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Novel 3-fluoro-6-methoxyquinoline derivatives as inhibitors of bacterial DNA gyrase and topoisomerase IV ☆

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ABSTRACT

Novel (non-fluoroquinolone) inhibitors of bacterial type II topoisomerases (NBTIs) are an emerging class of antibacterial agents. We report an optimized series of cyclobutylaryl-substituted NBTIs. Compound **14** demonstrated excellent activity both in vitro (*S. aureus* MIC_{90} = 0.125 μg/mL) and in vivo (systemic and tissue infections). Enhanced inhibition of Topoisomerase IV correlated with improved activity in *S. aureus* strains with mutations conferring resistance to NBTIs. Compound **14** also displayed an improved hERG IC_{50} of 85.9 μM and a favorable profile in the anesthetized guinea pig model.

Multidrug-resistant bacteria represent a grave threat to human health.[1] In the absence of significant innovations, estimates suggest that ten million people may die annually as a result of drug-resistant infections by 2050.[2] In recent years, multidrug-resistant infections caused by Gram-negative bacteria have attracted particular attention.[3] For example, novel plasmid-mediated mechanisms such as MCR-1 (colistin resistance)[4] and NDM-1 (carbapenem resistance)[5] have elicited substantial concerns. Infections caused by Gram-positive organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA)[6] also remain a "serious threat" (CDC classification),[7] causing more than 11,000 deaths annually according to a 2013 report.[8] The approval of new therapies such as tedizolid[9] and dalbavancin,[10] as well as the persistent low level of resistance to linezolid in *S. aureus*,[11] give reason for optimism. Nevertheless, new options for the treatment of *S. aureus* (especially MRSA) infections are needed, given their highly significant role in both the healthcare-associated and community settings[6c]

Novel Bacterial Type II Topoisomerase Inhibitors (NBTIs) represent one promising approach to tackling this problem.[12–26] Compounds from this antibacterial class inhibit both DNA gyrase and topoisomerase IV (TopoIV) in *S. aureus*, with the available evidence indicating superior inhibition of DNA gyrase in this pathogen.[16a,18,21,22,23,24a,24h,27] Importantly, work from several

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groups has established the absence of cross-resistance to the fluoroquinolone class,[15a,16a,18,20c,21,22,23,24,25b,25c,26] offering the potential for a new therapeutic option without extensive preexisting resistance in the clinic. Groundbreaking structural efforts from GSK,[18] coupled with detailed analyses of mutations conferring resistance at Novexel[16a] and AstraZeneca,[27] have established the binding site for the NBTIs as well as demonstrated mechanistic differences from the fluoroquinolones. Efforts from numerous researchers have culminated in several highly optimized agents, for example AVE6971[14] (**1**, Fig. 1), NXL-101[16] (**2**), AZD9742[20c,28] (**3**), GSK945237[19c] (**4**), gepotidacin[25] (**5**, formerly GSK2140944), GSK966587[17] (**6**), AM8191[24a] (**7**), and ACT-387042[22b,22c] (**8**). Compounds **1**–**5** all advanced to clinical trials. Gepotidacin remains in active development,[25e] having completed a Phase 2 efficacy trial in acute bacterial skin and skin structure infections (ABSSSIs) caused by Gram-positive pathogens, in particular MRSA.[29] Recent reports also demonstrate substantial antibacterial activity for **5** for other bacterial pathogens.[30] An efficacy trial of **5** in uncomplicated urogenital gonorrhea caused by *Neisseria gonorrhoeae* has also been completed.[31]

We previously described a novel cyclobutyl-linked series of DNA gyrase inhibitors, typified by compounds **9**–**11** (Figure 2).[23] Our studies, as well as other published reports, identified several key issues for further optimization. hERG inhibition, with attendant concerns about QT prolongation and cardiac arrythmias, constitutes perhaps the most significant challenge in developing novel NBTIs.[17b,19,20,21,22,23,24,26] QT prolongation caused the attrition of **2** from clinical trials,[16c] and gepotidacin has also shown QT prolongation in human volunteers at maximal plasma concentrations of 7–13 μg/mL, despite minimal hERG inhibition (IC_{50} = 588 μg/mL).[25e] Additionally, single step mutations in DNA gyrase in *S. aureus* give rise to substantially reduced antibacterial activity for NBTIs against such mutant organisms.[16a,22,23,24h,27] Target-based mutations are the primary mechanism of fluoroquinolone resistance,[32] and resistance of MRSA to ciprofloxacin arose very rapidly,[33] illustrating the urgency of this issue. Our previous work[23] and that of other authors[22a,22b] demonstrates that this issue can be ameliorated through a dual-targeting[34] approach involving enhanced inhibition of the secondary NBTI target, TopoIV. In this paper, we describe utilization of a fluorinated "left hand side" (LHS), coupled with our previous learnings, to address these key objectives (compounds **12**–**26**).

