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Structure–activity relationships of 3-aminoquinazolinediones, a new class of bacterial type-2 topoisomerase (DNA gyrase and topo IV) inhibitors

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Abstract—A series of 3-aminoquinazolinediones was synthesized and evaluated for its antibacterial and DNA gyrase activity. The SAR around the quinazolinedione core was explored and the optimal substitutions were combined to give two compounds, **2r** and **2s**, with exceptional enzyme potency (IC_{50} = 0.2 μM) and activity against Gram-positive organisms (MIC's = 0.015–0.06 μ g/mL).

The continual emergence of bacterial resistance to most classes of antibiotics is a cause for great concern and creates a pressing need for new antibacterial agents. Despite much effort from the pharmaceutical industry to counter the resistance problem by finding new and effective antibacterial therapies, the discovery and development of new mechanistic classes of antibiotics has met with very little success. The difficulty of this task is demonstrated by the fact that only two antibiotics of new classes, linezolid (an oxazolidinone)[1] and daptomycin (a cyclic lipopeptide),[2] have been successfully developed in the last three decades.

As part of a program to discover new antibacterial agents, we have focused our attention on the well-studied bacterial type-2 topoisomerase (topo) enzymes, namely DNA gyrase and topo IV, as targets. Since the discovery of nalidixic acid in 1962[3] and the subsequent emergence of the fluoroquinolone antibiotics, for example, ciprofloxacin (Cipro™) (**1**, Fig. 1), as a prominent class of chemotherapeutic agents, these enzymes have proven to be clinically relevant targets. While much research has been conducted within this mechanistic class over the past forty years, these enzymes still remain as attractive targets in the search for new antibiotics.[4–7]

Previously we reported on the 3-aminoquinazolinediones (**2**, Fig. 1) as a novel class of bacterial gyrase and

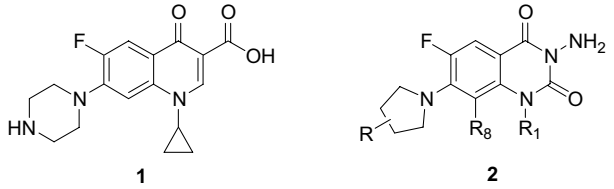

Figure 1. Structures of ciprofloxacin (**1**) and a generic 3-aminoquinazolinedione (**2**).

Keywords: 3-Aminoquinazolinediones; Antibacterial agents; DNA gyrase inhibitors; Topoisomerase inhibitors; Antibacterial activity.
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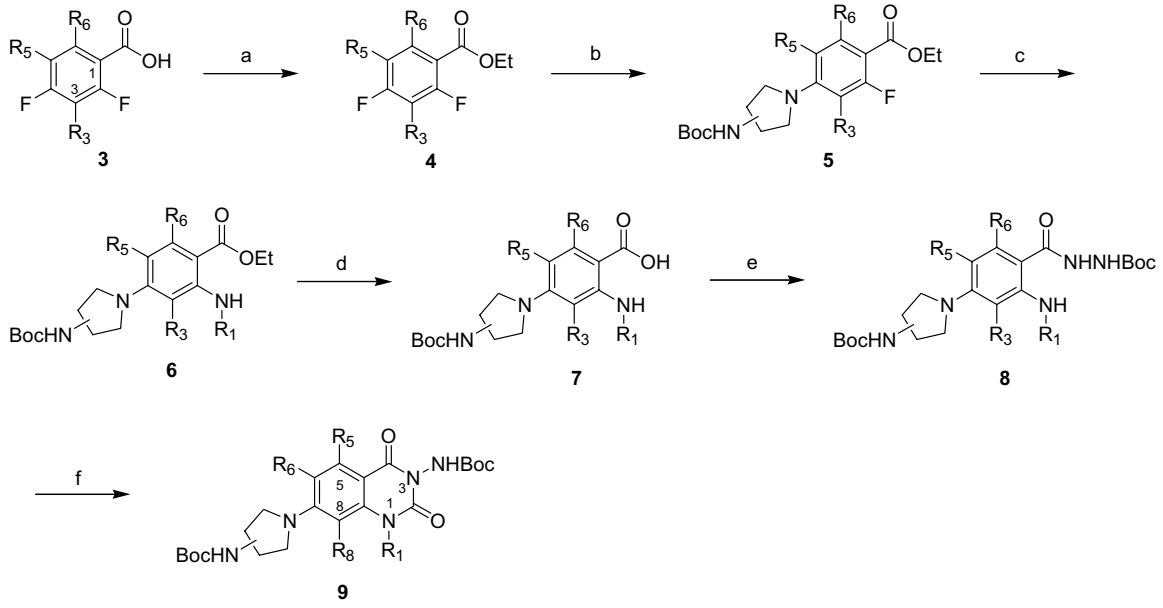
topo IV inhibitors that demonstrates outstanding in vivo efficacy, particularly against Gram-positive organisms, in murine infection models.[8] In this paper, we expand on our initial disclosure with further details on the synthetic pathways and structure–activity relationships (SAR) developed within this class. Because the 3-aminoquinazolinediones bear structural similarity to the fluoroquinolone antibiotics and inhibit the same biological targets via a similar mechanism of action, we sought to apply known SAR trends within the fluoroquinolones in an effort to explore and expand the SAR of our series.

