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1,3-Dioxane-Linked Novel Bacterial Topoisomerase Inhibitors: Expanding Structural Diversity and the Antibacterial Spectrum

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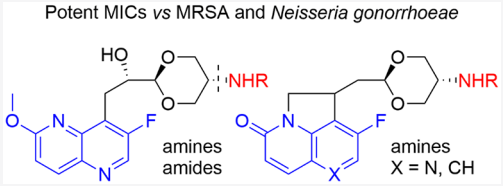
ABSTRACT: Antibacterial resistance continues its devastation of available therapies. Novel bacterial topoisomerase inhibitors (NBTIs) offer one solution to this critical issue. Two series of amine NBTIs bearing tricyclic DNA-binding moieties as well as amide NBTIs with a bicyclic DNA-binding moiety were synthesized and evaluated against methicillin-resistant *Staphylococcus aureus* (MRSA). Additionally, these compounds and a series of bicyclic amine analogues displayed high activity against susceptible and drug-resistant *Neisseria gonorrhoeae*, expanding the spectrum of these dioxane-linked NBTIs.



KEYWORDS: *MRSA, gonorrhea, antibacterial, gyrase, TopoIV, hERG*

Despite advances in public health, bacterial infections continue to extract a tremendous toll. Antibacterial-resistant infections present a particular threat because of their continued evolution and dissemination and limited treatment options and the frequently higher toxicity of available therapies. In the United States, the Centers for Disease Control and Prevention estimates nearly 3 million yearly cases of antibiotic-resistant infections, 35 000 deaths, and billions of dollars of attributable medical costs.[1] Novel bacterial topoisomerase inhibitors (NBTIs)[2,3] target DNA gyrase and topoisomerase IV (TopoIV), enzymes that are also targeted by fluoroquinolones.[4,5] The distinctive binding mode and differential pharmacology of the NBTIs[6−8] generally avoid cross-resistance with fluoroquinolones and other therapies, offering an exciting tool for antibiotic-resistant infections. The most advanced clinical candidate, gepotidacin, exhibits promising efficacy in patients against diverse pathogens, including *Escherichia coli*,[9] methicillin-resistant *Staphylococcus aureus* (MRSA),[10] and the "urgent" (highest CDC threat level) *Neisseria gonorrhoeae*.[11]

During our research, we synthesized a promising series of bicyclic fluoronaphthyridine NBTIs and identified **1c** as a lead with potent antibacterial activity and *in vivo* efficacy against MRSA (Figure 1).[12] However, **1c** lacked potent activity against Gram-negative pathogens and had a relatively short half-life in mice (38 min *in vivo* and 18−26 min *in vitro* in microsomes). We hypothesized that additional polarity and removal of one or more rotatable bonds would enhance the metabolic stability and might improve the activity[13] against the Gram-negative pathogen *N. gonorrhoeae* while preserving the activity against MRSA. To address these hypotheses, we report herein a series of NBTIs bearing a previously reported diazatricyclic DNA-binding moiety (compounds **2a−c**, **2e**, and **2f**).[14,15] Furthermore, our prior experience[12] and evidence from the literature[14,15] suggested that this modification would also reduce hERG inhibition, a cardiovascular safety liability common to NBTIs.[2,3,16] To discern whether structure−property relationships were intrinsic to the tricyclic DNA-binding motif or merely the result of increased polarity, we synthesized structure-matched analogues with a more lipophilic azatricyclic motif[17] (**3a−e**). Additionally, we prepared a small number of amide derivatives bearing the bicyclic fluoronaphthyridine DNA-binding moiety (**4a**, **4e**, and **4f**). Such amides have one less rotatable bond compared with amines, and other amides developed by our group have demonstrated reduced hERG inhibition.[18] We report the antistaphylococcal activity and topoisomerase inhibition of these new compounds. In addition, we determined their potency alongside earlier amines[12] against *N. gonorrhoeae*. Finally, we determined the *in vitro* metabolic stability and hERG inhibition of these new analogues.

The racemic synthesis of both tricyclic series (Scheme 1) followed the same general approach and employed a reductive amination in the final step to introduce diversity in the

