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5-(2-Pyrimidinyl)-imidazo[1,2-*a*]pyridines are antibacterial agents targeting the ATPase domains of DNA gyrase and topoisomerase IV

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ABSTRACT

Dual inhibitors of bacterial gyrB and parE based on a 5-(2-pyrimidinyl)-imidazo[1,2-*a*]pyridine template exhibited MICs (μg/mL) of 0.06–64 (Sau), 0.25–64 (MRSA), 0.06–64 (Spy), 0.06–64 (Spn), and 0.03–64 (FQR Spn). Selected examples were efficacious in mouse sepsis and lung infection models at <50 mg/kg (PO dosing).

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Fluoroquinolone (FQ) inhibitors of bacterial DNA gyrase and topoisomerase IV have a long history characterized by the clinical and commercial successes of Ciprofloxacin, Levofloxacin, and newer generations of the FQ class. However, resistance to FQs has emerged[1] and signals the need for discovery of medicines effective against fluoroquinolone resistant (FQR) variants in the Gram-positive manifold where methicillin-resistant *Staphylococcus aureus* (MRSA) is already a serious threat. FQs bind in the 'Quinolone Resistance Determining Region' (QRDR) at the interface of the gyrA and gyrB subunits in DNA gyrase, or the parC and parE subunits of topoisomerase IV,[2] inducing cell death by a complex cascade of events involving cleavage of DNA.[3a] By contrast, coumerin natural products such as novobiocin, inhibit type II topoisomerases by binding in the ATPase domains of the gyrB subunit of DNA gyrase and the parE subunit of topoisomerase IV, inhibiting the ATP dependent step in the catalytic cycle.[3b–d] This leads to cell death by loss of the respective supercoiling or decatenation functions of DNA gyrase and topoisomerase IV. Dual gyrB/parE inhibitors, therefore, present an alternative mechanistic approach to a classical enzyme target. Exploiting this mechanism would allow use of the gyrase/topoisomerase IV pair as a therapeutic target while retaining activity against FQR mutant organisms.

The opportunity for dual target inhibition and the availability of high quality crystal structures[4] of gyrB and parE has encouraged numerous efforts to discover a new antibacterial drug based on the gyrB/parE dual mechanism. Reports by many research groups, among them, Vertex,[5] Dainippon,[6] Evotec,[7] Quorex,[8] Prolysis,[9] Astra Zeneca[10] and others,[11] have yielded an array of novel antibacterial chemical classes beyond the prototypical coumerin natural products (Fig. 1). Our own work in this area identified triazolopyridines[12] similar to those reported by Evotec, and, 5-(2-pyrimidinyl)-imidazo[1,2-*a*]pyridines (**1**)[13] (Fig. 2), the subject of this report. The latter are a novel class of dual gyrB/parE inhibitors that exhibit excellent performance against important Gram-positive pathogens including wild-type and methicillin-resistant *Staphylococcus*, and wild-type and FQR *Streptococcus* and possess desirable in vivo pharmacokinetic and efficacy properties.

Initially, 5-(2-pyrimidinyl)-imidazo[1,2-*a*]pyridines were synthesized according to the route outlined in Scheme 1.[14] Conversion