These efforts were facilitated by a synthetic route that was readily amenable to multigram-scale synthesis (Scheme 1).[35] The known ester **27**[12] was reduced with DIBAL-H to afford the corresponding aldehyde **28**. Fluoroquinoline **30**, prepared by hydrogenolysis of the dichloroquinoline **29**,[36,37] was lithiated with lithium diisopropylamide (LDA) and reacted with **28** to afford the derivatized fluoroquinoline intermediate **31** in good yield (57% over 2 steps). Following chiral chromatography, the olefin was oxidized in 2 steps to the carboxylic acid and the BOC group deprotected to afford the secondary amine in 70% yield over three steps. Reductive amination afforded compound **14** in good yield on >25 g scale. While many analogs were studied as mixtures of *cis* and *trans* cyclobutyl isomers, the pure diastereomers could also be readily obtained via chiral chromatography.

As mentioned, several groups have demonstrated that *S. aureus* can evolve resistance to NBTIs by single-step mutations in DNA gyrase, for example at D83 or M121.[16a,22a,22b,23,24h,27] Additionally, two *S. aureus* strains carrying D83 N mutations were isolated *prior to therapy* during the Phase 2 ABSSSI trial of gepotidacin.[29b,29c] These isolates showed dramatically reduced susceptibility to gepotidacin, with minimum inhibitory concentrations (MICs) of 8 and >32 μg/mL.[29b,29c] Although these infections were successfully

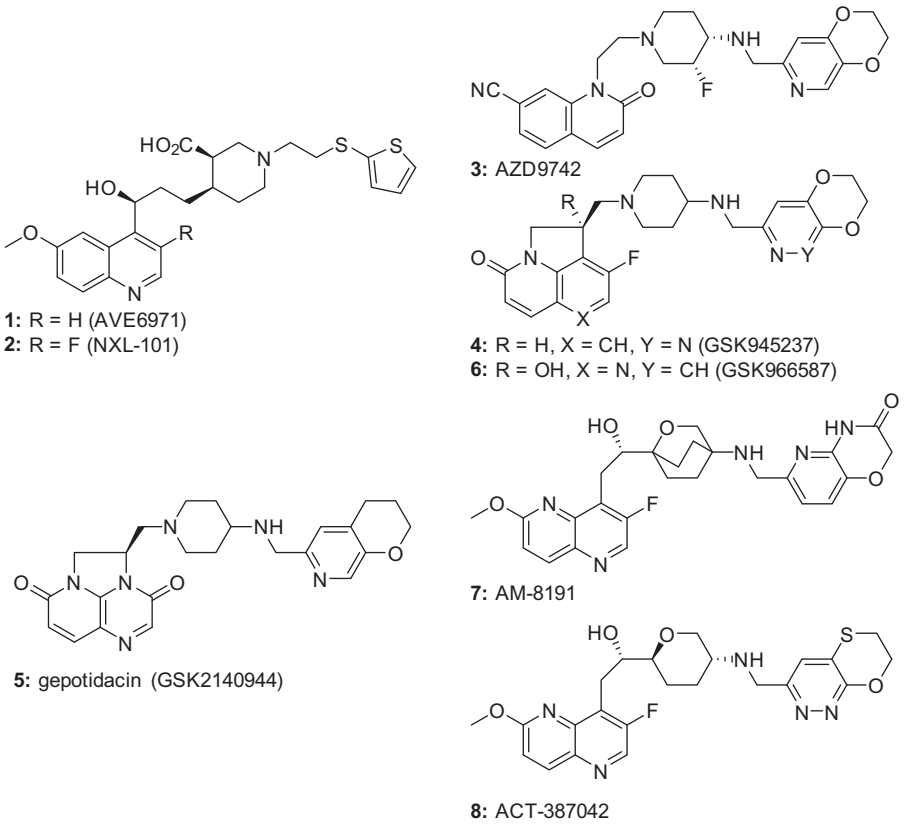


Fig. 1. Representative optimized Novel Bacterial Type II Topoisomerase Inhibitors (NBTIs).

