Letters

3-Aminoquinazolinediones as a New Class of Antibacterial Agents Demonstrating Excellent Antibacterial Activity Against Wild-Type and Multidrug Resistant Organisms

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Abstract: The 3-aminoquinzolinediones represent a new series of antibacterial agents structurally related to the fluoroquinolones. They are inhibitors of bacterial gyrase and topoisomerase IV and demonstrate clinically useful antibacterial activity against fastidious Gram-negative and Gram-positive organisms, including multidrug- and fluoroquinolone-resistant organisms. These agents also demonstrate in vivo efficacy in murine systemic infection models.

Antibiotic resistance, both in the community and in the hospital, is a growing public health concern due to the continual emergence of bacterial strains that demonstrate multidrug resistance.[1] Successful traditional therapies are proving to be ineffective, and clinicians are now switching to newer agents as a strategy to treat life-threatening infections. Consequently, the fluoroquinolones, as a newer antibacterial class, are filling an unmet medical need.

Since the discovery of the fluoroquinolones, a great body of knowledge has been accumulated for this class of bacterial type-2 topoisomerase (gyrase and topoisomerase IV) inhibitors.[2] Structure−activity relationships associated with both efficacy and safety are well-understood, and new fluoroquinolones[3] continue to be developed to overcome issues associated with existing agents. Prominent clinical agents are ciprofloxacin, indicated primarily for the treatment of Gram-negative and urinary tract infections, gatifloxacin, moxifloxacin, and levofloxacin (Figure 1) for the treatment of respiratory tract infections and skin and soft tissue infections caused by *S. aureus*, and garenoxacin, a new entry into the class, which is currently in clinical trials.[4]

Although the fluoroquinolones are highly effective in the clinic, increasing use places their future utility in jeopardy. In recent years, resistance to fluoroquinolones has begun to rise in both the hospital and community settings.[5]

The search for new classes of antibacterial agents to overcome growing bacterial resistance focuses largely on two approaches. One is to identify novel targets where no pre-existing resistance exists.[6] This approach, although sound in concept, has been met with little success in identifying quality lead matter due to issues associated with target validation and low hit rates from high throughput screening. The second approach is to explore existing clinically proven targets for new chemical matter or modify existing matter with limited or no cross-resistance to existing agents, eliminating the risk of target validation. This approach has been used successfully by a number of researchers to identify new series of antibacterial agents. This has been particularly effective toward the generation of newer classes of macrolide, tetracycline, and cephalosporin antibiotics and especially significant in the discovery of newer generations of vancomycin derivatives.[7]

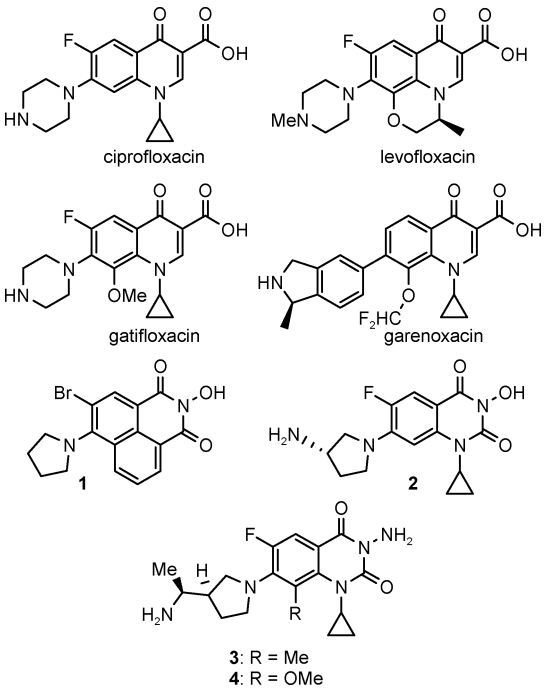

Figure 1. Structures of known inhibitors of bacterial gyrase/topo IV and 3-aminoquinazolinediones.

