac (3.6 μM)	16	**16aj** (2.0 μM)	64	**16bc** (6.6 μM)	32
16ad (8.6 μM)	64	**16ak** (2.1 μM)	64	**16bd** (2.0 μM)	32
16ae (1.0 μM)	64	**16al** (14.7 μM)	32	**16be** (3.6 μM)	16
16af (18.8 μM)	32	**16am** (1.8 μM)	32	**16bf** (4.2 μM)	16
16ag (2.1 μM)	64	**16an** (4.0 μM)	64	**16bg** (1.0 μM)	32
2 (50.0 μM)	64	**16ao** (2.0 μM)	32	Control[a]	64

[a] No compound was combined with oxacillin.

The ability of lead compounds to disperse preformed *A. baumannii* biofilms was also evaluated. EC_{50} values were determined and are summarized in Table 3. Here, we define EC_{50} as the concentration that elicits 50% dispersion of a preformed biofilm. All lead compounds were able to disperse preformed *A. baumannii* biofilms at low-micromolar concentrations, with **16ae** being the most active compound.

cis-2-Decenoic acid **2** was recently reported as a signaling molecule that possesses the ability to inhibit and disperse biofilms from both Gram-positive and Gram-negative bacterial strains as well as a representative fungal strain.[20] Given this activity, we were eager to evaluate how this naturally occurring molecule compares with our 2-AIT derivatives under identical conditions. Compound **2** was synthesized as described by Rappe[21] and evaluated for its antibiofilm activity by initially screening at 400 μM for biofilm inhibition/dispersion activity against *A. baumannii*, MRSA, and methicillin-sensitive *S. aureus* (MSSA). The ability of **2** to disperse preformed biofilms against all three bacterial strains and to inhibit biofilm formation against *A. baumannii* at 400 μM was <10%. Nonetheless, **2** displayed moderate biofilm inhibition activity against MRSA and MSSA. IC_{50} values were determined to be 220 ± 14 μM against MRSA and 121 ± 35 μM against MSSA. A stability study of **2** was performed by ^{1}H NMR spectroscopy and is described in detail in the Supporting Information.

Next, we studied the ability of our 2-AIT library to resensitize MRSA to oxacillin. This antibiotic was specifically chosen because the parent compound **6** had minimal effect on the ability to resensitize MRSA to oxacillin (about twofold). Therefore, it represented an ideal canvas to test whether our 4,5-disubstituted-2-AIT library possesses enhanced activity. We were also interested in determining whether, like 2-AI-derived antibiofilm agents, *cis*-2-decenoic acid modulates planktonic response to antibiotics. We first determined the minimum inhibitory concentration (MIC) values of each compound against MRSA. A fourth of each respective MIC value was subsequently used as the concentration to test antibiotic resensitization. This concentration was chosen because in previous studies, no microbicidal activity is typically observed at 25% of the MIC values of our 2-AI antibiofilm agents.

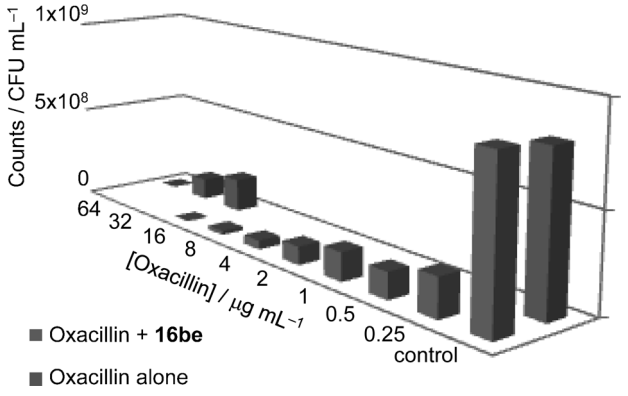
Bacteria were pretreated with each compound for 30 min, and the MIC of oxacillin was subsequently determined. All MIC values of oxacillin combined with each of the 4,5-disubstituted-2-AITs and *cis*-2-decenoic acid **2** are summarized in Table 4. Compounds **16ac**, **16be**, and **16bf** resensitized MRSA to the effects of oxacillin by fourfold (roughly twofold over parent molecule **6**). Next, the lead compounds were subjected to the checkerboard assay,[22] in which the synergistic activity of the combined agents was evaluated. The ΣFIC values of **16ac**, **16be**, and **16bf** were determined to be 0.25, 0.19, and 0.5 respectively, indicating that these three 2-AIT derivatives work synergistically with oxacillin ($\Sigma FIC = FIC_{Compd} + FIC_{Oxa}$, for which $FIC_{Compd} = [MIC_{Compd}$ in combination$]/[MIC_{Compd}$ alone$]$, and $FIC_{Oxa} = [MIC_{Oxa}$ in combination$]/[MIC_{Oxa}$ alone$]$).

In the resensitization and checkerboard assays we also observed that in between the thoroughly clear and turbid wells, there were wells that contained a minute amount of bacteria that were barely visible by the naked eye. This is in contrast to MIC assays with conventional antibiotics in which there is an abrupt transition between turbid and clear wells. Although all our MIC values were recorded from completely clear wells, we were interested in determining the bacterial density of these wells that contained the barely visible bacterial precipitate. We performed colony-count analysis on selected wells from the checkerboard assay of **16be**, and these results are summarized in Table 5. Comparing the activity of **16be** plus 16 $\mu g\,mL^{-1}$ oxacillin against 16 $\mu g\,mL^{-1}$ oxacillin alone reveals that the combination is 99.9% more effective. However, we note from growth-curve analysis that **16be** does alter early (0–8 h) MRSA growth characteristics, although bacterial CFUs are identical after 16 h.

Conclusions

In summary, we have successfully synthesized a variety of 4,5-disubstituted-2-aminoimidazole–triazole conjugates through a Weinreb amide approach. The antibiofilm activity of these compounds was evaluated, and compounds **16ae**, **16ak**, and **16am** were identified as the most active. Growth-curve and colony-count analyses indicated that lead compounds inhibit biofilm formation via a non-microbicidal mechanism. Further comparison of lead compounds against the naturally occurring dispersant *cis*-2-decenoic acid revealed that appropriately designed 2-AI derivatives are two orders of magnitude more potent. 2-AI-based antibiofilm agents can also be simultaneously augmented for both their antibiofilm properties and their antibiotic resensitization activity. The fact that not all lead compounds have the ability to resensitize MRSA to the effects

Table 5. Colony-count comparison between wells in checkerboard assay against MRSA.