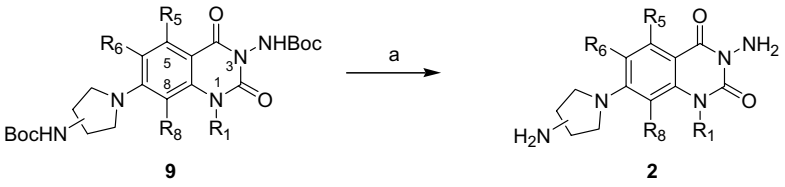
Initially our strategy was to prepare the targeted 3-aminoquinazolinediones from an appropriately substituted 2,4-difluorobenzoic acid **3** as outlined in Scheme 1. The acid was esterified with saturated hydrogen chloride in ethanol, followed by selective nucleophilic displacement of the 4-fluoro substituent with a functionalized pyrrolidine in acetonitrile to provide adduct **5**. Ester **5** was treated with a primary amine in dimethylsulfoxide at elevated temperature to give **6**, which was saponified with aqueous sodium hydroxide to form acid **7**. The functionalized acid **7** was coupled with *tert*-butyl carbazate and the subsequent acyl hydrazide **8** was cyclized into quinazolinedione **9** with triphosgene. The formation of the intended quinazolinedione ring by this method was directly dependent on the nature of R_3, where small groups such as the hydrido- or fluoro-substituents afforded the 1,4-dihydro-benzo[*d*][1,3]oxazin-2-one by-product.[9]

The last step involved the removal of the Boc-protecting groups, which was extensively utilized in our synthesis, by treatment of **9** with an acid, such as hydrogen chloride or trifluoroacetic acid, to afford the 3-aminoquinazolinedione target **2**. The general reaction is shown in Scheme 2.

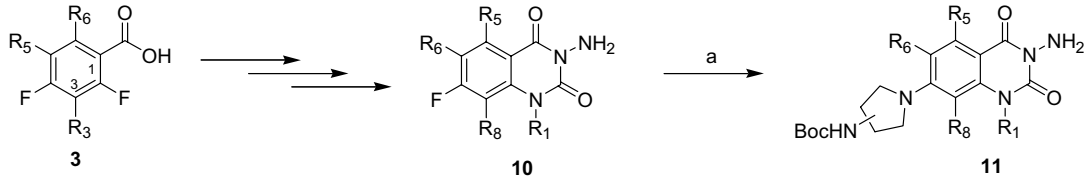
The limitations of the route above, along with its non-convergent nature, prompted further investigations that led to a more efficient synthesis summarized in Scheme 3.[9] The new synthesis provided intermediate 3-aminoquinazolinedione **10** from a substituted 2,4-difluorobenzoic acid **3** in five high-yielding steps. Next, nucleophilic displacement of the 7-fluoro substituent of **10** with an appropriate amine afforded the penultimate compound **11**.[6] Lastly, compound **11** was treated with an acid as described in Scheme 2 to generate the intended 3-aminoquinazolinedione target **2**. Target compounds of this paper incorporate amino-substituted pyrrolidines at R_7. The functionalized pyrrolidines were either commercially



Scheme 1. Reagents and conditions: (a) satd HCl–EtOH, 100%; (b) substituted pyrrolidine, TEA, MeCN, 60–87%; (c) primary amine, DMSO, 100 °C, 20–50%; (d) NaOH, THF, MeOH, 60 °C, 83–90%; (e) *tert*-butyl carbazate, EDCI, CH_2Cl_2, 60–87%; (f) triphosgene, K_2CO_3, THF, reflux, 30–85%.