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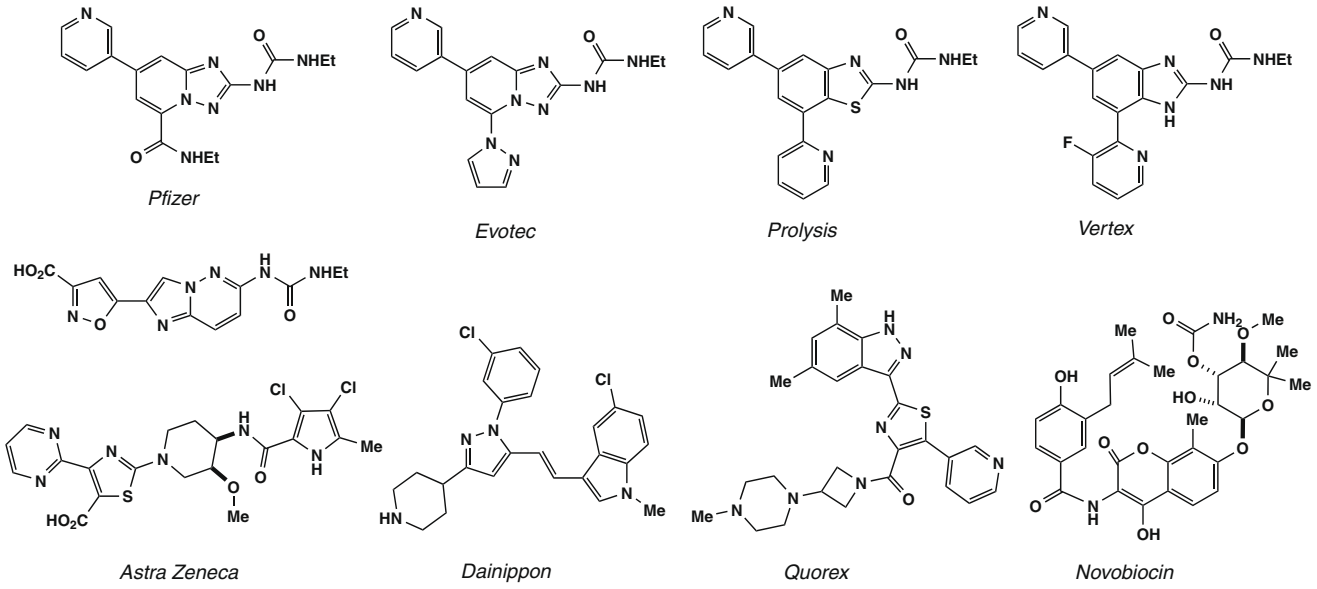


Figure 1. Examples of GyrB inhibitors.

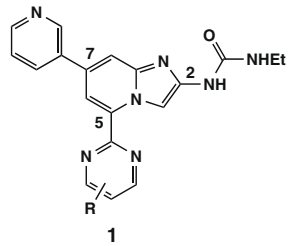
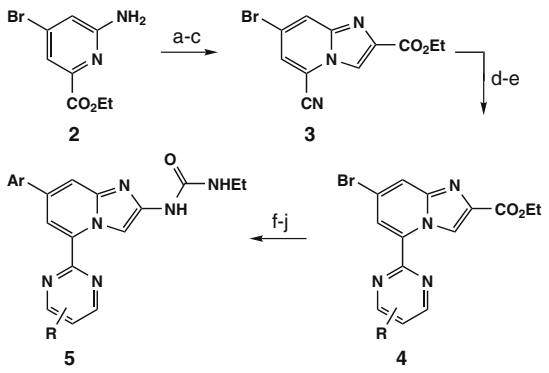


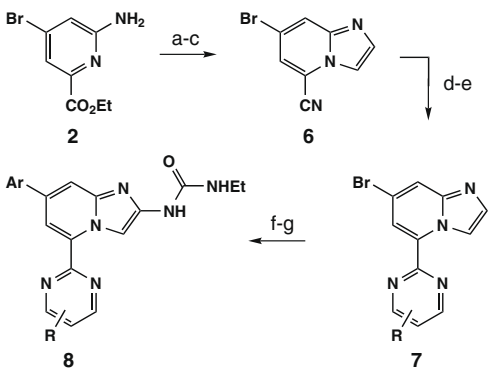
Figure 2. 5-(2-Pyrimidinyl)-imidazo[1,2-*a*]pyridines.



Scheme 1. Reagents and conditions: (a) ethylbrompyruvate, EtOH, 75–99%; (b) NH_3, MeOH, 60 °C, 74%; (c) TFAA, pyr., 82%; (d) NH_3, MeOH then NH_4Cl, reflux; (e) substituted β-aminoacrolein or 1,3-dicarbonyl, AcOH, reflux, 25–80%; (f) $ArB(OR)_2$, aq Na_2CO_3, $Pd(dppf)Cl_2$, 50–75%; (g) N_2H_4, MeOH, 88%; (h) $NaNO_2$, aq HCl, (i) trifluoroethanol, reflux; (j) 70% aq $EtNH_2$, DMSO, 80 °C, 25% over three steps.