MIC_{Oxa} [μg mL^{-1}]	Count [CFU mL^{-1}] Oxacillin + **16be**[a]	Oxacillin alone
64	–	$7.60 \pm 0.57 \times 10^5$
32	–	$1.11 \pm 0.06 \times 10^8$
16	$1.96 \pm 0.20 \times 10^5$	$1.75 \pm 0.16 \times 10^8$
8	$2.04 \pm 0.20 \times 10^7$	–
4	$4.70 \pm 0.57 \times 10^7$	–
2	$9.55 \pm 1.34 \times 10^7$	–
1	$1.50 \pm 0.26 \times 10^8$	–
0.5	$1.38 \pm 0.11 \times 10^8$	–
0.25	$2.03 \pm 0.42 \times 10^8$	–
Control	$8.35 \pm 0.64 \times 10^8$	$8.00 \pm 1.27 \times 10^8$

[a] [**16be**] in assay: 3.6 μM; values represent the mean ±SD as determined by at least three independent parallel experiments.

of oxacillin indicates that antibiofilm properties and resensitization potentially act through distinct mechanisms. However, we note that a single molecular scaffold based on the 2-AI framework can be designed to target both properties simultaneously.

Experimental Section

General experimental

All reagents used for chemical synthesis were purchased from commercially available sources were and used without further purification. Chromatography was performed with 60 Å mesh standard-grade silica gel from Sorbtech (Atlanta, GA, USA). Infrared spectra were collected on an FT/IR-4100 spectrophotometer (ν_{max} in cm^{-1}). UV absorbance was recorded on a Genesys 10 scanning UV/Vis spectrophotometer (λ_{max} in nm). NMR solvents were obtained from Cambridge Isotope Labs and used as is. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded at 25 °C on Varian Mercury spectrometers. Chemical shifts (δ) are given in ppm relative to tetramethylsilane or the respective NMR solvent; coupling constants (J) are in Hertz (Hz). Abbreviations used are: s = singlet, bs = broad singlet, d = doublet, dd = doublet of doublets, t = triplet, dt = doublet of triplets, td = triplet of doublets, tt = triplet of triplets, bt = broad triplet, q = quartet, m = multiplet, bm = broad multiplet. Mass spectra were obtained at the NCSU Department of Chemistry Mass Spectrometry Facility. Purity of tested compounds was confirmed to be >95% by LC–MS analysis unless otherwise stated.

MRSA (ATCC #BAA-44), MSSA (ATCC #29213) and *A. baumannii* (ATCC #19606) were obtained from the American Type Culture Collection. Mechanically defibrinated sheep blood (DSB100) was obtained from Hemostat Labs. Oxacillin sodium salt was purchased from Fluka (cat. #28221). All other reagents were purchased from commercially available sources.

Synthesis of *N*-methoxy-*N*-methylamide–triazole precursors (14a, 14b): Ethyl 2-(diphenylmethyleneamino)non-8-ynoate (10): *N*-(Diphenylmethylene)glycine ethyl ester **8** (4.010 g, 15 mmol) and anhydrous THF (110 mL) were added to a 250 mL round-bottomed flask. The solution was stirred under N_2 at −78 °C for 15 min. *t*BuOK (2.020 g, 18 mmol) was added, and the resulting solution was allowed to warm to 0 °C and stirred for 1 h. 7-Iodohept-1-yne **9** (4.329 g, 19.5 mmol) was prepared as described by Knapp et al.[23] and added to the mixture. The mixture was then warmed and stirred at room temperature for 69 h. The reaction was quenched by 100 mL H_2O and extracted with EtOAc (2×100 mL). The combined organic extracts were washed with 100 mL brine, dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. Purification of the residue was carried out on a silica gel column and eluted with CH_2Cl_2/MeOH (9:1) to give the desired product **10** (3.854 g, 71%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.65$ (m, 1H), 7.64 (m, 1H), 7.44 (m, 2H), 7.39 (m, 1H), 7.38 (m, 1H), 7.35 (m, 1H), 7.33 (m, 1H), 7.17 (m, 2H), 4.17 (m, 2H), 4.04 (t, $J = 6.8$ Hz, 1H), 2.13 (td, $J = 2.4$, 7.2 Hz, 2H), 1.92 (m, 2H), 1.91 (t, $J = 2.8$ Hz, 1H), 1.49 (m, 2H), 1.32 (m, 4H), 1.26 (t, $J = 7.6$ Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 172.7$, 170.6, 139.8, 136.8, 130.5, 129.0, 128.8, 128.7, 128.3, 128.1, 84.8, 68.4, 65.6, 61.1, 33.7, 28.7, 28.5, 25.7, 18.5, 14.5 ppm; IR $\nu_{max} = 3280$, 3136, 3064, 2923, 2855, 1955, 1735, 1660, 1599, 1447, 1317, 1278, 1177, 1073, 1030, 941, 920, 763, 700 cm^{-1}; $\lambda_{max} = 252$ nm; HRMS (FAB) calcd for $C_{24}H_{27}NO_2$ $[M+H]^+$: 362.2115, found: 362.2117.