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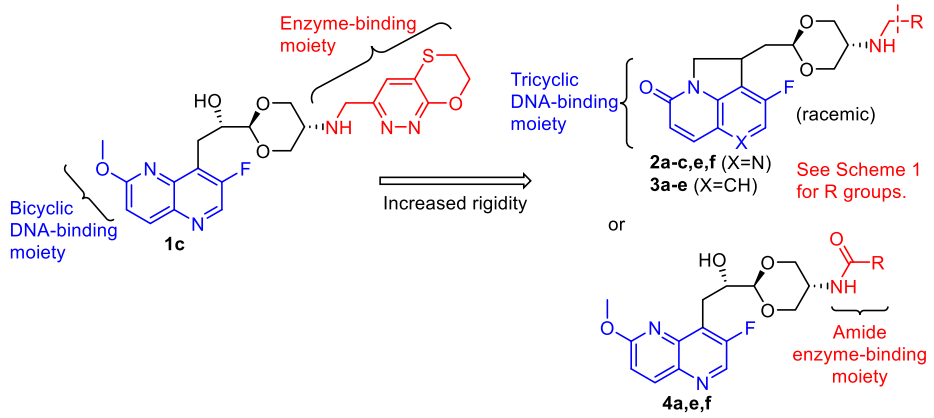
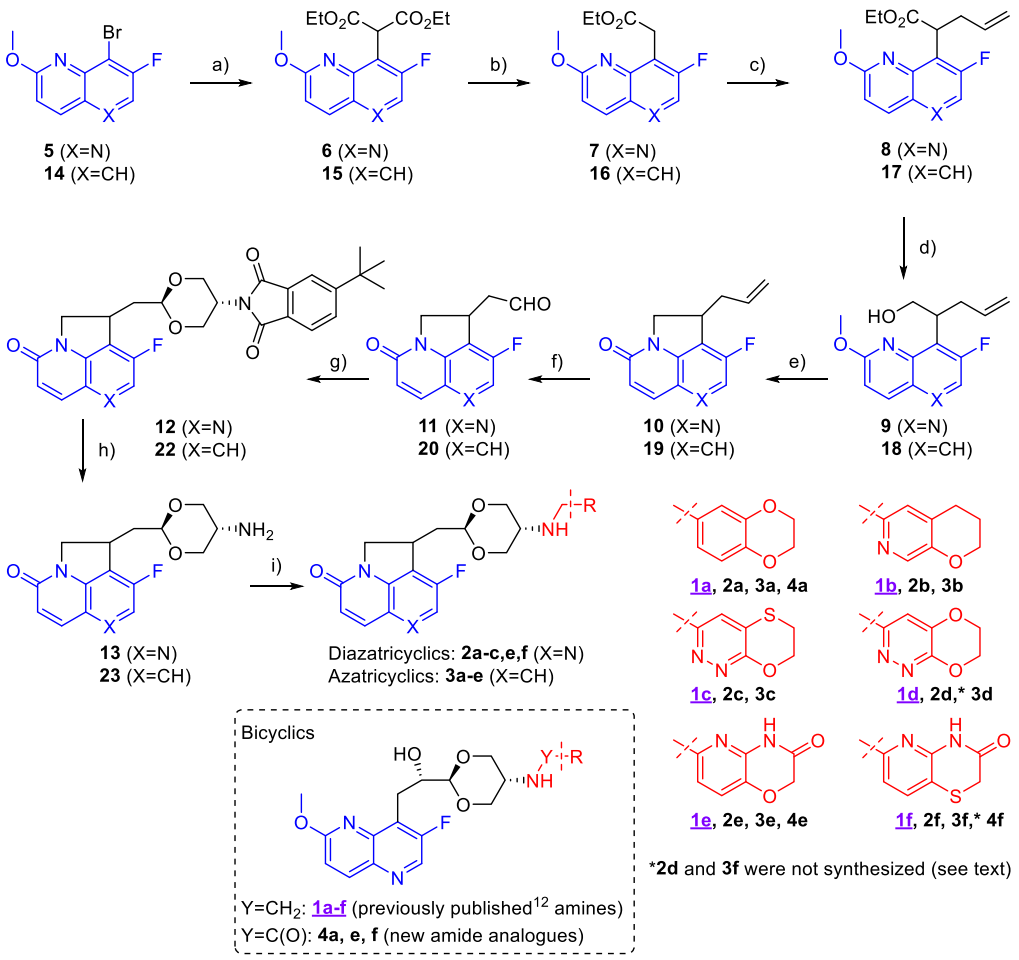


Figure 1. Anti-MRSA lead compound **1c**[12] and molecular design strategy.

Scheme 1. Synthesis of NBTIs with Tricyclic DNA-Binding Moieties[a]



[a]For azatricycles **3a−e**: (a) Diethyl malonate, NaH, dioxane, rt, 15 min, then 80 °C, 1 h followed by **14**, CuBr, 100 °C, overnight, 41.5−80.7%. (b) LiCl, DMSO, H_2O, 110 °C, overnight, 23.8−26.5%. (c) LHMDS, THF, −78 °C, 1 h, then allyl bromide, −78 °C to rt, overnight, 99.3%. (d) $LiAlH_4$, THF, rt, 1.5 h, 72.6%. (e) MsCl, NEt_3, $CHCl_3$, 0 °C, 1 h, then 60 °C, overnight, 92.2%. (f) $NaIO_4$, THF, H_2O, rt, 15 min, then OsO_4 in *tert*-butanol, rt, overnight, 78.1%. (g) 5-(*tert*-Butyl)-2-(1,3-dihydroxypropan-2-yl)isoindoline-1,3-dione (**21**), *p*-TsOH, toluene, 110 °C, overnight; (h) ethylenediamine, EtOAc, 70 °C, overnight, 46.9% over two steps. (i) RCHO, 4 Å molecular sieves, THF, MeOH, AcOH, rt, 4 h, then $NaBH_3CN$. For diazatricycles **2a−c**, **2e**, and **2f** and amides **4a**, **4e**, and **4f**, see the Supporting Information.