of 2-amino-4-bromo-6-ethoxycarbonylpyridine **2** to the 2-ethoxycarbonyl-imidazopyridine derivative was accomplished by reaction with ethyl bromopyruvate in EtOH at 60 °C. The diethylester was selectively aminolyzed with ammonia in MeOH at the C5 position then dehydrated with TFAA in THF to give the corresponding C5 nitrile **3**. Formation of the derived methyl imidate under basic conditions (NH_3 or Et_3N in MeOH) followed by refluxing with ammonium chloride in MeOH gave the amidine intermediate which reacted with appropriately substituted 1,3-dicarbonyl synthons in refluxing AcOH to give pyrimidines **4**. Suzuki coupling with the appropriate boronic acid or boronate ester installed the desired heteroaryl substituent at C7. Hydrazinolysis of the remaining ethyl ester (N_2H_4, MeOH) followed by azidation ($NaNO_2$, aq HCl), Curtius rearrangement in trifluoroethanol, and aminolysis with ethylamine afforded final compounds **5**.

Some analogs were synthesized by the shorter route shown in Scheme 2.[15] Conversion of 2-amino-4-bromo-6-ethoxycarbonylpyridine **2** to the 2-H-imidazopyridine derivative was accomplished by reaction with chloroacetaldehyde (50% aq) in EtOH at 60 °C. The ethyl ester was aminolyzed with ammonia in MeOH at the C5 position then dehydrated with TFAA in THF to give the corresponding C5 nitrile **6**. Formation of the derived methyl imidate under basic conditions (DBU in MeOH) followed by refluxing with ammonium chloride in MeOH gave the amidine intermediate which reacted with appropriately substituted 1,3-dicarbonyl synthons in refluxing AcOH to give pyrimidines **7**. Suzuki coupling with the appropriate boronic acid or boronate ester installed the desired heteroaryl substituent at C7. Direct installation of the ethyl urea group at C2 by oxidation with Br_2 or NBS in the presence of an excess of ethyl urea completed the synthesis of final compounds **8**.



Scheme 2. Reagents and conditions: (a) chloroacetaldehyde, EtOH, 87%; (b) NH_3, MeOH, 60 °C, 85%; (c) TFAA, pyr., 60%; (d) DBU, MeOH then NH_4Cl, reflux, 76%; (e) substituted β-aminoacrolein or 1,3-dicarbonyl, AcOH, reflux, 65–85%; (f) $ArB(OR)_2$, aq. Na_2CO_3 or KF, $Pd(dppf)Cl_2$, THF, 60–90%; (g) Br_2 or NBS, $EtNHCONH_2$, ACN then aq $NaHSO_3$, 10–50%.

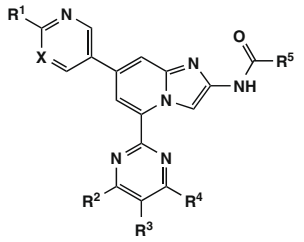
Yields for this one pot procedure for direct installation of the urea were typically modest (10–50%) but were competitive with the overall yields for the four step Curtius rearrangement sequence employed in the initial route (see Scheme 1). In practice, the direct route became the preferred method due to the fewer number of steps and greater tolerance of the 2-H intermediates for diverse chemistry en route to final compounds.

The MIC[16] and enzyme[17] potencies of 5-pyrimidinyl-imidazopyridines against Spn gyrB and parE, and five Gram positive organisms (Sau, MRSA, Spy, Spn, and FQR Spn), are shown in Tables 1 and 2. Variation of the 4, 5, and 6 positions of the C5-pyrimidine (R2, R3, R4), the heteroaryl group at C7 (R1, X), and the urea at C2 (R5) were investigated. Table 1 contains data for the C7-pyridyl and C7-pyrimidinyl analogs. In general, deviation from ethyl urea at C2 was found to be poorly tolerated (entries **22–26**, **30**) resulting in >4-fold loss of enzyme potency in Spn gyrB and parE and >4-fold loss of whole-cell activity across all the bacteria strains, compared with the ethyl urea analogs. Substitution at R3 (entries **10–16, 22–26, 28–29, 33, 35–37**) conveyed improved whole-cell potency over substitution at either R2 or R2 plus R4 (entries **17–21, 30–31**). However, tolerated substitution at R3 was limited to smaller axially symmetric groups or groups that could orient in plane with the heterocycle. Polar functional groups at R3 or those with forced 3-dimensional geometry (entries **12, 15–16**) exhibited reduced enzyme inhibition. These observations are consistent with docking in the active site of Eco gyrB where a lipophilic surface associated with the ILE-64 residue in the region of the ribose binding pocket interacts with the planar C5 substituent. This region is similar in the analogous domain of Eco parE.[4e]