In an effort to discover new classes of antibacterial agents that inhibit bacterial gyrase and topo IV, we have previously reported on the discovery of the 2-hydroxyisoquinolines **1**[8] and, subsequently, the 3-hydroxyquinazolinediones **2**.[9] Both series exhibit moderate antibacterial activity and can be differentiated from the fluoroquinolones by di-acyl hydroxamic acid functionality (pK_a = 6.3−7.0), which replaces the 3-position

Scheme 1. Preparation of 3-Amino-quinazolinedione Antibacterial Agents

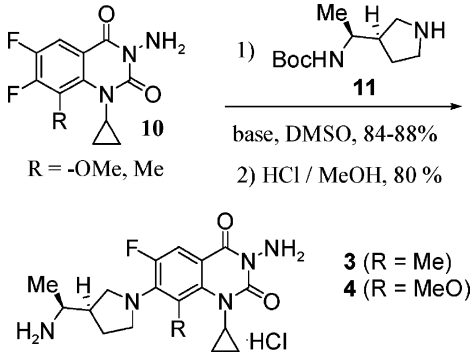

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Table 1. In Vitro Gyrase and Antibacterial Activity Against Selected Pathogens

	ciprofloxacin	garenoxacin	gatifloxacin	levofloxacin	**3**	**4**
E. coli gyrase IC_{50} (μM)[12]	0.2		0.5		0.2	0.6
organism			MIC (μg/mL)			
			Gram-negatives			
E. cloacae EC1-10	0.03	0.25	0.06	0.06	1.0	2
K. pneumoniae KP-2	0.03	0.06	0.06	0.03	1.0	8
K. pneumoniae ESBL KP-3729		32	8	8	2	8
P. rettgeri PR-9	0.06	0.06	0.50	0.50	2.0	4
P. aeruginosa PA-7	0.06	1.0	0.50	0.50	4.0	8
E. coli EC-2026	0.06	0.03	<0.06	0.015	1.0	4.0
E. coli EC-2549 (TolC)	0.004	0.008	<0.06	0.008	0.03	0.13
			Fastidious Gram-negatives			
H. influenzae HI-3542	0.008	0.015	0.015	0.008	0.125	0.25
M. catarrhalis BC-3531	0.03	0.03	0.03	0.03	0.25	1.0
			Gram-positives			
S. aureus SA-1417	0.5	0.06	0.125	0.25	0.125	0.5
S. aureus UC-76 SA-1	0.13	.015	.03	0.125	0.06	0.13
S. aureus SA-2552 (nor A)	2.0	0.03	0.125	0.25	0.25	0.5
S. aureus SA- 2554 (nor A, grlA)	2.0	0.03	0.125	0.50	0.13	0.25
S. aureus SA-2558 (nor A, grlA, gyrA)	64.0	2.0	4.0	8.0	0.25	0.5
S. aureus MRSA SA-1417	0.5	0.03	0.125	0.25	0.125	0.25
S. aureus CMRSA SA-2017	>64	2	8	8	0.5	1
E. faecalis MGH-2 EF1-1	0.5	0.125	0.50	1.0	0.125	0.13
E. faecalis (VanA) EFI-3524	32	4	16	32	0.25	0.25
*E. faecium*VRE (van A) EF4-3489	>64	8	32	64	1	1
S. pneumoniae SVI SP-3	1.0	0.03	0.125	0.50	0.015	.03
S. pneumoniae QRSP SP-3763	64	1	4	16	0.06	0.06
S. pyogenes C-203 SP1-1	0.5	0.06	0.50	0.50	0.015	.03

carboxylic acid moiety ($pK_a = 5.6-6.4$) observed with the fluoroquinolones. The acidic functionality has historically been viewed as essential for binding to bacterial gyrase and topo IV.[10]

We now report on the discovery of the 3-aminoquinazolinediones, exemplified by **3** and **4**, as a new class of bacterial gyrase and topo IV inhibitors. The 3-aminoquinazolinediones challenge the historic dogma that 3-position acidic functionality is required for antibacterial activity.