enzyme-binding domain. The first two steps utilized chemistry reported by Singh *et al.*[15] The preparation of **3a−e** is representative. Deprotonation of diethyl malonate with NaH and coupling to known bromoquinoline **14** under promotion by CuBr afforded diester **15**. Krapcho decarboxylation[19] provided ester **16**, which was deprotonated with LiHMDS and alkylated with allyl bromide to afford **17**. Reduction with $LiAlH_4$ yielded alcohol **18**, which underwent activation and subsequent cyclization with methanesulfonyl chloride to form the azatricyclic system in **19**. Lemieux−Johnson oxidation[20]

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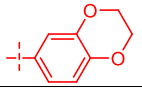
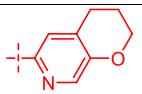
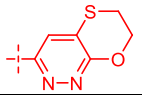
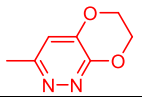
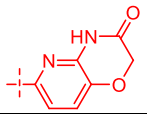
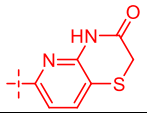
Table 1. *S. aureus* Minimum Inhibitory Concentrations (μg/mL)[a]

Cmpd.	R group	ATCC 29213	MRSA USA300 strain	CF isolate	Strain 3527	1st-step mutant (GyrA D83N)	ChemDraw cLogP
							
2a		0.125-0.5	0.125-0.5	0.25-0.5	0.25-2	2-8	1.95
3a		0.125	0.25	0.25	0.25	4	2.82
4a		0.25	0.25	0.25	0.25	2	2.25
							
2b		0.25-0.5	1	0.5	1	8	1.50
3b		≤0.25	0.5	≤0.25	0.5	2	2.37
							
2c		2	4	2	4	>8	0.73
3c		0.5	1	0.5	1	8	1.60
							
3d		2	4	1	4	>8	1.33
							
2e		2	1	2	2	8	0.45
3e		0.5	0.25	0.5	0.25	4	1.32
4e		0.125	≤0.06	≤0.06	≤0.06	1	1.62
							
2f		1	1	1	1	8	0.68
4f		≤0.06-0.125	≤0.06	≤0.06-0.125	≤0.06	0.25-1	1.88
gepotidacin	--	0.25-1	0.25-1	0.25-1	0.25-1	8-32	0.006
ciprofloxacin	--	0.25-0.5	16	0.25-0.5	>64	64	-0.73
vancomycin	--	1	1-2	1	1-2	1	NC[b]

[a]Determined in triplicate (at a minimum) according to CLSI guidelines.[22] Observed ranges are reported where appropriate. [b]NC = not calculated.

provided aldehyde **20**, and cyclization with known[12] diol **21** afforded dioxane **22**. Deprotection of the phthalimide with ethylenediamine[12,21] yielded amine **23**. Finally, reductive amination afforded azatricyclic analogues **3a**–**e**. The synthesis of the diazatricyclic analogues **2a**–**c**, **2e**, and **2f** proceeded similarly, beginning with commercially available bromonaphthyridine **5**. The synthesis of amides **4a**, **4e**, and **4f** is described in the Supporting Information.

Minimum inhibitory concentrations (MICs) were determined in triplicate under CLSI guidelines[22] against several strains of *S. aureus* (Table 1), as previously reported for other compounds.[12] Ciprofloxacin, gepotidacin, and vancomycin were positive controls. ATCC 29213 is a drug-susceptible lab strain, and the CF isolate is a susceptible respiratory isolate obtained from an individual with cystic fibrosis. USA300 is a ciprofloxacin-resistant MRSA strain, and 3527 is a previously reported[23] multidrug-resistant strain. MICs were also obtained for a first-step mutant daughter strain of 3527 with a GyrA D83N amino acid substitution.[23] The D83N substitution has been reported as an important determinant of resistance to NBTIs by several authors,[10,24−26] including us.[12,18,23] MICs for bicyclic analogues **1a**–**f** were previously reported.[12]