Scheme 2. Reagents: (a) HCl or TFA, CH_2Cl_2, 80–90%.



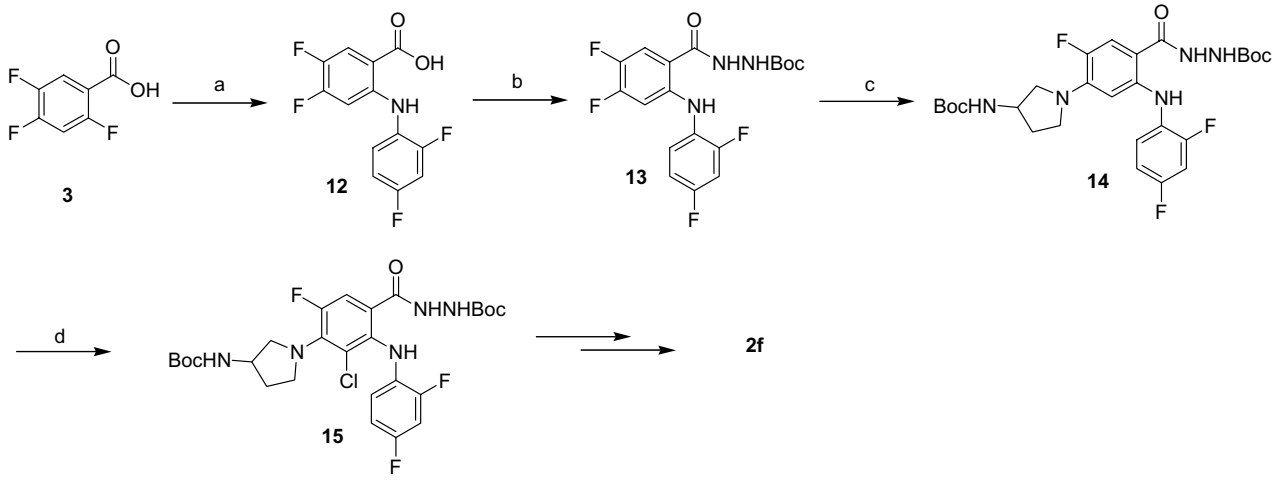
Scheme 3. Reagents and condition: (a) 3-substituted pyrrolidines, TMG, DMSO, 110 °C, 35–80%.

available or prepared according to reported procedures.[10–12]

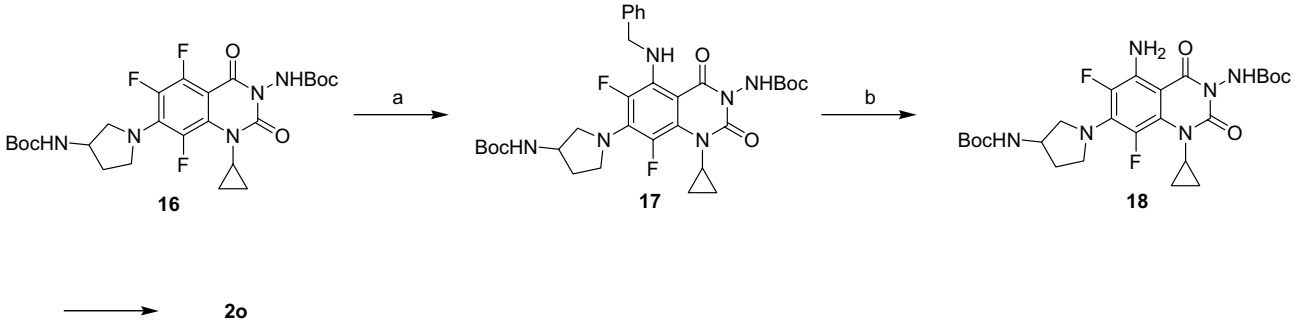
While many of the compounds reported in this study were synthesized according to Schemes 1 or 3 directly from their respective commercially available benzoic acids **3**, targets **2f**, **2k**, **2o**, **2q**, **2s**, and **2t** required additional steps to incorporate the appropriate substitution. The corresponding precursors for compounds **2k** and **2s** were prepared as reported in the literature.[12,13] The required intermediate of target **2f** was prepared as shown in Scheme 4. Trifluorobenzoic acid **3** was treated with a preformed anion of 2,4-difluoroaniline[14] and the resulting **12** was coupled with *tert*-butyl carbazate to form hydrazide **13**. Treatment of **13** with Boc-protected 3-aminopyrrolidine in dimethylsulfoxide at elevated temperature gave adduct **14**. Chlorination of **14** with *N*-chlorosuccinimide in acetic acid afforded intermediate **15**, which was carried forward to target **2f** according to procedures outlined in Schemes 1(step f) and 2.