The 3-aminoquinazolinediones were prepared according to Scheme 1. For the analogues highlighted herein, the syntheses of the 3-aminoquinazolinedione cores **5** have been reported previously.[11] These were coupled with side chain **6** in good yields, followed by BOC deprotection, which provided target compounds **3** and **4**.

The 3-aminoquinazolinediones (**3** and **4**) demonstrate significant inhibition of *E. coli* gyrase activity, similar to that observed for the fluoroquinolones (Table 1).[12] They can be characterized, however, as having only moderate activity against Gram-negative pathogens (Table 1). This is demonstrated by comparing the activities of **3** and **4** relative to the comparator agents against *E. cloacae*, *K. pneumoniae*, *P. rettgeri*, *P. aeruginosa*, and *E. coli*. One reason for the diminished activity can be ascertained from the activities of **3** and **4** against the *E. coli* Tol C strain, where an efflux pump has been knocked out, clearly demonstrating that these agents are substrates for efflux.

A clue that the 3-aminoquinazolinediones represent a class of agents dramatically different from the fluoroquinolones is observed against the *K. pneumoniae* extended spectrum β-lactamase (ESBL[a]), producing strain KP-3729. Against this strain, the fluoroquinolones lost significant antibacterial activity as compared to the corresponding sensitive strain KP-2 (>100-fold). Both strains were equally sensitive to **3** or **4**.

[a] Abbreviations: ESBL, extended spectrum β-lactamase; QRDR, quinolone-resistant determining regions; MRSA, methicillin-resistant; CMRSA, ciprofloxacin- and methicillin-resistant; strains of *S. aureus*; VRE, vancomycin-resistant Enterococci.

Against the fastidious Gram-negative strains *H. influenzae* and *M. catarrhalis*, reasonable levels of antibacterial activity can be achieved, especially for **3**, despite the efflux mechanism.

Against Gram-positive organisms, the 3-aminoquinazolinediones demonstrate exceptional antibacterial activity. Similar antibacterial activity is exhibited for comparator agents and analogues **3** and **4** against the *S. aureus* UC-76 strain. This changes, however, when all five compounds are examined against *S. aureus* resistant strains. It is well-established that clinical fluoroquinolone resistance results from multiple target mutations, the sum of up-regulation of efflux pumps and multiple mutations in the quinolone-resistant determining regions (QRDR) of both gyrase and topo IV.[13] As shown in Table 1, *S. aureus* strains SA-2552, SA-2554, and SA-2558, raised as strains resistant (each from UC-76) to ciprofloxacin, demonstrate reduced susceptibility to the comparator fluoroquinolones to varying degrees. SA-2552 has an efflux pump (norA) that has been up-regulated and is observed clinically.[13] Against this strain, ciprofloxacin loses significant activity. The remaining comparators and the 3-aminoquinazolinediones demonstrate a 2- to 4-fold reduction with the resultant MICs still of clinical utility. Against the SA-2554 strain, which has a mutation in norA and a single mutation in topo IV (*grlA*), none of the agents lose significant activity versus that observed with SA-2552. However, when a second mutation (SA-2558 strain) is introduced into the QRDR of gyrase (*gyrA*), all the fluoroquinolone comparators lose significant antibacterial activity (100- to 500-fold vs SA-1). This same strain, however, is still highly sensitive to the 3-aminoquinazolinediones, apart from the 2- to 4-fold loss in activity due to efflux.

The comparators and **3** and **4** were also tested against methicillin-resistant (MRSA) and ciprofloxacin- and methicillin-resistant (CMRSA) strains of *S. aureus*. As shown in Table 1, these organisms are highly sensitive to the 3-aminoquinazolinediones, whereas the fluoroquinolones are only effective against MRSA.