Representative analogues bearing either the more polar diazatricyclic moiety (such as **2a**, **2b**, and **2f**) or the more lipophilic azatricycle (**3a**–**c** and **3e**) had potent antistaphylococcal activity, with MICs of ≤1 μg/mL, the upper end of the observed range for gepotidacin. While we would expect potent activity from **3f**, we did not prepare it on the basis of anticipated potent hERG inhibition. Bicyclic amides **4a**, **4e**, and **4f** were synthesized based on the structure−activity relationships (SAR) gleaned from our earlier amide NBTIs.[18] Indeed, each of these amides had excellent MICs (0.06−0.25 μg/mL), with **4e** and **4f** being especially potent. There were no meaningful differences between the ciprofloxacin-susceptible

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and -resistant strains, consistent with previous experience with NBTIs.[6,12] Lipophilicity positively influenced the antibacterial activity for the tricyclic analogues; compounds bearing the most lipophilic benzodioxane enzyme binding moiety (**2a** and **3a**) were the most potent of the tricyclic analogues. In matched-pair comparisons of the two tricyclic series, the more lipophilic azatricycle derivatives were routinely 2- to 4-fold more potent (**3b** vs **2b**, **3c** vs **2c**, and **3e** vs **2e**). Compounds **3a** and **2a** with the lipophilic benzodioxane enzyme-binding group constitute an exception, with azatricycle **3a** showing activity similar to that of diazatricycle **2a**. Interestingly, analogues **2a** and **3a** matched the potency of the direct comparator (**1a**) from the previously reported bicyclic fluoronaphthyridine series,[12] but all of the other tricyclics were less potent than their bicyclic counterparts. Azatricycle **3d** with the very polar dioxinopyridazine and diazatricycle **2c** with the polar oxathiinopyridazine were the least active of the new molecules, with MICs ranging from 1 to 4 μg/mL. We opted not to synthesize the even more polar compound **2d** as a result. As expected,[10,12,18,23−25] all of the compounds displayed less activity (elevated MICs) against the NBTI-resistant strain bearing the gyrase D83N amino acid substitution. However, azatricyclic compound **3b**, with the same enzyme-binding moiety as gepotidacin, is among the most potent amino-dioxane-linked NBTIs we have tested against the NBTI-resistant strain (MIC = 2 μg/mL), and amide **4a** was equally active. Impressively, amides **4e** and **4f** were even more potent, with MICs of 1 and 0.25−1 μg/mL, respectively.

We also measured inhibition of *S. aureus* DNA gyrase supercoiling and TopoIV decatenation activity (Table 2). The tricyclic NBTIs were generally modest inhibitors of both DNA gyrase and TopoIV. The most potent antistaphylococcal agents, **3a**, **2a**, and **3b**, displayed submicromolar inhibition of DNA gyrase, as did all three amide NBTIs. Compared with earlier bicyclic naphthyridine-type amine NBTIs,[12] inhibition of DNA gyrase and TopoIV was more balanced: eight of the 10 tricyclic compounds and one amide (**4f**) showed gyrase/TopoIV IC_{50} ratios of ≤5. All compounds exhibited weaker inhibition of the D83N mutant compared with wild-type DNA gyrase (Table S5). Surprisingly, three compounds (**2b, 2e**, and **3e**) showed slightly more potent inhibition of TopoIV than DNA gyrase. However, these compounds still displayed a modest loss of activity in the *S. aureus* strain carrying the gyrase D83N amino acid substitution (Table 1), suggesting that gyrase remains their primary target in *S. aureus*. The fundamental mechanism of bacterial killing by NBTIs in *S. aureus* has not yet been fully elucidated and remains an active area of investigation in our laboratories.

Table 2. Target Inhibition by NBTIs[a]

compd	DNA gyrase IC_{50} (μM)[b]	TopoIV IC_{50} (μM)[c]	TopoIV/gyrase ratio
2a	0.047 (3)	0.54 (3)	11
3a	0.23 (2)	0.60 (2)	2.6
4a	0.23 (2)	1.7 (2)	7.6
2b	2.1 (2)	1.6 (2)	0.76
3b	0.58 (3)	2.9 (3)	5.0
2c	1.8 (3)	10 (2)	5.6
3c	1.7 (2)	7.4 (2)	4.4
3d	3.5 (2)	9.7 (2)	2.8
2e	4.2 (2)	1.4 (2)	0.33
3e	1.6 (2)	1.3 (5)	0.81
4e	0.19 (1)	1.1 (2)	5.8
2f	1.4 (2)	2.1 (2)	1.5
4f	0.15 (1)	0.42 (1)	2.8
gepotidacin (racemic)	0.17 (2)	2.8 (3)	16
ciprofloxacin	28 (2)	8.6 (2)	0.31

[a]Average IC_{50} values from full concentration−response curves performed on separate days, with the number of replicate experiments in parentheses. [b]Inhibition of supercoiling activity. [c]Inhibition of decatenation activity.