(*E*)-Ethyl-7-[1-(3-bromo-2-methylallyl)-1*H*-1,2,3-triazol-4-yl]-2-(diphenylmethyleneamino)heptanoate (12): Compound **10** (3.354 g, 9.29 mmol) was dissolved in a solvent mixture of 18 mL CH_2Cl_2, 36 mL *t*BuOH, and 36 mL H_2O in a 150 mL round-bottomed flask. (*E*)-3-Azido-1-bromo-2-methylprop-1-ene **11** (1.950 g, 11.14 mmol) was prepared as described by Handa et al.[24] and added to the solution at room temperature. With vigorous stirring, $CuSO_4$ (0.222 g, 1.39 mmol) and sodium ascorbate (0.736 g, 3.71 mmol) were added, and the resulting solution was stirred at room temperature for 12 h. The mixture was extracted with CH_2Cl_2 (3×20 mL). The combined organic extracts were washed with 20 mL brine, dried over Na_2SO_4, and the solvent was removed in vacuo. The resulting residue was purified by column chromatography (hexane/EtOAc, 1:1) to afford the target compound **12** (4.105 g, 82%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.50$ (d, $J = 8.0$ Hz, 2H), 7.29 (m, 3H), 7.18 (m, 4H), 7.03 (d, $J = 6.8$ Hz, 2H), 6.14 (s, 1H), 4.74 (s, 2H), 4.01 (m, 2H), 3.93 (m, 1H), 2.51 (t, $J = 7.2$ Hz, 2H), 1.80 (m, 2H), 1.56 (s, 3H), 1.50 (m, 2H), 1.14 (m, 4H), 1.10 ppm (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 172.4$, 170.4, 148.7, 139.6, 136.5, 136.5, 130.5, 128.9, 128.8, 128.7, 128.2, 127.9, 121.0, 108.0, 65.5, 60.9, 55.6, 33.7, 29.3, 29.1, 25.8, 25.7, 17.2, 14.4 ppm; IR $\nu_{max} = 3136$, 3061, 3024, 2929, 2856, 2110, 1959, 1896, 1732, 1623, 1445, 1369, 1289, 1181, 1030, 957, 781, 699 cm^{-1}; $\lambda_{max} = 250$ nm; HRMS (FAB) calcd for $C_{28}H_{33}BrN_4O_2$ $[M+H]^+$: 537.1860, found: 537.1867.

(*E*)-Ethyl-2-(diphenylmethyleneamino)-7-[1-(2-methyl-3-phenylallyl)-1*H*-1,2,3-triazol-4-yl]heptanoate (13a): Compound **12** (0.802 g, 1.50 mmol) and THF (8 mL) were added to a vial (23× 85 mm). $PhB(OH)_2$ (0.367 g, 3.00 mmol) was then added and subsequently treated with dichlorobis(triphenylphosphine)palladium(II) (0.056 g, 0.08 mmol) and an aqueous solution of Na_2CO_3 (2 M,

2.38 mL) under vigorous stirring. The resulting mixture was initially sonicated for 15 s and then stirred at 69 °C for 20 h. The reaction was extracted with EtOAc (3×3 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 and purified by column chromatography (hexane/EtOAc, 1:1) to give the desired compound **13a** (0.771 g, 96%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.65 (d, J = 7.2 Hz, 2H), 7.42 (m, 3H), 7.31 (m, 9H), 7.17 (m, 2H), 6.46 (s, 1H), 4.95 (s, 2H), 4.15 (m, 2H), 4.05 (t, J = 7.2 Hz, 1H), 2.68 (t, J = 7.6 Hz, 2H), 1.94 (m, 2H), 1.77 (s, 3H), 1.65 (m, 2H), 1.31 (m, 4H), 1.23 ppm (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.7, 170.6, 148.8, 139.8, 136.7, 132.5, 130.6, 130.3, 130.0, 129.2, 129.1, 128.9, 128.8, 128.5, 128.3, 128.1, 127.4, 120.8, 65.7, 61.1, 58.7, 33.8, 29.5, 29.2, 26.0, 25.9, 15.9, 14.5 ppm; IR ν_{max} = 3277, 3136, 3097, 2931, 2858, 2092, 1948, 1713, 1593, 1563, 1452, 1437, 1369, 1298, 1248, 1024, 986, 742, 700 cm^{-1}; λ_{max} = 229 nm; HRMS (FAB) calcd for $C_{34}H_{38}N_4O_2$ $[M+H]^+$: 535.3068, found: 535.3056.

(*E*)-Ethyl-2-(diphenylmethyleneamino)-7-[1-(2-methyl-3-(naphthalen-1-yl)allyl]-1*H*-1,2,3-triazol-4-yl)heptanoate (13b): Compound **12** (0.856 g, 1.60 mmol) and 1-naphthylboronic acid (0.549 g, 3.19 mmol) was treated with dichlorobis(triphenylphosphine)palladium(II) (0.056 g, 0.08 mmol) and an aqueous solution of Na_2CO_3 (2 M, 2.40 mL) according to the procedure above. Purification by column chromatography gave the desired compound **13b** (0.919 g, 98%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.87 (m, 2H), 7.78 (d, J = 8.4 Hz, 1H), 7.63 (d, J = 7.2 Hz, 2H), 7.42 (m, 8H), 7.32 (m, 3H), 7.15 (m, 2H), 6.87 (s, 1H), 5.12 (s, 2H), 4.16 (m, 2H), 4.04 (t, J = 7.2 Hz, 1H), 2.71 (t, J = 7.6 Hz, 2H), 1.93 (m, 2H), 1.67 (m, 5H), 1.34 (m, 4H), 1.25 ppm (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.7, 170.6, 149.0, 139.8, 136.7, 134.6, 133.8, 133.7, 131.9, 130.5, 129.1, 128.9, 128.8, 128.3, 128.1, 128.0, 128.0, 127.8, 127.0, 126.4, 126.2, 125.5, 124.8, 120.8, 65.7, 61.1, 58.1, 33.8, 29.6, 29.3, 26.0, 25.9, 16.1, 14.5 ppm; IR ν_{max} = 3137, 3058, 2926, 2855, 1958, 1732, 1621, 1445, 1369, 1286, 1180, 1044, 1029, 783, 698 cm^{-1}; λ_{max} = 234, 287 nm; HRMS (FAB) calcd for $C_{38}H_{40}N_4O_2$ $[M+H]^+$: 585.3224, found: 585.3226.