Table 2 contains data obtained for C7-4-(2-pyridone) substituted analogs. Generally, C7 pyridones performed comparably to or slightly better than C7 pyridyl or pyrimidine analogs**,** however, greater disparity between Spn gyrB and parE inhibition was observed with this substitution. The C7 pyridone having an anionic group attached at the nitrogen (entry **42**) was atypical in that enzyme potency was not translated to whole-cell activity. Poor cell penetration may be prohibitive in this case.

Overall, achieving the goal of robust dual target pharmacology was challenging across the imidazopyridines shown here and balanced activity was seen only in isolated cases (entries **27, 29**). Typically, Spn parE inhibition trailed Spn gyrB inhibition by >4-fold and this gulf widened to >10-fold in the case of the C7-pyridones. Activity against MRSA (SA-1417) was similar to WT *Staphylococcus aureus* (UC76) for most compounds that were tested. It is noteworthy

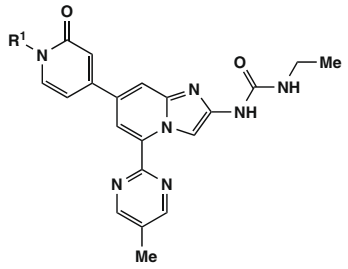
Table 1
Activity of selected C7-pyridyl and C7-pyrimidinyl examples



No.	Substitution						Spn IC_{50} (μM)		MIC[a] (μg/mL)				
	X	R1	R2	R3	R4	R5	gyrB	parE	S. au.	MRSA	S. py.	S. pn.	FQR S. pn.
Novobiocin							0.037	2.03	0.125	0.125[b]	8[b]	4[b]	4
9	CH	H	H	H	H	NHEt	0.048	0.447	0.5	0.5	0.25	0.125	0.25
10	CH	H	H	Me	H	NHEt	0.053	0.250	0.5	0.5	0.5	0.125	0.125
11	CH	H	H	CF_3	H	NHEt	0.163	0.367	1	1	1	0.5	0.25
12	CH	H	H	Et	H	NHEt	0.353	0.265	0.25	1	0.125	0.06	0.06
13	CH	H	H	F	H	NHEt	0.056	0.364	1		0.5	0.125	0.125
14	CH	H	H	Cl	H	NHEt	0.149	0.419	0.25	0.25	0.125	0.06	0.03
15	CH	H	H	SO_2Me	H	NHEt	0.433	1.83	64		64	8	16
16	CH	H	H	*i*Pr	H	NHEt	1.91	1.38	1		0.5	0.25	0.25
17	CH	H	CO_2Me	H	H	NHEt	0.052	0.911	8	8	32	16	16
18	CH	H	cPr	H	H	NHEt	0.33	3.75	0.5	0.5	1	0.125	0.25
19	CH	H	Me	H	Me	NHEt	0.198	0.541	1	4	1	0.5	0.5
20	CH	H	CF_3	H	Me	NHEt	0.216	0.546	0.5	1	0.5	0.25	0.125
21	CH	H	CF_3	H	CF_3	NHEt	0.242	0.745	2	4	1	0.25	0.125
22	CH	H	H	Me	H	NHMe	0.092	0.629	4		2	1	0.25
23	CH	H	H	Me	H	Et	0.255	5	64	>64	16	16	16
24	CH	H	H	Me	H	NHcPr	0.295	0.571	64	>64	16	1	0.25
25	CH	H	H	Me	H	NH*t*Bu	0.261	4.75	8		2	2	0.125
26	CH	H	H	Me	H	$NHCH_3CF_3$	0.368	0.504	8	4	16	0.25	0.125
27	CH	Me	H	H	H	NHEt	0.044	0.119	0.25		0.25	0.06	0.06
28	CH	Me	H	Me	H	NHEt	0.096	0.373	0.5	1	0.25	0.5	0.25
29	CH	OMe	H	Me	H	NHEt	0.117	0.147	0.5	2	0.5	0.25	0.125
30	N	Me_2N	Me	H	H	OEt	1.38	6.22	64		0.25	0.5	0.125
31	N	Me_2N	Me	H	H	NHEt	0.113	0.424	0.5		0.25	0.25	0.125
32	N	Me_2N	H	H	H	NHEt	0.137	0.611	1		0.25	0.125	0.125
33	N	Me_2N	H	Me	H	NHEt	0.524	1.38	1	4	0.5	0.25	0.125
34	N	H	H	H	H	NHEt	0.075	0.28	0.5		0.5	0.125	0.125
35	N	H	H	Me	H	NHEt	0.036	0.258	0.25	1	0.5	0.25	0.06
36	N	MeO	H	Me	H	NHEt	0.132	3.64	16	>64	32	16	16
37	N	C_4H_8N	H	Me	H	NHEt	0.244	0.366	0.5		0.25	0.125	0.06