Given the promising results described above, we investigated the antibacterial spectrum of our NBTIs. MICs were promising against *N. gonorrhoeae* (see below) but modest at best against *E. coli*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* (Table S6). The rise of sexually transmitted infections by drug-resistant *N. gonorrhoeae* constitutes an urgent threat to human health.[1,27] Gepotidacin[11] is currently in Phase 3 clinical trials for uncomplicated urogenital gonorrhea. Nevertheless, despite decades-long research on NBTIs, very few published studies on NBTIs[28−30] (apart from gepotidacin[31,32]) report antibacterial activity for *N. gonorrhoeae*. This paucity of data represents a key deficit in the NBTI field.

Using previously reported[12] lead **1c**, MICs against *N. gonorrhoeae* were determined by agar dilution using ATCC strain 49226 (MIC > 4 μg/mL) and 110 clinical isolates (see the Supporting Information for full results and discussion). Compound **1c** demonstrated encouraging activity against *N. gonorrhoeae*: 45% of the isolates showed MICs of ≤4 μg/mL, and no obvious cross-resistance to ciprofloxacin was observed (Table S1).

A follow-up study (Table 3) employing structurally diverse compounds was carried out by broth microdilution with *N. gonorrhoeae* ATCC strain 49226 and seven WHO reference strains[33] (F, G, K, O, P, V, and Y). Ceftriaxone and ciprofloxacin served as comparators and controls. Strains K, V, and Y display high-level resistance to ciprofloxacin, and Y also has high-level resistance to ceftriaxone. Key amino acid substitutions (if any) in DNA gyrase and/or TopoIV are listed for each isolate.[33]

Of the DNA-binding motifs, the bicyclic fluoronaphthyridine was generally superior in matched-pair comparisons. Among the tricyclic moieties, the more lipophilic azatricycle afforded more potent MICs. Of the enzyme-binding groups, polar moieties lacking a hydrogen-bond donor such as the oxathiinopyridazine (**1c**, **2c**, **3c**) and dioxinopyridazine (**1d** and **3d**) afforded weak inhibitory activity, whereas the more lipophilic benzodioxane showed lower MICs, especially for bicyclic **1a** and azatricycle **3a**. In contrast, benzodioxane amide **4a** lacked activity against four of eight strains. Intriguingly, the pyridooxazinone moiety in bicyclic compound **1e** and azatricycle **3e** was very potent, as was the analogous bicyclic amide **4e**. Bicyclic amine compound **1f** with its pyridothiazinone moiety was consistently potent across all eight strains (MICs ≤ 0.03 μg/mL), and amide **4f** likewise showed excellent MICs (0.12 μg/mL against WHO G and ≤0.015 μg/mL against the remaining strains). Among *N. gonorrhoeae* strains, the highly ciprofloxacin-resistant WHO V was the most susceptible to these NBTIs, whereas WHO G with the unusual ParE G410V mutation was the least susceptible.

Amine compounds **1c** and **1f** demonstrated a divergent SAR between *S. aureus*, where they are essentially equipotent across several strains (range of 0.125−0.5 μg/mL[12]), and *N. gonorrhoeae*, where **1f** is 16- to 1000-fold more potent against

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Table 3. Minimum Inhibitory Concentrations (μg/mL) of NBTIs against *N. gonorrhoeae*[a,b]

compd	ATCC 49226 (WT[c])	WHO F (WT)	WHO G (GyrA S91F; ParE G410V)	WHO K (GyrA S91F, D95N; ParC S87R, S88P)	WHO O (WT)	WHO P (WT)	WHO V (GyrA S91F, D95G; ParC S87R)	WHO Y (GyrA S91F, D95G; ParC S87R)
1a	≤0.03	≤0.03	0.5	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03
2a	0.25	0.12	4	1	2	2	≤0.03	≤0.03
3a	0.12	≤0.03	0.5	0.12	0.25	0.25	≤0.03	≤0.03
4a	4	0.25	>16	>16	>16	>16	0.25	0.5
1b	NT[d]	NT	NT	NT	NT	NT	NT	NT
2b	2	0.12	8	1	2	2	≤0.03	0.25
3b	0.25	≤0.03	2	0.5	0.5	1	≤0.03	≤0.03
1c	8	1	32	16	16	16	≤0.03	0.5
2c	32	8	>32	32	32	32	4	8
3c	16	2	32	32	16	16	4	2
1d	32	8	>32	>32	32	32	8	16
3d	32	16	>32	>32	32	32	32	32
1e	≤0.03	0.06	0.25	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03
2e	1	0.12	2	1	1	2	0.5	1
3e	0.12	0.06	0.25	0.12	0.25	0.25	≤0.03	0.12
4e	≤0.015	≤0.015	0.25	0.12	0.06	≤0.015	≤0.015	≤0.015
1f	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03
2f	NT	NT	NT	NT	NT	NT	NT	NT
4f	≤0.015	≤0.015	0.12	≤0.015	≤0.015	≤0.015	≤0.015	≤0.015
CIP[e]	0.002	0.002	0.12	>1	0.03	0.004	>1	>1
CRO[f]	0.004	≤0.001	≤0.001	0.008	0.004	≤0.001	0.002	1