(*E*)-2-(Diphenylmethyleneamino)-*N*-methoxy-*N*-methyl-7-[1-(2-methyl-3-phenylallyl)-1*H*-1,2,3-triazol-4-yl]heptanamide (14a): Compound **13a** (0.441 g, 0.83 mmol) and anhydrous THF (2 mL) were added to a vial (23×85 mm). The solution was stirred under N_2 at −20 °C until **13a** was dissolved. The solution was treated with *N*,*O*-dimethylhydroxyamine·HCl (0.242 g, 2.48 mmol), and *i*PrMgCl (2 M, 2.48 mL) was then added dropwise in order to keep the reaction temperature below −5 °C. The resulting solution was stirred at 0 °C for 2 h. The reaction was quenched with NH_4Cl (20%, 2 mL) and then extracted with EtOAc (3×2 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, and the solvent was removed in vacuo. Purification of the residue took place on a silica gel column and was eluted with EtOAc to give the desired product **14a** (0.311 g, 69%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.64 (d, J = 7.6 Hz, 2H), 7.42 (m, 3H), 7.30 (m, 9H), 7.16 (m, 2H), 6.48 (s, 1H), 4.98 (s, 2H), 4.31 (t, J = 6.4 Hz, 1H), 3.22 (s, 3H), 3.13 (s, 3H), 2.67 (t, J = 7.2 Hz, 2H), 2.02 (m, 2H), 1.79 (s, 3H), 1.64 (m, 2H), 1.29 ppm (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.6, 169.7, 149.0, 139.8, 137.2, 136.7, 132.5, 130.4, 130.0, 129.2, 129.0, 128.7, 128.7, 128.5, 128.2, 128.1, 127.4, 120.7, 63.1, 61.1, 58.7, 33.8, 32.5, 29.4, 29.2, 26.2, 25.9, 15.9 ppm; IR ν_{max} = 3057, 3024, 2933, 2856, 1666, 1621, 1597, 1576, 1445, 1386, 1285, 1178, 1046, 1021, 990, 782, 699 cm^{-1}; λ_{max} = 246 nm; HRMS (FAB) calcd for $C_{34}H_{39}N_5O_2$ $[M+H]^+$: 550.3177, found: 550.3177.

(*E*)-2-(Diphenylmethyleneamino)-*N*-methoxy-*N*-methyl-7-{1-[2-methyl-3-(naphthalen-1-yl)allyl]-1*H*-1,2,3-triazol-4-yl}heptanamide (14b): Compound **13b** (0.664 g, 1.14 mmol) was treated with *N*,*O*-dimethylhydroxyamine·HCl (0.444 g, 4.55 mmol) and *i*PrMgCl (2 M, 4.55 mL) according to the procedure above. Purification by column chromatography gave the desired compound **14b** (0.378 g, 56%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.83 (d, J = 8.0 Hz, 1H), 7.76 (d, J = 7.2 Hz, 1H), 7.69 (d, J = 8.4 Hz, 1H), 7.62 (d, J = 8.4 Hz, 2H), 7.38 (m, 7H), 7.24 (m, 4H), 7.11 (m, 2H), 6.79 (s, 1H), 5.03 (s, 2H), 4.30 (t, J = 5.6 Hz, 1H), 3.16 (s, 3H), 3.07 (s, 3H), 2.67 (t, J = 7.2 Hz, 2H), 2.02 (m, 2H), 1.64 (m, 2H), 1.60 (s, 3H), 1.29 ppm (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.3, 169.7, 148.8, 139.7, 137.1, 134.7, 133.8, 133.7, 131.9, 130.4, 129.0, 128.7, 128.7, 128.5, 128.2, 128.0, 128.0, 127.6, 126.9, 126.4, 126.2, 125.5, 124.9, 121.1, 63.0, 61.1, 57.9, 33.8, 32.4, 29.5, 29.2, 26.2, 25.9, 16.0 ppm; IR ν_{max} = 3396, 3135, 3058, 2935, 2857, 1682, 1623, 1594, 1577, 1506, 1445, 1286, 1045, 955, 784, 700 cm^{-1}; λ_{max} = 259, 287 nm; HRMS (FAB) calcd for $C_{38}H_{41}N_5O_2$ $[M+H]^+$: 600.3333, found: 600.3329.

Procedure for one-pot synthesis of 4,5-disubstituted-2-aminoimidazole–triazole conjugates from the Weinreb amide (16aa and 16ba): **(*E*)-5-Methyl-4-{5-[1-(2-methyl-3-phenylallyl)-1*H*-1,2,3-triazol-4-yl]pentyl}-1*H*-imidazol-2-amine (16aa)**: Compound **14a** (0.046 g, 0.084 mmol) was dissolved in 1.5 mL anhydrous THF in a vial (23×85 mm). The solution was stirred under N_2 at −20 °C, and MeMgBr (3 M, 0.09 mL) was added dropwise. The resulting mixture was allowed to warm to room temperature and stirred for 4 h. The reaction was cooled to 0 °C and quenched with NH_4Cl (20%, 2 mL), then extracted with EtOAc (3×2 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The residue was redissolved in 1.5 mL EtOH, and HCl (2 M, 0.75 mL) was added and stirred at room temperature for 12 h. The solution was then adjusted to pH 4.3 with NaOH (0.1 M). Cyanamide (0.016 g, 0.38 mmol) was then added, and the resulting mixture was heated at 95 °C for 3 h. The EtOH was removed in vacuo and the residue was purified by column chromatography (CH_2Cl_2/MeOH satd. NH_3 4:1) to afford the target product **16aa** (0.025 g, 82%) in its free-base form. Addition of concentrated HCl to a MeOH solution (2 mL) of the free base followed by solvent evaporation under reduced pressure delivered the corresponding **16aa** as its HCl salt: ^{1}H NMR (400 MHz, CD_3OD): δ = 8.56 (s, 1H), 7.33 (m, 4H), 7.26 (m, 1H), 6.78 (s, 1H), 5.33 (s, 2H), 2.92 (t, J = 7.6 Hz, 2H), 2.47 (t, J = 7.2 Hz, 2H), 2.06 (s, 3H), 1.85 (s, 3H), 1.81 (m, 2H), 1.63 (m, 2H), 1.43 ppm (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ = 146.2, 145.3, 136.4, 132.4, 130.2, 128.9, 128.2, 127.4, 126.4, 121.7, 117.6, 60.9, 28.3, 28.0, 27.9, 23.2, 22.8, 14.7, 7.6 ppm; IR ν_{max} = 3399, 2920, 2850, 2110, 1682, 1457, 1403, 1180, 1052, 797, 699 cm^{-1}; λ_{max} = 202, 237 nm; HRMS (FAB) calcd for $C_{21}H_{28}N_6$ $[M+H]^+$: 365.2448, found: 365.2438.