Table 2. In Vivo Efficacy of 3-aminoquinazolinediones **3** and **4** in Systemic Murine Infection Models

		3	
organism	route of admin.	MIC (μg/mL)	PD_{50} (mg/kg)
S. pyogenes	PO	0.015	1.4
	SC		0.27
E. faecalis	PO	0.06	19
	SC		4.2
		4	
S. pyogenes	PO	0.03	3.1
	SC		1.2
E. faecalis	PO	0.25	38
	SC		11

Against the Enterococci strains (*E. faecalis* and *E. faecium*), both wild-type (EF1-1) and VRE (EF1-3524, vancomycin-resistant Enterococci), the 3-aminoquinazolinediones demonstrate reasonable antibacterial activity, especially when compared to the fluoroquinolones. Particularly noteworthy is the sensitivity of *E. faecium* VRE to **3** and **4**, considering the rise of resistance to vancomycin in the hospital setting, which represents a significant medical need.[1]

Streptococci organisms are also highly sensitive to **3** and **4**. Of particular interest is the activity against quinolone-resistant *S. pneumoniae* (QRSP), where the fluoroquinolones are essentially devoid of clinical utility (garenoxacin is borderline).

Although the 3-aminoquinazolinediones have striking structural similarity to the fluoroquinolones and demonstrate enzymatic activity against gyrase (vide supra), fluoroquinolone-resistant organisms are still highly susceptible to this novel class. To better understand this phenomenon, a number of studies with compound **3** were carried out to validate its mechanism of action. Biochemical studies reveal that the conformational change in DNA gyrase in the ternary complex of gyrase/DNA/**3** is identical to that induced by ciprofloxacin.[14] Compound **3** also inhibits the ability of purified *E. coli* DNA gyrase to supercoil DNA at concentrations comparable to ciprofloxacin and, like ciprofloxacin, produces a linear cleavage product.[15] It was also found in studies against *E. coli* gyrase that unlabeled ciprofloxacin and compound **3** each displaced bound [^{14}C]-ciprofloxacin in competition assays, suggestive of similar or overlapping binding regions on the enzyme for the two classes.[16]

To further assess the mechanism of action of compound **3**, the compound was tested in whole cell macromolecular synthesis analysis[17] to determine the effect of the compound **3** on the synthesis of DNA, RNA, fatty acids, and protein. These experiments demonstrated that only the synthesis of DNA was specifically inhibited, consistent with the in vitro DNA gyrase assay data.

To help identify the precise molecular target, *N. gonorrheae* mutants resistant to at least four times the MIC of **3** were isolated, and the mutations responsible for resistance were identified by genetic "backcross" experiments.[18] The mutations were identified to be in or near the quinolone-resistant determining regions (QRDR) in either *gyrA*, resulting in D95G or E161K alterations of the DNA gyrase A-subunit, or *gyrB*, resulting in E469V, K450N, or D419N substitutions in the DNA gyrase B-subunit.[19] These data suggest that the mechanism of action of the 3-aminoquinazolinediones is due to specific inhibition of DNA gyrase and topo IV in a quinolone-like manner.

To further assess the utility of the 3-aminoquinazolinediones, **3** and **4** have also been examined for in vivo efficacy in murine bacteremia infection models (Table 2).[20] Both compounds exhibit significant activity in both models (*S. pyogenes* and *E. faecalis*) via oral and subcutaneous dosing, demonstrating the potential of the 3-amino-quinazolinediones as antibacterial agents.

In summary, we have detailed the synthesis and in vitro and in vivo activity of selected compounds of the 3-aminoquinazolinediones, a novel class of antibacterial agents. This class is shown to be inhibitory against bacterial gyrase and topo IV in a fluoroquinolone-like manner and demonstrates significant antibacterial activity against wild-type and multidrug-resistant organisms. The greatest utility of the 3-aminoquinazolinediones appears to be against Gram-positive organisms that have reduced sensitivity to the known antibacterials, including the fluoroquinolones. This class of compounds also demonstrates excellent in vivo activity in murine infection models. In future publications, we will extensively detail in vitro structure−activity relationships, in vivo activity, and PK/PD relationships for this novel class.

Supporting Information Available: Synthesis procedures, supporting analytical data, testing protocols, and data associated with mechanism of action studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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