[a]Determined at Micromyx, LLC (Kalamazoo, MI) by microbroth dilution. [b]Mutations (if any) to type II topoisomerase enzymes are indicated in parentheses.[33] [c]WT = wild type. [d]NT = not tested. [e]CIP = ciprofloxacin. [f]CRO = ceftriaxone.

all strains except WHO V (Table 3). Since agar MICs represent the gold-standard method for *N. gonorrhoeae*,[34] we compared the ATCC 49226 agar-dilution MICs for compounds **1c** (>4 μg/mL) and **1f** (0.25 μg/mL) (Table 4). The

Table 4. Agar Dilution MICs (μg/mL) of Select NBTIs against *N. gonorrhoeae*[a,b]

compd	ATCC 49226 (WT[c])	WHO G (GyrA S91F; ParE G410V)	WHO M (GyrA S91F, D95G)	WHO L (GyrA S91F, D95N; ParC D86N, S88P)
1f	0.25	1	0.5–1	1 to >1
4f	0.12	0.25–0.5	0.12	0.5
CIP[d]	0.004–0.008	0.12	2	>8

[a]Determined in triplicate at Micromyx, LLC (Kalamazoo, MI) by agar dilution; ranges are shown where appropriate. [b]Mutations (if any) to type II topoisomerase enzymes are indicated in parentheses.[33] [c]WT = wild type. [d]CIP = ciprofloxacin.

>16-fold more potent activity for **1f** over **1c** by agar dilution is considerably less than the 256-fold difference observed by broth microdilution, but it is nevertheless clear that **1f** is much more potent than **1c**.

Compounds **1c** and **1f** have very similar physicochemical properties (Table S7). If these properties are relevant for penetration into or accumulation in *N. gonorrhoeae*, variations in antibacterial activity might be attributable to differences in inhibition of the topoisomerase enzymes. Consequently, inhibition of supercoiling and decatenation by *N. gonorrhoeae* DNA gyrase and TopoIV, respectively, was assayed (Figure 2). **1f** is a substantially more potent inhibitor of gyrase (IC_{50} = 0.39 μM) and TopoIV (IC_{50} = 3.6 μM) than is **1c** (gyrase IC_{50} = 33 μM, TopoIV IC_{50} = 83 μM), consistent with the observed differences in *N. gonorrhoeae* MICs. By contrast, these compounds demonstrate similar inhibition of *S. aureus* DNA gyrase (IC_{50} = 0.16 and 0.22 μM, respectively[12]), consistent with their equivalent antistaphylococcal activity. Taken together, these findings suggest that the disparity in *N. gonorrhoeae* MICs for **1f** and **1c** is at least partially attributable to inherent differences in the potency of target inhibition. Amide **4f** displayed potent inhibition of supercoiling by DNA gyrase (IC_{50} = 0.64 μM), similar to amine **1f** (see the Figure 2A inset). In contrast, inhibition of decatenation of TopoIV by **4f** (IC_{50} = 27 μM) was considerably weaker. The potent *N. gonorrhoeae* MICs for **4f** (Tables 3 and 4) suggest that DNA gyrase may be its primary target.

While *N. gonorrhoeae* broth MICs serve as a useful tool for rapidly delineating the SAR,[35] agar dilution is the standard method for susceptibility testing of this pathogen, and compound activity in agar is required for spontaneous mutation frequency determination. Given the relatively strong antimicrobial activity of **1f** and **4f**, we determined agar MICs against ATCC 49226 and WHO strains G, M, and L (Table 4), with ciprofloxacin as a control. In the earlier study (Table 3), WHO G was the least susceptible strain to our NBTIs and is of intermediate susceptibility to ciprofloxacin. WHO M is ciprofloxacin-resistant with S91F and D95G mutations to GyrA. WHO L has further-reduced susceptibility to ciprofloxacin with two substitutions in GyrA (S91F and D95N) and two in ParC (D86N and S88P). ParC D86N is analogous to the GyrA D83N substitution commonly seen in NBTI-resistant *S. aureus* and has been deemed a "stepping stone" to gepotidacin resistance in *N. gonorrhoeae*.[36]

The MICs on agar for **1f** and **4f** against the ATCC strain (0.25 and 0.12 μg/mL, respectively) were elevated compared with those obtained by microbroth dilution (Table 3) but were nevertheless relatively low. The agar MIC of gepotidacin against ATCC 49226 has been reported as 0.25–0.5 μg/mL.[31,37] Amide **4f** was slightly more active (~2-fold) than amine **1f** across the four strains tested, and **4f** retained a potent