(*E*)-5-Methyl-4-{5-[1-(2-methyl-3-{naphthalen-1-yl}allyl)-1*H*-1,2,3-triazol-4-yl]pentyl}-1*H*-imidazol-2-amine (16ba): According to the procedure above, **14b** (0.082 g, 0.14 mmol) was treated with MeMgBr (3 M, 0.09 mL) followed by deprotection with HCl (2 M, 1.2 mL) and cyclization with cyanamide (0.027 g, 0.65 mmol). Purification by column chromatography gave the desired product **16ba** (0.050 g, 88%) in its free-base form. Addition of concentrated HCl to a MeOH solution (2 mL) of the free base followed by solvent evaporation under reduced pressure delivered the corresponding **16ba** as its HCl salt: ^{1}H NMR (400 MHz, CD_3OD): δ = 7.95 (s, 1H), 7.88 (m, 1H), 7.84 (m, 1H), 7.79 (d, J = 8.4 Hz, 1H),7.48 (m, 3H), 7.31 (d, J = 7.6 Hz, 1H), 6.86 (s, 1H), 5.22 (s, 2H), 2.73 (t, J = 7.2 Hz, 2H), 2.37 (t, J = 7.2 Hz, 2H), 1.97 (s, 3H), 1.71 (m, 2H),1.62 (s, 3H),

1.56 (m, 2H), 1.34 ppm (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ = 148.2, 146.2, 134.8, 133.9, 133.9, 131.9, 128.3, 127.6, 127.2, 126.5, 126.0, 125.8, 125.1, 124.6, 122.7, 121.7, 117.5, 57.3, 29.0, 28.4, 28.1, 24.9, 22.8, 14.8, 7.5 ppm; IR ν_{max} = 3410, 2925, 2853, 2104, 1958, 1680, 1402, 1261, 1180, 1021, 954, 805, 698 cm^{-1}; λ_{max} = 207, 286 nm; HRMS (FAB) calcd for $C_{25}H_{30}N_6$ $[M+H]^+$: 415.2605, found: 415.2600.

Synthesis of selected 4,5-disubstituted-2-aminoimidazole–triazole conjugates (16ae, 16ak, and 16am): General procedure for the preparation of α-diphenylmethyleneamino ketones: Compound **14a** (or **14b**) was dissolved in 1.5 mL anhydrous THF in a vial (23×85 mm). The solution was stirred under N_2 at −20 °C, and the appropriate Grignard reagent was added dropwise. The resulting mixture was allowed to warm to room temperature and stirred for 2–4 h until completion of the reaction was confirmed by TLC analysis. The reaction was cooled to 0 °C and quenched with NH_4Cl (20%, 2 mL), then extracted with EtOAc (3×2 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 and purified by column chromatography (hexane/EtOAc, 1:1) to give the desired α-diphenylmethyleneamino ketones. All α-diphenylmethyleneamino ketones were then subjected to deprotection/cyclization (detailed below).

(*E*)-2-(Diphenylmethyleneamino)-7-[1-(2-methyl-3-phenylallyl)-1*H*-1,2,3-triazol-4-yl]-1-(4-propylphenyl)heptan-1-one (15ae): Compound **14a** (0.050 g, 0.091 mmol) was treated with (4-propylphenyl)MgBr (0.5 M, 1.82 mL) according to the general procedure. Purification by column chromatography gave the desired product **15ae** (0.049 g, 88%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.80 (d, J = 8.4 Hz, 2H), 7.66 (m, 2H), 7.28 (m, 12H), 7.19 (d, J = 8.0 Hz, 2H), 7.07 (m, 2H), 6.47 (s, 1H), 4.96 (s, 2H), 4.76 (dd, J = 5.6, 7.6 Hz, 1H), 2.66 (t, J = 7.6 Hz, 2H), 2.60 (t, J = 7.6 Hz, 2H), 1.98 (m, 2H), 1.78 (s, 3H), 1.63 (m, 4H), 1.30 (m, 4H), 0.93 ppm (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 200.0, 169.6, 148.8, 148.4, 139.6, 136.8, 136.6, 133.9, 132.4, 130.3, 129.8, 129.2, 129.0, 128.9, 128.7, 128.7, 128.6, 128.4, 128.1, 127.8, 127.3, 120.6, 69.9, 58.6, 38.1, 35.2, 29.3, 29.0, 26.4, 25.7, 24.3, 15.7, 13.9 ppm; IR ν_{max} = 3058, 2926, 2855, 1958, 1661, 1604, 1447, 1277, 1047, 941, 741, 700 cm^{-1}; λ_{max} = 248 nm; HRMS (FAB) calcd for $C_{41}H_{44}N_4O$ $[M+H]^+$: 609.3588, found: 609.3571.

(*E*)-2-(Diphenylmethyleneamino)-1-(4-fluorophenyl)-7-[1-(2-methyl-3-phenylallyl)-1*H*-1,2,3-triazol-4-yl]heptan-1-one (15ak): Compound **14a** (0.063 g, 0.11 mmol) was treated with (4-fluorophenyl)MgBr (1 M, 1.15 mL) according to the general procedure. Purification by column chromatography gave the desired product **15ak** (0.053 g, 79%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.01 (m, 2H), 7.65 (m, 2H), 7.41 (m, 3H), 7.32 (m, 5H), 7.28 (m, 4H), 7.05 (m, 4H), 6.47 (s, 1H), 4.97 (s, 2H), 4.69 (dd, J = 6.0, 8.4 Hz, 1H), 2.67 (t, J = 8.0 Hz, 2H), 1.98 (m, 2H), 1.78 (d, J = 1.2 Hz, 3H), 1.63 (m, 2H), 1.29 ppm (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 198.9, 169.9, 166.9, 164.4, 148.7, 139.4, 136.6, 136.6, (132.4, 132.4, 132.4), (132.1, 132.0), 130.5, 129.9, 129.0, 128.9, 128.8, 128.4, 128.2, 127.8, 127.3, 120.6, 115.7, 115.5, 70.6, 58.6, 35.2, 29.3, 29.0, 26.3, 25.7, 15.8 ppm; IR ν_{max} = 3077, 2918, 2849, 1961, 1682, 1597, 1446, 1230, 1156, 1049, 781, 698 cm^{-1}; λ_{max} = 248 nm; HRMS (FAB) calcd for $C_{38}H_{37}FN_4O$ $[M+H]^+$: 585.3024, found: 585.3026.