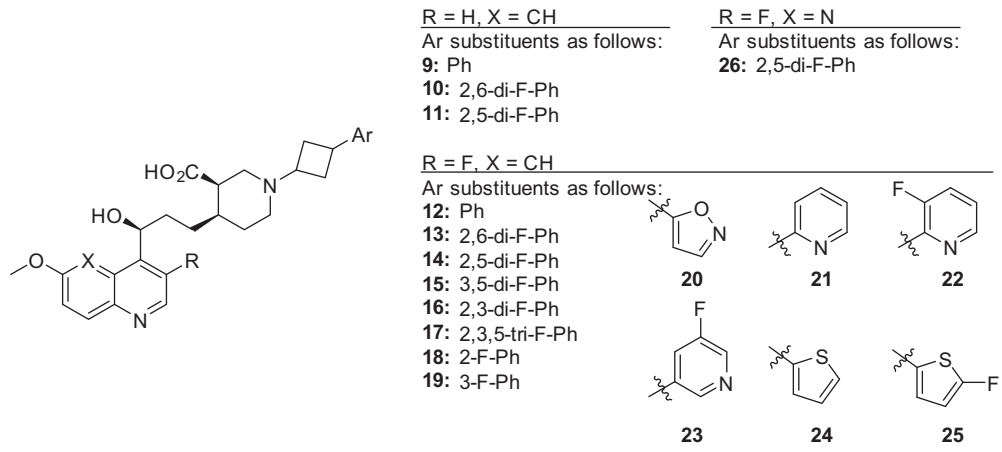
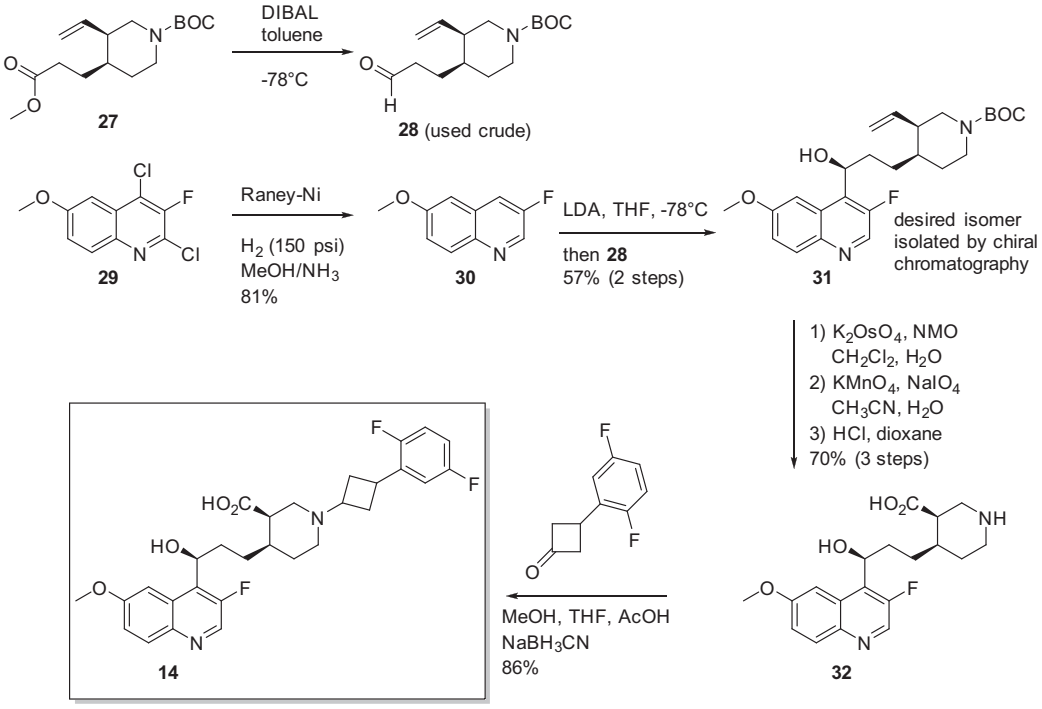


Fig. 2. Arylcyclobutyl series of NBTIs.



Scheme 1.

treated, the presence of *S. aureus* isolates in the clinic with this mutation highlights the need for improved potency versus TopoIV.