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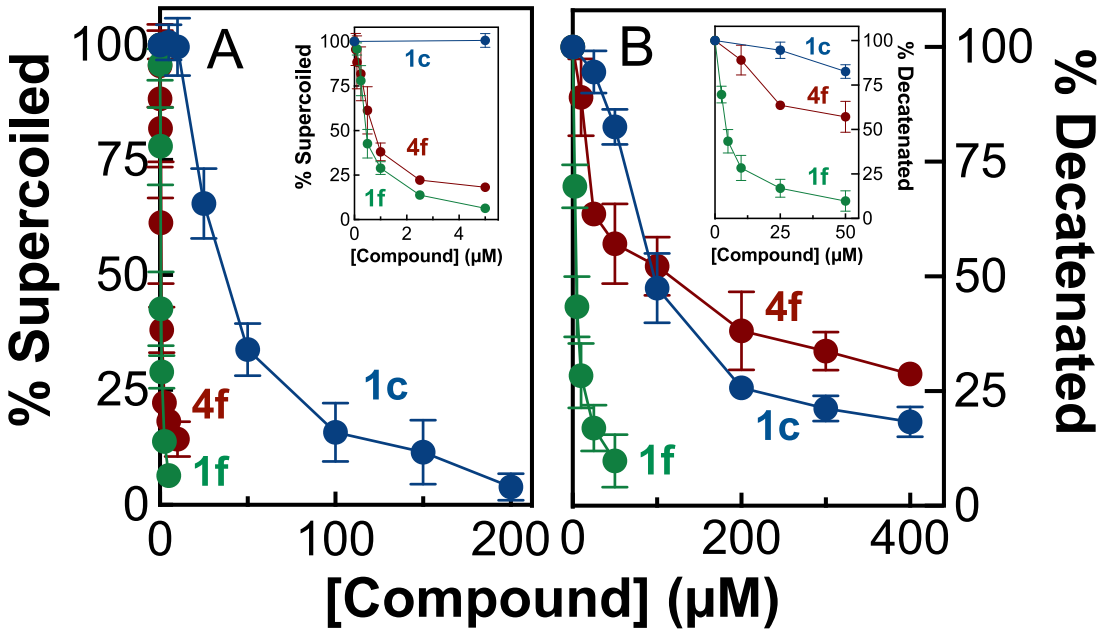

Figure 2. Inhibition of *N. gonorrhoeae* (A) DNA gyrase and (B) TopoIV by **1c**, **1f**, and **4f**. Compounds **1c**, **1f**, and **4f** inhibit DNA supercoiling and decatenation catalyzed by *N. gonorrhoeae* gyrase and topoisomerase IV, respectively. Panel (A) shows the effects of **1c** (blue), **1f** (green), and **4f** (maroon) on supercoiling of relaxed DNA by *N. gonorrhoeae* gyrase, with an expanded scale in the inset. Panel (B) shows the effects of **1c**, **1f**, and **4f** on decatenation of kDNA by *N. gonorrhoeae* topoisomerase IV, with an expanded scale in the inset. Error bars represent the standard deviation of at least three independent experiments.

MIC of 0.5 μg/mL against the quadruple mutant WHO L strain.

We also evaluated compounds **1f** and **4f** in a spontaneous frequency of resistance (FoR) assay with the same four strains. No resistant mutants were obtained for either compound at concentrations of 4 × MIC or 8 × MIC (Table S8), including against the WHO L strain. The FoR on the comparator, ciprofloxacin, led to resistant colonies with a mutation frequency of >9.6 × 10^8 (WHO G strain). Gepotidacin has shown low resistance frequencies in *N. gonorrhoeae*,[31] although resistant mutants at 4 × MIC were recovered.[11,37] The low FoR results for **1f** and **4f** further evince their quality as early leads for *N. gonorrhoeae*.

While potent antibacterial activity is required for new antibacterials, further progression requires careful attention to ADMET properties. Given our earlier experience,[12] we directed particular attention to the metabolic stability in mouse microsomes and inhibition of the hERG cardiac ion channel (Table 5). As we observed for earlier NBTIs,[12] the microsomal stability was correlated well with the polarity for tricyclic analogues. The more polar diazatricyclic series was superior in each matched-pair comparison. Unfortunately, amide compounds **4e** and **4f** had short half-lives compared with the previously reported analogous amines[12] and the tricyclic amines, illustrating a need for continued optimization. Within the amine analogues, hERG inhibition was correlated with the lipophilicity, although the tricyclic analogues appear to be intrinsically superior to the previously reported[12] bicyclic analogues. The members of the azatricyclic series are slightly more lipophilic than the bicyclic amines, yet hERG inhibition is reduced in matched-pair comparisons. All of the diazatricyclic compounds achieved ADMET properties similar to or better than those of our anti-MRSA lead **1c** (microsomal $t_{1/2}$ = 18.1 min; hERG IC_{50} = 103 μM),[12] as did azatricyclic compounds **3c** and **3d**. Thus, such tricyclic DNA-binding moieties constitute promising components for the continued development of dioxane-linked NBTIs. Solubility limits of approximately 3 μM for some of our previously reported amides impacted hERG assay determinations.[18] However, solubility issues were overcome with the current amides; **4a**, **4e**, and **4f** gratifyingly showed hERG IC_{50} values greater than 100 μM.