The penultimate intermediate of target **2o** was formed as outlined in Scheme 5. Intermediate **16** was made from the starting pentafluorobenzoic acid as described in Scheme 1(steps a–f). Treatment of **16** with benzylamine at elevated temperature afforded **17** and hydrogenolysis of the benzyl-protecting group generated the required intermediate **18**. Removal of the Boc-groups as usual gave the intended target **2o**.

The intermediate for target **2q** was synthesized as indicated in Scheme 6. Compound **19** was prepared from



Scheme 4. Reagents and condition: (a) 2,4-difluoroaniline, LiHMDS, THF, 45%; (b) *tert*-butyl carbazate, EDCI, CH_2Cl_2, 72%; (c) 3-Boc-aminopyrrolidine, TEA, DMA, 110 °C, 46%, (d) NCS, HOAc, 80%.



Scheme 5. Reagents and condition: (a) benzylamine, TEA, DMSO, 100 °C, 86%; (b) H_2, $Pd(OH)_2/C$, THF, 76%.

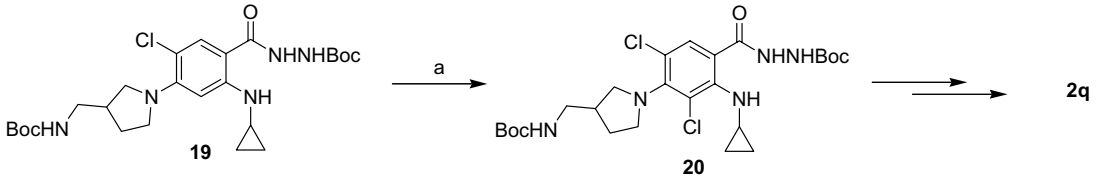
5-chloro-2,4-difluorobenzoic acid according to Scheme 1(steps a–e). Chlorination of **19** with *N*-chlorosuccinimide in acetic acid gave the desired dichloro **20**, which was taken on to target **2q** as shown in Schemes 1(step f) and 2.

The corresponding intermediate of target **2t** was prepared via the chemistry shown in Scheme 7. Quinazolindione **21**[9] was treated with slightly more than two equivalents of lithium diisopropylamide, and the resulting dianion was reacted with ethylformate to form aldehyde **22**. The aldehyde was converted to the difluoromethyl group by treatment with (diethylamino)sulfur trifluoride (DAST). Compound **23** was then aminated with *o*-(2,4-dinitrophenyl)-hydroxylamine to give the intended intermediate **24**.[9] Intermediate **24** was carried onto target **2t** as described in Schemes 3 and 2.

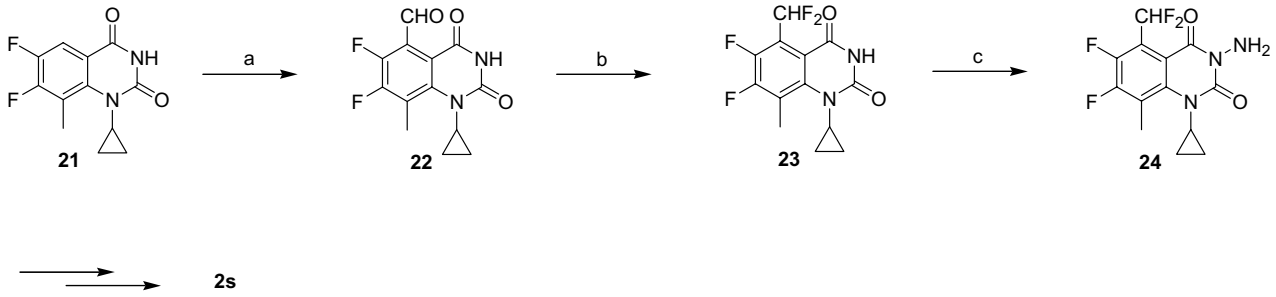
Compounds **2v** and **2w**, bearing substituents on the 3-amino group, were synthesized as summarized in Schemes 8 and 9, respectively. Compound **25**, made from chemistry shown in Scheme 1, was treated with sodium hydride and the resulting anion was quenched with iodomethane to generate the penultimate compound **26**. Removal of the Boc-protecting group afforded target **2v**.