Fluorination is a commonly explored strategy in medicinal chemistry,[38] including for NBTI LHS moieties.[16a,17,19b,19c,22,23,24,26] Compound **2** with its 3-fluoroquinoline LHS shows moderately improved TopoIV inhibition versus quinoline **1** as well as a modest but reproducible improvement in minimum inhibitory concentrations (MICs) against the mutant organisms (Table 1). Surivet and coworkers have also demonstrated such improvements using a fluorinated 1,5-naphthyridine LHS.[22b] Given that this substitution did not cause any notable undesirable changes in properties, we synthesized a series of fluoroquinoline analogs within our cyclobutylaryl series of inhibitors.

Our work began with 3-fluoroquinoline analogs of several of the more promising compounds we previously reported.[23] Given our focus on developing dual inhibitors of DNA gyrase and TopoIV, we assayed both targets in early screening. In order to ensure continued whole cell activity, we also utilized previously reported[23] fluoroquinolone-sensitive (1146) and fluoroquinolone-resistant (1095) strains of *S. aureus* for MIC determination. MICs against mutant strains of *S. aureus* possessing either the D83 N or M121 K mutations to DNA gyrase were also routinely determined. Our earlier work demonstrated that these mutations completely abrogated inhibition of DNA gyrase,[23] so assaying MICs against these mutant organisms enabled a direct assessment of the impact of TopoIV inhibition on whole cell antibacterial activity.

Initial results are shown in Table 1. By comparing matched pairs of analogs (**1** and **2**, **9** and **12**, **10** and **13**, and **11** and **14**), it is clear that the 3-fluoroquinoline substitution usually improves inhibitory activity against both targets and provides superior MICs against the first-step mutant organisms. Nevertheless, the preferred target in every case remained DNA gyrase. The comparator agent GSK966587 (**6**) also shows preferential inhibition of DNA gyrase,

Table 1
Target inhibition and minimum inhibitory concentrations in *Staphylococcus aureus*.[a]

Compound	*S. aureus* TopoIV IC_{50} (μM)	*S. aureus* DNA gyrase IC_{50} (μM)	*S. aureus* 1146 MIC (μg/mL)	*S. aureus* 1095 MIC (μg/mL)	*S. aureus* D83N MIC (μg/mL)	*S. aureus* M121K MIC (μg/mL)
1	84.6 (n = 11)	2.42 (n = 39)	0.068 (n = 58)	0.10 (n = 58)	>64 (n = 9)	>64 (n = 7)
2	30.4 (n = 2)	1.56 (n = 2)	≤0.063 to 0.125 (n = 2)	0.125 (n = 2)	32 (n = 3)	64
6	4.96 (n = 3)	0.448 (n = 3)	0.125 (n = 3)	0.31 (n = 3)	4 (n = 4)	8
9	32.1 (n = 60)	1.19 (n = 77)	0.09 (n = 68)	0.20 (n = 68)	≥64 (n = 59)	≥64 (n = 16)
10	100 to >120 (n = 3)	2.93 (n = 4)	0.125	0.25	32 (n = 4)	23 (n = 2)
11	9.62 (n = 5)	3.00 (n = 4)	0.25	0.5	10 (n = 3)	5.7 (n = 2)
12	15.9 (n = 4)	1.15 (n = 4)	0.069 (n = 7)	0.125 (n = 7)	22.6 (n = 6)	8 (n = 3)
13	18.3 (n = 4)	4.61 (n = 4)	≤0.063 (n = 3)	0.079 (n = 3)	3.2 (n = 3)	2.8 (n = 2)
14	5.47 (n = 5)	0.977 (n = 5)	0.088 (n = 6)	0.11	3.4 (n = 4)	2
15	6.25	1.2	0.125 (n = 2)	0.35 (n = 2)	5.7 (n = 2)	4
16	7.5	0.78 (n = 3)	≤0.0625	≤0.0625	8	4
17	4.7	1.56	≤0.0625	≤0.0625	1	2
18	19.0	0.78	≤0.0625	0.125	16 (n = 2)	16
19	19.0	0.78	≤0.0625	0.25	16 (n = 2)	16
20	35.1 (n = 3)	2.68 (n = 2)	0.5	4	>64	NT[b]
21	50	3.13	1	16	>64	NT
22	30	6.25	0.125	0.5	32 (n = 2)	32
23	>120	4.7	2	32	>64	NT
24	9.4	0.78	≤0.0625	0.125	11.3 (n = 2)	16
25	19	0.78	≤0.0625	≤0.0625	16 (n = 2)	16
26	5.09 (n = 3)	1.43 (n = 3)	≤0.0625 (n = 6)	0.088 (n = 6)	2.5 (n = 10)	2.3 (n = 5)

[a] Data from multiple runs are given as geometric means unless otherwise indicated.
[b] NT = not tested.