[a] Strains: S. au. = *Staphylococcus aureus* UC76; MRSA = *Staphylococcus aureus* SA-1417; S. py. = *Streptococcus pyogenes* SP1-1; S. pn. = *Streptococcus pneumoniae* SP-3; FQR S. pn. = *Streptococcus pneumoniae* SP-3765. 'MIC' refers to minimum inhibitory concentration and is the lowest concentration at which bacterial growth is inhibited.
[b] MRSA = *Staphylococcus aureus* 01A-1095; S. py. = *Streptococcus pyogenes* C203; S. pn. = *Streptococcus pneumoniae* D39.

Table 2
Activity of selected C7-pyridone examples



No.	Substitution	Spn IC_{50} (μM)		MIC (μg/mL)				
	R1	gyrB	parE	S. au.	MRSA	S. py.	S. pn.	FQR S. pn.
38	Me			0.5		0.125	0.06	0.06
39	Et	0.051	0.624	0.25	0.5	1	0.06	0.125
40	*n*Pr	0.097	1.02	0.125	0.25	0.25	0.06	0.06
41	$MeOC_2H_4$	0.401	1.64	0.06	0.25	0.06	0.06	0.125
42	HO_2CCH_2	0.098	2.14	64	32	64	64	64
43	FC_2H_4	0.376	10	0.5	1	0.5	0.125	0.5

Table 3
In vivo activity and pharmacokinetics of selected compounds

No.	Mouse		Rat		
	Sepsis[a] (oral) PD_{50} (mg/kg)	Lung[b] (oral) PD_{50} (mg/kg)	Bioavailability (%F)	Clearance (mL/min/kg)	Volume of distribution (L/kg)
9	24	35	56	33	1.6
10	24	21	99	18	1.3

Infecting organism: (a) *Streptococcus pyogenes* SP1-1; (b) *Streptococcus pneumoniae* SV-1.

that some potentiation of effect in an FQR resistant strain (SP-3765) compared to wild-type occurred. Many compounds exhibited >2-fold lower MIC against a FQR Spn strain compared to WT, consistent with a fitness penalty associated with the FQR resistant DNA gyrase or topoisomerase IV enzymes. An agent possessing this property may be advantageous in patients at higher risk of FQR infection, in coadministration with a FQ agent, or as follow-on therapy in cases that are refractory to FQ treatment.

The 5-(2-pyrimidinyl)-imidazopyridines were efficacious in vivo against murine Spy sepsis and Spn Lung models. Table 3 shows selected examples with oral PD_{50} and rat PK parameters. Compound **10** was selected for additional study and will be highlighted individually in a future publication.