Target **2w** was derived from acid **27** (Scheme 9), which was prepared from 3-chloro-2,4,5-trifluorobenzoic acid according to Scheme 1(steps a–d). The acid was coupled with acethydrazide to form hydrazide derivative **28**, which was then converted to its corresponding target **2w** as described in Schemes 1(steps e–f) and 2.

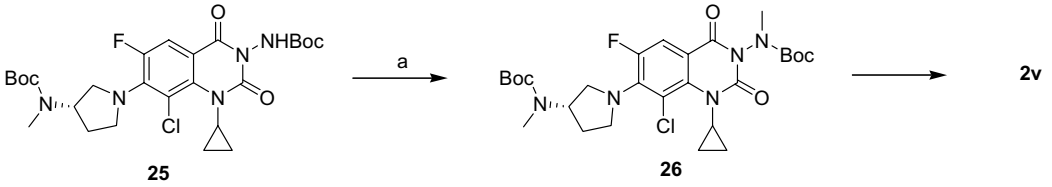
Our series of prepared 3-aminoquinazolinediones was tested against a representative panel of Gram-negative



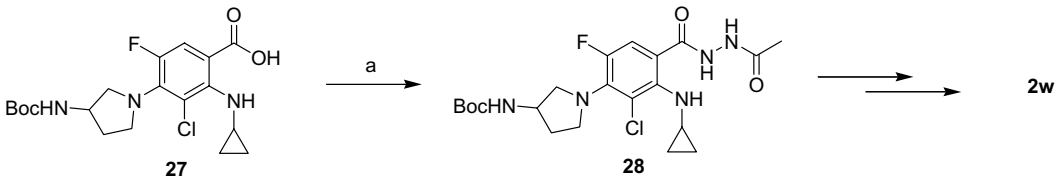
Scheme 6. Reagents and condition: (a) NCS, HOAc, 31%.



Scheme 7. Reagents and condition: (a) i—LDA, THF; ii—HCO_2Et, 60%; (b) DAST, CH_2Cl_2, 20%; (c) NaH, 2,4-dinitrophenylhydroxylamine, THF, 76%.



Scheme 8. Reagents and conditions: (a) NaH, MeI, DMF, 48%.



Scheme 9. Reagents and conditions: (a) acethydrazide, EDCI, CH_2Cl_2, 87%.

and Gram-positive organisms and their minimum inhibitory concentrations (MICs, μg/mL) were determined using standard microtitration techniques.[15] The Gram-negative strains reported in this paper are *Escherichia coli* MC4100 (wild-type) and *E. coli* Tol C (membrane efflux-pump deficient); the Gram-positive panel includes *Enterococcus faecalis*, *Staphylococcus aureus*, and *Streptococcus pyogenes*. Additionally, all compounds were tested for their ability to inhibit the target enzyme DNA gyrase, isolated and purified from *E. coli* H560.[16] The assay measures the concentration of drug needed to cleave DNA, as visualized by agarose gel electrophoresis and staining with ethidium bromide (ciprofloxacin control).

In general, the 3-aminoquinazolinediones displayed excellent activity against both Gram-negative and Gram-positive organisms. The best compounds of this series, **2r** and **2s**, although having only modest activity against the Gram-negative organisms when compared to ciprofloxacin, display far superior activity against Gram-positive bacteria. These two compounds also exhibit better *E. coli* gyrase inhibition than ciprofloxacin. In addition, most of 3-aminoquinazolinediones tested are multiple-fold more potent against the *E. coli* Tol C strain than the *E. coli* wild-type strain, clearly demonstrating that they, unlike ciprofloxacin, are substrates for efflux.

The quinazolinedione core offers multiple opportunities for SAR exploration. In general, subtle modification to the substitution at any position on the quinazolinedione ring has a significant impact on the antibacterial and enzymatic activity. This paper examines the SAR trends specifically at R_1, R_3, R_5, R_6, and R_8. Because of the number of sites investigated, the SAR effect of each position was determined by varying the substitution at that site and evaluating the effects of change with comparable substitutions at all other positions.