(*E*)-1-(Biphenyl-4-yl)-2-(diphenylmethyleneamino)-7-[1-(2-methyl-3-phenylallyl)-1*H*-1,2,3-triazol-4-yl]heptan-1-one (15am): Compound **14a** (0.066 g, 0.12 mmol) was treated with biphenyl-4-yl-MgBr (0.5 M, 2.4 mL) according to the general procedure. Purification by column chromatography gave the desired product **15am** (0.059 g, 77%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.98 (d, J = 8.4 Hz, 2H), 7.68 (m, 2H), 7.59 (m, 4H), 7.42 (m, 5H), 7.27 (m, 10H), 7.09 (m, 2H), 6.45 (s, 1H), 4.94 (s, 2H), 4.79 (t, J = 7.2 Hz, 1H), 2.67 (t, J = 8.0 Hz, 2H), 2.03 (m, 2H), 1.77 (s, 3H), 1.64 (m, 2H), 1.32 ppm (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 199.8, 169.6, 148.6, 145.4, 139.8, 139.4, 136.6, 136.4, 134.7, 132.2, 130.3, 129.7, 129.6, 128.9, 128.9, 128.7, 128.7, 128.6, 128.2, 128.1, 128.0, 127.7, 127.2, 127.1, 127.0, 120.4, 70.1, 58.4, 35.0, 29.2, 28.9, 26.2, 25.6, 15.6 ppm; IR ν_{max} = 3062, 2928, 2854, 1948, 1683, 1600, 1447, 1277, 1120, 1044, 856, 748, 697 cm^{-1}; λ_{max} = 229, 252 nm; HRMS (FAB) calcd for $C_{44}H_{42}N_4O$ $[M+H]^+$: 643.3431, found: 643.3422.

Deprotection/cyclization procedure to access 2-aminoimidazoles from diphenylmethyleneamino ketones: The appropriate α-diphenylmethyleneamino ketone was dissolved in 1.5 mL EtOH. HCl (2 M) was added to the solution, which was stirred at room temperature for 12 h. The resulting solution was adjusted to pH 4.3 with NaOH (0.1 M). Then the mixture was treated with cyanamide and heated at 95 °C for 3 h. The EtOH was removed under reduced pressure, and the resulting solid was washed with Et_2O (1×3 mL). Purification by column chromatography (CH_2Cl_2/MeOH satd. NH_3 4:1) afforded the desired compound in its free-base form. Addition of concentrated HCl to a MeOH solution (2 mL) of the free base followed by solvent evaporation under reduced pressure delivered the corresponding 2-aminoimidazole as its HCl salt.

(*E*)-4-{5-[1-(2-Methyl-3-phenylallyl)-1*H*-1,2,3-triazol-4-yl]pentyl}-5-(4-propylphenyl)-1*H*-imidazol-2-amine (16ae): Compound **15ae** (0.053 g, 0.087 mmol) was treated with HCl (2 M, 0.44 mL), then reacted with cyanamide (0.110 g, 2.61 mmol) according to the general procedure. Purification by column chromatography afforded the target compound **16ae** (0.030 g, 74%) over two steps as a yellow oil: ^{1}H NMR (400 MHz, CD_3OD): δ = 7.94 (s, 1H), 7.33 (m, 4H), 7.28 (m, 5H), 6.57 (s, 1H), 5.11 (s, 2H), 2.72 (t, J = 7.6 Hz, 2H), 2.64 (t, J = 8.0 Hz, 2H), 2.59 (t, J = 8.0 Hz, 2H), 1.77 (d, J = 1.2 Hz, 3H), 1.70 (m, 4H), 1.64 (m, 2H), 1.39 (m, 2H), 0.92 ppm (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ = 148.8, 148.1, 144.6, 138.0, 133.3, 131.5, 130.4, 130.1, 129.5, 128.4, 128.3, 127.1, 124.5, 124.0, 123.7, 60.0, 38.8, 29.9, 29.8, 29.5, 25.8, 25.7, 25.0, 15.9, 14.2 ppm; IR ν_{max} = 3360, 2919, 2849, 1955, 1682, 1457, 1128, 1081, 702 cm^{-1}; λ_{max} = 204, 250 nm; HRMS (FAB) calcd for $C_{29}H_{36}N_6$ $[M+H]^+$: 469.3074, found: 469.3086.

(*E*)-5-(4-Fluorophenyl)-4-{5-[1-(2-methyl-3-phenylallyl)-1*H*-1,2,3-triazol-4-yl]pentyl}-1*H*-imidazol-2-amine (16ak): Compound **15ak** (0.053 g, 0.091 mmol) was treated with HCl (2 M, 0.91 mL), then reacted with cyanamide (0.153 g, 3.63 mmol) according to the general procedure. Purification by column chromatography afforded the target compound **16ak** (0.038 g, 95%) over two steps as a colorless oil: ^{1}H NMR (400 MHz, CD_3OD): δ = 8.50 (s, 1H), 7.48 (m, 2H), 7.33 (m, 4H), 7.22 (m, 3H), 6.76 (s, 1H), 5.31 (s, 2H), 2.87 (t, J = 7.6 Hz, 2H), 2.66 (t, J = 7.2 Hz, 2H), 1.85 (d, J = 1.2 Hz, 3H), 1.76 (m, 4H), 1.44 ppm (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ = 165.4, 162.9, 148.2, 146.6, 137.7, 133.7, 131.5, 130.8, 130.7, 130.2, 129.5, 128.7, 127.5, 126.0, 124.4, 122.8, (117.3, 117.1), 62.1, 29.6, 29.5, 29.0, 24.8, 24.5, 16.0 ppm; IR ν_{max} = 3349, 2923, 2855, 1683, 1515, 1456, 1226, 1159, 1066, 838, 701 cm^{-1}; λ_{max} = 204, 242 nm; HRMS (FAB) calcd for $C_{26}H_{29}FN_6$ $[M+H]^+$: 445.2510, found: 445.2508.