Acknowledgment

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14. For example, the synthesis of compound **32** is described in Ref. 13.
15. *For example, the synthesis of compound* **9**: (a) To a stirring solution of ethyl 6-amino-4-bromopicolinate (**2**) (30.11 g, 123 mmol) in 200 mL EtOH was added 50% aq chloroacetaldehyde (19 mL) and the reaction was heated to reflux. After 3 h, the solvent volume was reduced to ca. 100 mL in vacuo then the brown solution was slowly poured into dil. aq sodium bicarbonate. The resulting slurry was then vacuum filtered, the filter cake was rinsed 3 × water, 1 × ether, and dried to give 28.90 g (87%) of ethyl 7-bromoimidazo[1,2-*a*]pyridine-5-dicarboxylate as an amorphous powder. (b) A solution of ethyl 7-bromoimidazo[1,2-*a*]pyridine-5-dicarboxylate (15.0 g, 55.7 mmol) in 150 mL 2:1 THF/MeOH was treated with 150 mL 7 N NH_3/MeOH and the reaction was stirred at 23 °C for 3 h. The resulting copious precipitate was collected by vacuum filtration and the filtrate was allowed to continue reacting. The filter cake was rinsed 1 × MeOH and 1 × ether then dried to give a first crop of 7.09 g of amide product. After 24 h, additional precipitate had formed in the filtrate and an additional 4.33 g was repetition of the collecting procedure described above to give a combined yield of 11.42 g (85%) of 7-bromo-5-carboxamidoimidazo[1,2-*a*]pyridine. (c) To a suspension of 7-bromo-5-carboxamidoimidazo[1,2-*a*]pyridine (7.09 g, 29.8 mmol) in 200 mL dichloromethane at 0 °C was added triethylamine (16.5 mL, 118 mmol) followed by dropwise addition of trifluoroacetic anhydride (5.2 mL, 37 mmol). The reaction was allowed to warm to 23 °C and it gradually became a homogeneous bright yellow solution. After 2 h, the reaction was washed 2 × 1 N NaHSO4, 2 × satd aq sodium bicarbonate and dried over sodium sulfate. Evaporation in vacuo gave 5.59 g of crude product that was recrystallized from ethylacetate to give 3.96 g (60%) of 7-bromo-5-cyanoimidazo[1,2-*a*]pyridine (**6**). (d) To a suspension of 7-bromo-5-cyanoimidazo[1,2-*a*]pyridine (**6**) (3.15 g, 14.2 mmol) in 75 mL MeOH was added DBU (2.20 g, 14.5 mmol) and the reaction was stirred at 23 °C for 30 min. To the reaction was then added 2.60 g ammonium chloride and the reaction was heated to reflux. After 3 h, the reaction was uncapped and allowed to evaporate to 50% volume. It was then cooled to 23 °C and poured into water. The resulting slurry was vacuum filtered and the wetcake was rinsed 3 × water. To the filtrate was then added solid NaCl and the mixture was heated until the salt (and all other solids) dissolved. Cooling in the freezer resulted in copious precipitation. Vacuum filtration gave 1.31 g of tan solid after drying the filtercake. The mother liquor was allowed to stand in the freezer for two more days and a second crop of 1.27 g of tan solid was obtained. The combined solids were dried to give a total yield of 2.58 g (76%) of 7-bromoimidazo[1,2-a]pyridine-5-carboximidamide. (e) A mixture of 7-bromoimidazo[1,2-*a*]pyridine-5-carboximidamide (1.10 g, 4.64 mmol) and dimethylaminoacrolein (0.6 mL, 6 mmol) in 20 mL AcOH was refluxed for 3 h. The dark brown solution was then evaporated to a viscous residue that was diluted with water and poured into dil. aq sodium bicarbonate. The resulting yellow slurry was vacuum filtered and the collected solid was dried to give 0.824 g (65%) of 7-bromo-5-(pyrimidin-2-yl)imidazo[1,2-*a*]pyridine. (f) To a degassed solution of 7-bromo-5-(pyrimidin-2-yl)imidazo[1,2-*a*]pyridine (0.820 g, 2.98 mmol) and