Table 2
hERG IC_{50} values for selected compounds.

Compound	hERG IC_{50} of 3-F-quinoline (μM)	Compound	hERG IC_{50} of 3-H-quinoline (μM)
2	23.6	**1**	19.7
6	>300	**N/A**	N/A
12	22.3	**9**	24.9
13	45.3	**10**	47.6
14	85.9	**11**	9.97
15	14.1	**N/A**	7.18 (compound **31** from reference 23)
16	25.0	**N/A**	N/A
20	>300	**N/A**	N/A
22	10.7	N/A	N/A
26	45.0	N/A	N/A

Table 3
MIC_{90} values for representative Gram-positive pathogens.

Compound	*S. aureus* MIC_{90} (μg/mL) (n = 11 strains)	*S. pyogenes* MIC_{90} (μg/mL) (n = 11 strains)
9	0.25–1 (5 separate experiments)	1–2 (5 separate experiments)
10	0.25	1
11	0.25	1
2	0.125	0.25
12	0.25	1
13	0.125	0.5
14	0.125 (2 separate experiments)	0.25–0.5 (2 separate experiments)
15	0.25	1
18	0.125	0.5
19	0.25	0.5
22	1	2
24	0.125	0.5
25	0.125	0.5
26	0.125–0.25 (3 separate experiments)	0.5–1 (3 separate experiments)
6	0.5	0.125

and the diminished potency against the D83N mutant organism is consistent with data in the literature.[24h]

Our prior results suggested that fluorination of the "right hand side" (RHS) aryl ring was particularly promising.[23] Accordingly, we synthesized a variety of derivatives changing both the position and degree of fluorination of the RHS. Monofluorination did not confer any advantage (**12** versus **18** or **19**). Four regioisomeric difluorinated analogs were prepared (**13**–**16**), and each demonstrated improved whole cell activity against the mutant organisms, generally consistent with improved TopoIV inhibition. Compound **13** appears to be an outlier in this regard, but the underlying reasons are not understood. The 2,3,5-trifluorophenyl analog **17** demonstrated the most potent inhibition of TopoIV and the best MIC values against the first step mutant organisms. Unfortunately, the additional lipophilicity led to unacceptable rates of metabolism in vitro (data not shown).

We also explored isosteric replacements of the RHS phenyl ring with pyridine (**21**) or thiophene (**24**) rings. These compounds, as well as monofluorinated analogs (**22**, **23**, and **25**) proved inferior. Similarly, replacement of the phenyl RHS with an isoxazole (**20**) resulted in diminished potency at the enzyme and whole cell levels, consistent with our earlier report.[23] Finally, having identified the 2,5-difluorophenyl RHS (**14**) as particularly promising, we synthesized the corresponding fluorinated 1,5-naphthyridine LHS analog (**26**). This compound proved essentially equipotent to **14.**

The results from enzymatic and microbiological screening demonstrated improved TopoIV inhibition and better whole cell activity versus the first step mutant organisms. Given the critical importance of minimal hERG inhibition for the NBTIs, we profiled a large number of compounds in a patch clamp assay using the PatchXpress platform (Table 2). GSK966587 (**6**) showed minimal hERG inhibition (IC_{50} > 300 μM), similar to the previously reported IC_{50} (239 μM).[17b] While it negatively impacted potency, the more polar isoxazole showed an attractive hERG profile (IC_{50} > 300 μM), whereas the fluoropyridine **22** did not. In general, hERG IC_{50} values were not substantially different between quinoline and 3-fluoroquinoline analogs, with the exception being compound **14** (IC_{50} 85.9 μM versus 9.97 μM for **11**). Compound **14** was also superior to the analogous 1,5-naphthyridine **26**. Compound **14** was consequently tested in an anesthetized guinea pig model[39,40] to further assess the potential for QT prolongation via measurements of monophasic action potential duration (MAPD) and cardiac alternans (ALT). At the highest concentration studied ($C_{unbound}$ = 27.5 μM), there was no evidence of prolonged MAPD

Table 4
Plasma protein binding for representative compounds.[a]

Compound	Fu mouse	Fu rat	Fu human
9	0.129–0.246	0.296–0.437	0.079–0.155
12	0.150–0.158	0.277–0.334	0.122–0.211
14	0.201	0.197	0.054
26	0.131–0.242	0.121–0.189	0.036–0.037

[a] Fu = fraction unbound.