(*E*)-5-(Biphenyl-4-yl)-4-{5-[1-(2-methyl-3-phenylallyl)-1*H*-1,2,3-triazol-4-yl]pentyl}-1*H*-imidazol-2-amine (16am): Compound **15am** (0.059 g, 0.092 mmol) was treated with HCl (2 M, 0.92 mL), then reacted with cyanamide (0.154 g, 3.67 mmol) according to the general procedure. Purification by column chromatography afforded the target compound **16am** (0.026 g, 57%) over two steps as a color-

less oil: ^{1}H NMR (400 MHz, CD_3OD): $\delta = 8.41$ (s, 1H), 7.72 (d, $J =$ 8.4 Hz, 2H), 7.64 (m, 2H), 7.54 (d, $J = 8.0$ Hz, 2H), 7.46 (t, $J = 8.0$ Hz, 2H), 7.30 (m, 6H), 6.70 (s, 1H), 5.23 (s, 2H), 2.84 (t, $J = 8.0$ Hz, 2H), 2.74 (t, $J = 7.6$ Hz, 2H), 1.80 (d, $J = 1.2$ Hz, 3H), 1.74 (m, 4H), 1.45 ppm (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 148.3$, 146.8, 142.5, 141.3, 137.7, 133.4, 131.6, 130.2, 130.2, 129.5, 129.0, 128.8, 128.7, 128.7, 128.6, 128.0, 127.2, 124.6, 123.4, 61.8, 29.6, 29.4, 29.1, 25.0, 24.6, 15.9 ppm; IR $\nu_{max} = 3394$, 2918, 2847, 1957, 1683, 1456, 1121, 1024, 702 cm^{-1}; $\lambda_{max} = 204$, 240, 289 nm; HRMS (FAB) calcd for $C_{32}H_{34}N_6$ $[M+H]^+$: 503.2918, found: 503.2917.

Biological screening

Inhibition assays of selected compounds against MRSA and *A. baumannii*: Inhibition assays were performed by taking overnight cultures of MRSA or *A. baumannii* and subculturing at an OD_{600} of 0.01 into tryptic soy broth with a 0.5% glucose supplement (TSBG) for MRSA or Luria–Bertani (LB) media for *A. baumannii*. Stock solutions of predetermined concentrations of the test compound were prepared in the resulting bacterial suspension, and aliquots of 100 μL from stock solutions were distributed to the wells of a 96-well plate. Plates were covered, sealed (GLAD Press'n Seal film) and incubated under stationary conditions at 37 °C for 24 h. The media was then discarded, and the plates were washed thoroughly with H_2O. Each well was stained with crystal violet (CV; 110 μL, 0.1% solution) at room temperature for 30 min. After thorough washing with H_2O again, the remaining stain was dissolved in 95% EtOH (200 μL) and 125 μL was transferred to corresponding wells of a polystyrene microtiter dish. Biofilm inhibition was quantified by measuring the OD_{540} value of each well. Blank wells were employed as background controls.

Dispersion assays of selected compounds against *A. baumannii*: Dispersion assays were performed by taking overnight cultures of *A. baumannii* and subculturing at an OD_{600} of 0.01 into LB media. Aliquots (100 μL) of the resulting bacterial suspension were distributed to the wells of a 96-well plate. Plates were then wrapped (GLAD Press'n Seal film) followed by incubation under stationary conditions at 37 °C for 24 h. The media was then discarded from the wells, and the plates were washed thoroughly with H_2O. Stock solutions of predetermined concentrations of the test compound were prepared in LB, and 100 μL was transferred from stock solutions into the 96-well plate with preformed biofilms. Media alone was added as a control. Sample plates were incubated at 37 °C for 24 h. The media was discarded from the wells after incubation, and the plates were washed thoroughly with H_2O. Each well was stained with CV (110 μL, 0.1% solution) at room temperature for 30 min and then washed with H_2O again. The remaining stain in each well was dissolved in 95% EtOH (200 μL) and 125 μL was transferred to corresponding wells of a polystyrene microtiter dish. The biofilm dispersal effect was quantified by measuring the OD_{540} value of each well. Blank wells were employed as background controls.

Broth microdilution method for antibiotic resensitization: Mueller–Hinton broth (MHB) was inoculated (5×10^5 CFU mL^{-1}) with MRSA. Aliquots (4 mL) of the resulting bacterial suspension were distributed to culture tubes and compound, from 100 mM DMSO stock, was added to give the final testing concentration. Bacteria not treated with the tested 2-AI derivative served as the control. After sitting for 30 min at room temperature, 1 mL of each sample was transferred to a new culture tube and oxacillin sodium salt was added from 128 mg mL^{-1} H_2O stock to give a final concentration of 128 μg mL^{-1}. Rows 2–12 of a 96-well microtiter plate were filled (100 μL per well) from the remaining 3 mL bacterial subcultures, allowing the concentration of compound to be kept uniform throughout the antibiotic dilution procedure. After standing for 10 min, aliquots (200 μL) of the samples containing antibiotic were distributed to the corresponding first-row wells of the microtiter plate. Row 1 wells were mixed six to eight times, and then 100 μL were transferred to row 2. Row 2 wells were mixed six to eight times, followed by a 100 μL transfer from row 2 to row 3. This procedure was repeated to serially dilute the rest of the rows of the microtiter plate, with the exception of the final row, to which no antibiotic was added (to check for growth of bacteria in the presence of compound alone). The plate was then covered and incubated under stationary conditions at 37 °C. After 16 h, MIC values were recorded as the lowest concentration of antibiotic at which no visible growth of bacteria was observed.