pyridine-3-boronic acid (0.550 g, 4.47 mmol) in 20 mL THF and 7.5 mL 2 N KF was added 0.041 g of $Pd(dppf)Cl_2$ and the reaction was heated to reflux for 3 h. The reaction was then cooled to 23 °C and evaporated in vacuo to give a brown residue that was partitioned between 1 M HCl and dichloromethane. The aqueous layer was collected and the organic layer was extracted 2 × 1 M HCl then the combined aqueous layers were washed 1 × dichloromethane. The combined aqueous layers were then neutralized with sodium bicarbonate and extracted 3 × dichloromethane. The combined organic extracts were dried over sodium sulfate, evaporated in vacuo, and purified by silica gel chromatography (2–10% isopropanol/dichloromethane) to give 0.540 g (66.3%) of 7-(pyridin-3-yl)-5-(pyrimidin-2-yl)imidazo[1,2-*a*]pyridine. (g) To a preformed solution of ethylurea (1.74 g, 19.7 mmol) and bromine (1.26 g, 7.90 mmol) in 20 mL dichloromethane was added 7-(pyridin-3-yl)-5-(pyrimidin-2-yl)imidazo[1,2-*a*]pyridine (0.540 g, 1.97 mmol) and the resulting red solution was stirred at rt for 1 h. It was then poured into ice-cold aqueous sodium hydrogen sulfite (200 mL × 0.050 g/ml) and the bright yellow reaction was neutralized with sodium bicarbonate and extracted 3 × 10% isopropanol/dichloromethane. The combined organic extracts were dried over sodium sulfate and evaporated then purified by silica gel chromatography (5–30–60% isopropanol/dichloromethane). Fractions containing desired product were combined and washed 2 × water to remove ethylurea and dried over sodium sulfate. The solution was filtered and to this was added a solution of ca. 0.3 mL concd HCl in 10 mL IPA. Evaporation in vacuo and drying under hivac overnight gave 0.318 g (37%) of compound **9** as the HCl salt. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.76 (s, 1H), 9.36 (s, 1H), 9.26 (s, 1H), 9.14 (s, 1H), 9.13 (s, 1H), 8.87 (d, J = 5.66 Hz, 2H), 8.59 (d, J = 1.95 Hz, 1H), 8.29 (d, J = 1.76 Hz, 1H), 8.02 (dd, J = 7.81, 5.85 Hz, 1H), 7.68 (t, J = 4.88 Hz, 1H), 3.16 (q, J = 7.22 Hz, 2H), 1.06 (t, 7.22, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 159.55, 158.56, 154.58, 143.95, 142.83, 142.80, 141.58, 141.08, 136.00, 134.01, 131.68, 127.22, 121.90, 115.95, 113.83, 101.39, 34.79, 16.05; LCMS purity: 95%, MS(API): M+H = 360.1.
16. Clinical and Laboratory Standards Institute *Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria*; Approved Standard, 6th ed.; NCCLS Document M11-A6; Vol. 24, No. 2.
17. For gyrB see: (a) Miller, J. R.; Herberg, J. T.; Tomilo, M.; McCroskey, M. C.; Feilmeier, B. J. *Anal. Biochem.* **2007**, *365*, 132; (b) For parE: Compounds to be tested were solvated at 10–30 mM in DMSO then diluted into 10 mM Tris pH 7.5, 9 mM $MgCl_2$, and 0.02% (v/v) Tween 20 and serial diluted. The serially diluted compounds (10 μL) were then transferred into a clear 384-well plate. To each well, 10 μL of a solution consisting of 20 mM Tris, pH 7.5, 120 mM KCl, 1.5 mM $MgCl_2$, 0.02% (v/v) Tween 20, 0.018% polyethyleneimine, 0.03% butylperflorosulfonate, and 66 μg/mL *Streptococcus pneumoniae* ParE. The reaction was initiated by addition of 10 μL of a solution containing 3 mM ATP and 0.02% Tween 20 and allowed to proceed for 30 min. The ATPase activity was quenched and orthophosphate detected by addition of 30 μL of a solution consisting of equal volumes of 4.2% (w/v) ammonium molybdate in 4 M HCl and 0.135% (w/v) malachite green in 20% (w/v) glycerol. The plate was briefly shaken and the absorbance at 650 nm was determined using a Spectramax plate reader (Molecular Devices).