The 3-aminoquinazolinediones show an explicit preference for the cyclopropyl moiety at R_1, as shown in Table 1. For example, comparing compound clusters with R_8 = H or Cl, it is clear that R_1 = cyclopropyl is superior (**2c** vis-à-vis **2a** and **2b**; **2g** vis-à-vis **2d** through **2f**). While **2c** has only marginal activity against the three Gram-positive organisms, it is more potent against *E. coli* and its enzymatic activity is more than ten times better than **2a** and **2b**. This trend is reinforced by the superior overall activity of compound **2g** over compounds **2d**–**2f**.[17] Despite their structural similarities, this is where the 3-aminoquinazolinediones diverge considerably from their fluoroquinolone counterparts. Whereas compounds **2a** and **2f** display no significant activity, the ethyl- and 2,4-difluorophenyl components at R_1 confer outstanding activity in the fluoroquinolone series.[18]

Table 1 also shows that substitutions at R_8 can offer significant improvement in potency. The chloro- (**2g**), fluoro- (**2h**), methyl- (**2i**), and methoxy- (**2j**) analogues all display significantly greater activity than their unsubstituted analogue (**2c**). It is interesting to note that while **2c** has better enzymatic activity than **2j** (1.6 vis-à-vis 4.3 μg/mL, respectively), **2j** actually displays a two- to eightfold improvement in antibacterial activity against various strains over **2c**. This atypical trend also has been often observed within the fluoroquinolone SAR.[19] In particular, the 8-methyl analogue (**2i**) offers the best overall antibacterial and enzymatic activity profile of any compound in this group. These four R_8 substituents represent both withdrawing and donating functional groups. Other R_8 substituents were examined (**2k** and **2l**), but those substitutions result in a significant loss of activity. Thus, the antibacterial and enzymatic activity is not due to the electronics at this position, but likely to be directed by steric factor imposed by the substituent at R_8.

Having established the optimum substituents for R_1 and R_8, we set out to examine the relative importance of R_5 and R_6 (Table 2). Comparing compounds with identical substitutions elsewhere, it is apparent that fluorine substitution at R_6 provides the best antibacterial and gyrase activity in this series. For example, compound **2n** is more active than its unsubstituted analogue, **2m**, while **2p** displays significantly better antibacterial and enzymatic activity than its chloro-analogue, compound **2q**. The preference for the R_6 fluoro-substituent is also shared by many of the fluoroquinolone antibiotics.

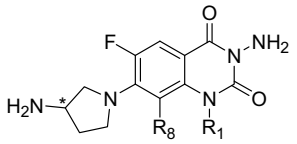
The data in Table 2 also indicate that there is opportunity for improvement of activity at R_5. While the 5-amino (**2o**) and 5-difluoromethyl analogues (**2t**) are significantly less active than their unsubstituted comparators, **2h** and **2r**, respectively, the 5-methyl analogue (**2s**) provides antibacterial and gyrase activity comparable to **2r**. This limited data set suggests that while some R_5 substituents can be detrimental to antibacterial activity, there exists some potential at R_5 for either maintaining or improving potency.

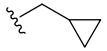
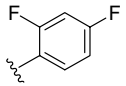
As can be ascertained from the data shown in Tables 1 and 2, substitution at each site of the 3-aminoquinazolinedione core can independently influence the SAR of the series. Moreover, combining the optimal substitution at each site affords compounds with the best antibacterial and enzymatic activity of the series (**2r** and **2s**). Thus, there is an additive effect among the substitutions on the quinazolinedione core. Undeniably, the functionalized pyrrolidines at R_7 also play a central role in the antibacterial SAR of the 3-aminoquinazolinediones, as clearly observed when comparing compounds **2p** to **2g** and, more profoundly, **2r** to **2i**. In fact, much of our effort was geared toward optimizing the SAR at R_7 for this series. Extensive details of these findings will be disclosed in future publications.

Lastly, we also probed the effects of 3-amino substitution on antibacterial and gyrase activity. As shown in Table 3, both mono-alkylation (**2v** vis-à-vis **2u**) and acylation (**2w** vis-à-vis **2g**) of the 3-amino moiety result in completely inactive compounds, along with a corresponding reduction in DNA gyrase activity. Hence, the unsubstituted 3-amino moiety has proven to be optimal for both antibacterial and enzymatic activity.