Checkerboard assay: MHB was inoculated with MRSA (5×10^5 CFU mL^{-1}) and 100 μL aliquots were distributed to all wells of a 96-well plate except well 1A. Inoculated MHB (200 μL) containing compound (at 2× the highest concentration being tested) was added to well 1A, and 100 μL of the same sample was placed in each of wells 2A–12A. Column A wells were mixed six to eight times, and then 100 μL were withdrawn and transferred to column B. Column B wells were mixed six to eight times, followed by a 100 μL transfer to column C. This procedure was repeated to serially dilute the rest of the columns of the plate up to column G (column H was not mixed to allow determination of the MIC of antibiotic alone). Inoculated media (100 μL) containing antibiotic at 2× the highest concentration being tested was placed in wells A1–H1 and serially diluted in the same manner to row 11 (row 12 was not mixed to allow determination of the MIC of compound alone). The plates were incubated for 16 h at 37 °C. The MIC values (lowest concentration at which there was no visible growth of bacteria) of both compound and antibiotic in the combination were recorded, as well as the MIC values of compound alone (from row 12) and antibiotic alone (from column H). The ΣFIC values were calculated as follows: $\Sigma FIC = FIC_{Compd} + FIC_{Antibiotic}$, for which $FIC_{Compd} = [MIC_{Compd}$ in combination$]/[MIC_{Compd}$ alone$]$, and $FIC_{Antibiotic} = [MIC_{Antibiotic}$ in combination$]/[MIC_{Antibiotic}$ alone$]$. The combination is considered synergistic if $\Sigma FIC \leq 0.5$, indifferent if $0.5 < \Sigma FIC < 2$, and antagonistic if $\Sigma FIC \geq 2$.

Acknowledgements

The authors thank the UNCGA competitive research fund and the US DoD Defense Medical Research and Development Program (DMRDP, # W81XWH-11-2-0115) for support of this work. The DMRDP is administered by the Department of the Army; the US Army Medical Research Acquisition Activity [820 Chandler Street, Fort Detrick, MD 21702-5014 (USA)] is the awarding and administering acquisition office. The content of this manuscript does not necessarily reflect the position or the policy of the US Government, and no official endorsement should be inferred.

Keywords: aminoimidazoles · antibiotic resensitization · biofilms · MRSA · synergism

[1] J. A. Alanis, *Arch. Med. Res.* **2005**, *36*, 697–705.
[2] B. Spellberg, R. Guidos, D. Gilbert, J. Bradley, H. W. Boucher, W. M. Scheld, J. G. Bartlett, J. Edwards, *Clin. Infect. Dis.* **2008**, *46*, 155–164.
[3] T. C. Mah, G. A. O'Toole, *Trends Microbiol.* **2001**, *9*, 34–39.
[4] J. S. Dickschat, *Nat. Prod. Rep.* **2010**, *27*, 343–369.

[5] T. B. Rasmussen, M. Givskov, *Int. J. Med. Microbiol.* **2006**, *296*, 149 – 161.
[6] D. Davies, *Nat. Rev. Drug Discovery* **2003**, *2*, 114 – 122.
[7] N. Fusetani, *Nat. Prod. Rep.* **2004**, *21*, 94 – 104.
[8] J. J. Richards, R. W. Huigens, T. E. Ballard, A. Basso, J. Cavanagh, C. Melander, *Chem. Commun.* **2008**, 1698 – 1700.
[9] J. J. Richards, T. E. Ballard, C. Melander, *Org. Biomol. Chem.* **2008**, *6*, 1356 – 1363.
[10] S. A. Rogers, C. Melander, *Angew. Chem.* **2008**, *120*, 5307 – 5309; *Angew. Chem. Int. Ed.* **2008**, *47*, 5229 – 5231.
[11] S. Reyes, R. W. Huigens, Z. Su, M. L. Simon, C. Melander, *Org. Biomol. Chem.* **2011**, *9*, 3041 – 3049.
[12] J. J. Richards, S. Reyes, S. D. Stowe, A. T. Tucker, T. E. Ballard, J. Cavanagh, C. Melander, *J. Med. Chem.* **2009**, *52*, 4582 – 4585.
[13] T. E. Ballard, J. J. Richards, A. Aquino, C. S. Reed, C. Melander, *J. Org. Chem.* **2009**, *74*, 1755 – 1758.
[14] T. E. Ballard, J. J. Richards, A. L. Wolfe, C. Melander, *Chem. Eur. J.* **2008**, *14*, 10745 – 10761.
[15] J. J. Richards, C. S. Reed, C. Melander, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4325 – 4327.
[16] J. J. Richards, T. E. Ballard, R. W. Huigens, C. Melander, *ChemBioChem* **2008**, *9*, 1267 – 1279.
[17] S. A. Rogers, R. W. Huigens, J. Cavanagh, C. Melander, *Antimicrob. Agents Chemother.* **2010**, *54*, 2112 – 2118.
[18] Z. Su, S. A. Rogers, W. S. McCall, A. C. Smith, S. Ravishankar, T. Mullikin, C. Melander, *Org. Biomol. Chem.* **2010**, *8*, 2814 – 2822.
[19] G. A. O'Toole, R. Kolter, *Mol. Microbiol.* **1998**, *30*, 295 – 304.
[20] D. G. Davies, C. N. H. Marques, *J. Bacteriol.* **2009**, *191*, 1393 – 1403.
[21] C. Rappe, *Org. Synth.* **1973**, *53*, 123 – 126.
[22] G. Orhan, A. Bayram, Y. Zer, I. Balci, *J. Clin. Microbiol.* **2005**, *43*, 140 – 143.
[23] F. F. Knapp, P. C. Srivastava, A. P. Callahan, E. B. Cunningham, G. W. Kabalka, K. A. R. Sastryt, *J. Med. Chem.* **1984**, *27*, 57 – 63.
[24] M. Handa, K. A. Scheidt, M. Bossart, N. Zheng, W. R. Roush, *J. Org. Chem.* **2008**, *73*, 1031 – 1035.

Received: June 28, 2011
Revised: August 26, 2011
Published online on September 16, 2011