Table 5
Pharmacokinetic data.[a]

Compound	Mouse Cl[b] (mL/min/kg)	Mouse Vdss L/kg	Mouse $t_{1/2}$ (h)[c]	Rat Cl[d] (mL/min/kg)	Rat Vdss L/kg	Rat $t_{1/2}$ (h)[c]
9	36.0	1.06	0.34	43.1	1.24	0.33
12	17.3	1.01	0.67	18.4	1.82	1.14
14	13.7	1.24	1.05	30.9	1.82	0.68
26	34	0.825	0.28	32.7	1.89	0.67

[a] Cl = clearance, Vdss = volume of distribution at steady state.
[b] Dosed IV at 10 mg/kg.
[c] Half-life calculated using $t_{1/2}$ = 0.693*Vdss/Cl.
[d] Dosed IV at 2 mg/kg.

Table 6
In vivo efficacy results.[a]

Compound	FQ^R-MRSA septicemia (PD_{50}, mg/kg)	FQ^R-MRSA neutropenic thigh (static dose, mg/kg/day)
9	6.9–8.1 (n = 2)	123.1
12	9.2–12.5 (n = 2)	92.1
14	12.5–39.4 (n = 3)	79.7–82.8 (n = 2)
26	9.9–15.9 (n = 3)	80.7
vancomycin	6.3–7.7 (n = 2)	29.0

[a] FQ^R = fluoroquinolone-resistant.

or increased ALT (data not shown).[41] Data for gepotidacin in this model have not been published, but evidence for QT prolongation was observed in a rabbit left ventricular wedge *ex vivo* model at concentrations ≥135 μg/mL and in a cardiovascular study carried out in cynomolgus monkeys at 1.2- to 7.0-fold the expected human C_{max}.[25e] NXL-101 (**2**) exhibited more significant QT_c prolongation in clinical trials than had been seen in preclinical studies.[16c] Given the modest hERG improvement for **14** as compared to **2** (3.6-fold), the potential translation of our promising preclinical data to the clinical setting is uncertain.

Compound **14** was identified as a promising lead in terms of TopoIV inhibition, MICs against first step mutants of *S. aureus*, and hERG, and we profiled it and several related analogs in MIC_{90} studies. Compound **14** as well as **13**, **18**, **19**, and **24**–**26** displayed sub-single digit MIC_{90} values against both *S. aureus* and *S. pyogenes* (Table 3), demonstrating excellent potency against these critical Gram-positive bacteria.

Pharmacokinetic data were also obtained for **14** and other compounds. Protein binding was moderate across species for several analogs (**9**, **12**, **14**, and **26**), although typically somewhat higher for humans (Table 4). Compound **14** displayed moderate clearance and modest volume of distribution in both mouse and rat,[39] consistent with other analogs from this series (Table 5).

Finally, several compounds were profiled in murine efficacy studies,[39] demonstrating translation from in vitro to in vivo activity. Compound **14** demonstrated in vivo efficacy in a systemic model of infection[23] with fluoroquinolone-resistant *S. aureus*, with a PD_{50} of 12.5–39.4 mg/kg over multiple trials (Table 6). Compound **14** was assayed twice in the murine neutropenic thigh model,[42] again using fluoroquinolone-resistant *S. aureus*. We (see Article footnote) and others have previously reported fAUC/MIC as the primary PK/PD driver for the NBTIs. The dose required for bacterial stasis in this model was 79.7–82.8 mg/kg, corresponding to an fAUC/MIC of 39. This value is similar to that reported for other NBTIs, notably 36 for AZD9742[28] and the mean value of 43 for ACT-387042 in a study of five *S. aureus* isolates.[22c] The median value for gepotidacin in this model (six isolates) was somewhat lower (13.4).[25d] Compounds **12** and **26** were similarly efficacious to **14** in this tissue infection model.

In summary, we have reported a series of cyclobutylaryl NBTIs possessing fluorinated quinoline and 1,5-naphthyridine LHS moieties. Extensive profiling both in vitro and in vivo enabled the identification of the 2,5-difluorophenyl analog **14** as the most promising lead compound. Improved activity against both the primary (DNA gyrase) and secondary (TopoIV) targets was achieved, and improved inhibition of TopoIV translated into superior whole cell activity against *S. aureus* mutants possessing either D83 N or M121 K mutations in DNA gyrase. To further diminish the potential for resistance in the clinical setting, additional improvements in TopoIV potency are desirable. Finally, the hERG activity of compound **14** was attenuated relative to earlier analogs, and this finding translated into a lack of MAPD prolongation or cardiac alternans in the anesthetized guinea pig.

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