Table 5. *In Vitro* Metabolic Stability, hERG Inhibition, and Cellular Growth Inhibition

compd	microsomal $t_{1/2}$ (min)[a]	hERG IC_{50} (μM)[b]	K562 IC_{50} (μM)	K/VP.5 IC_{50} (μM)
2a	13.9	100	34.8	39.2
3a	3.26	35	29.1	33.9
4a	14.2	>100	1.6[d]	6.0[d]
2b	18.3	125	268	187
3b	3.64	53	109	85.0
2c	113	>200 (23%[c])	98.0[d]	197, >200
3c	21.9	200	38.7	103
3d	102	>200 (21%[c])	156	171
2e	66.3	97	>200[d]	>200[d]
3e	18.4	30	101[d]	127[d]
4e	5.56	>100	0.49[d]	3.1[d]
2f	36.4	55	81.7	128
4f	5.42	>100	7.5[e]	>100[e]

[a]Charles River (Worcester, MA). [b]Charles River (Cleveland, OH). [c]Percent inhibition at 200 μM in parentheses. [d]n = 2. [e]n = 3.

Growth inhibition assays were also conducted using human leukemia K562 cells and an acquired etoposide-resistant clonal subline (K/VP.5) with reduced levels of human topoisomerase IIα (hTopoIIα).[38] While the tricyclic amine analogues exhibited potent antistaphylococcal activity (Table 1), they were relatively weak inhibitors of cell growth in both K562 and K/VP.5 cells, with comparable IC_{50} values in the high micromolar range (Table 5). Bicyclic amine **1f** exhibited

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similar results (IC_{50} = 57.8 and 46.5 μM for K562 and K/VP.5, respectively), consistent with our experience with bicyclic amines **1a**–**e**.[12] Amide NBTIs **4a**, **4e**, and **4f** were surprisingly potent inhibitors of K562 cell growth (IC_{50} = 1.6, 0.49, and 7.5 μM, respectively), with 3.8- to >13-fold reduced activity in K/VP.5 cells, which suggested the possibility of hTopoIIα targeting. In contrast, previous results with other amide NBTIs yielded similar IC_{50} values for K562 compared with K/VP.5 cells.[18] While the low hERG inhibition and outstanding antibacterial activity of **4e** and **4f** make them attractive starting points for further studies, additional optimization will be needed to reduce mammalian cytotoxicity.

In summary, we have incorporated tricyclic DNA-binding motifs into 1,3-dioxane-linked NBTIs and diversified our earlier series[12] by the use of amide enzyme-binding moieties. Such compounds display potent antistaphylococcal activity and reduced hERG inhibition compared with earlier bicyclic fluoronaphthyridine NBTIs.[12] *In vitro* metabolism in mouse microsomes was driven by lipophilicity for tricyclic compounds. Diazatricyclic NBTIs were superior to other analogues, while amides suffered generally rapid metabolism. Representative compounds with both bicyclic and tricyclic DNA-binding motifs demonstrated potent activity against *N. gonorrhoeae*, thus broadening the antibacterial spectrum of our dioxane-linked NBTIs. Amine **1f** and amide **4f** emerged as potent antigonorrheal early leads with low resistance frequencies, paving the way for future studies against this critical pathogen.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.2c00111.

> Full data set, strain characterization, and discussion of MICs for **1c** against 110 clinical isolates of *N. gonorrhoeae*; D83N mutant gyrase IC_{50} values, Gram-negative MICs, and microsomal intrinsic clearance results for all new compounds; physicochemical properties of **1c** and **1f** and frequencies of spontaneous resistance for **1f** and **4f**; assay methods; synthesis, characterization, and NMR spectra of test compounds **2a**–**c**, **2e**, **2f**, **3a**–**e**, **4a**, **4e**, and **4f** (PDF)

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■ ABBREVIATIONS

NBTI, novel bacterial topoisomerase inhibitor; MRSA, methicillin-resistant *Staphylococcus aureus*; TopoIV, topoisomerase IV; hERG, human ether-related-a-go-go gene; MIC, minimum inhibitory concentration; CLSI, Clinical and Laboratory Standards Institute; ATCC, American Type Culture Collection; WHO, World Health Organization; CDC, Centers for Disease Control and Prevention

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