In summary, we have prepared and evaluated a series of 3-aminoquinazolinediones as antibacterial agents. The

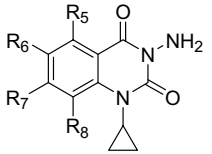
Table 1. In vitro antibacterial and enzyme activity: SAR of R_1 and R_8



Compound	Stereo-chemistry at*	R_8	R_1	Minimum inhibitory concentrations (MICs, μg/mL)					*Escherichia coli* gyrase DNA cleavage (IC_{50}, μM)
				G− organisms		G+ organisms			
				Escherichia coli MC4100 EC-2026	*Escherichia coli* Tol C EC-2549	*Enterococcus faecalis* MGH-2 EF1-1	*Staphylococcus aureus* UC-76 SA-1	*Streptococcus pyogenes* C-203 SP1-1	
2a	S	–H		64	16	>64	>64	>64	35
2b	S	–H		>64	8	>64	>64	>64	20
2c	S	–H		8	1	32	64	16	1.6
2d	Racemic	–Cl		>64	>64	>64	>64	>64	98
2e	Racemic	–Cl		16	2	>64	>64	>64	23
2f	Racemic	–Cl		>64	64	>64	>64	>64	>100
2g	S	–Cl		0.5	0.13	4	2	2	0.4
2h	Racemic	–F		2	0.25	8	8	4	1.3
2i	Racemic	$–CH_3$		1	0.06	2	4	0.5	0.1
2j	Racemic	$–OCH_3$		4	0.5	8	8	4	4.3
2k	Racemic	$–OCH_2CH_3$		32	4	>64	>64	64	11
2l	S	$–CF_3$		8	0.25	32	32	4	5.2
Ciprofloxacin				0.06	0.06	0.4	4	2	0.4

* refers to stereochemistry of drawn molecule.

Table 2. In vitro antibacterial and enzyme activity: SAR of R_5 and R_6



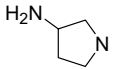
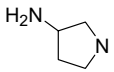
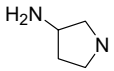
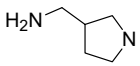
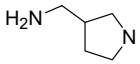
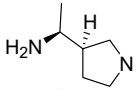
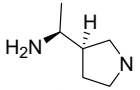
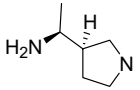
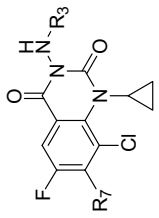
Compound	R_7	R_5	R_6	R_8	Minimum inhibitory concentrations (MICs, μg/mL)					*Escherichia coli* Gyrase DNA cleavage (IC_{50}, μM)
					G− organisms		G+ organisms			
					Escherichia coli MC 4100 EC-2026	*Escherichia coli* Tol C EC-2549	*Enterococcus faecalis* MGH-2 EF1-1	*Staphylococcus aureus* UC-76 SA-1	*Streptococcus pyogenes* C-203 SP1-1	
2m		–F	–H	–F	32	4	>64	>64	>64	17
2n		–F	–F	–F	2	0.5	32	16	16	3
2o		–NH_2	–F	–F	8	1	64	32	32	21
2p		–H	–F	–Cl	4	0.25	0.25	0.5	0.13	0.3
2q		–H	–Cl	–Cl	16	0.5	4	4	2	1.2
2r		–H	–F	–CH_3	1	0.03	0.06	0.06	0.015	0.2
2s		–CH_3	–F	–CH_3	2	0.03	0.06	0.06	0.03	0.24
2t		–CHF_2	–F	–CH_3	64	1	1	1	0.25	8.2

Table 3. In vitro antibacterial and enzyme activity: SAR of R_3



Compound	R_7	R_3	Minimum inhibitory concentrations (MICs, μg/mL)					*Escherichia coli* gyrase DNA cleavage (IC_{50}, μM)
			G− organisms		G+ organisms			
			Escherichia coli MC 4100 EC-2026	*Escherichia coli* Tol C EC-2549	*Enterococcus faecalis* MGH-2 EF1-1	*Staphylococcus aureus* UC-76 SA-1	*Streptococcus pyogenes* C-203 SP1-1	
2u		–H	4	0.5	16	16	8	6.1
2v		$–CH_3$	>64	8	>64	>64	>64	NT
2w		$–C(O)CH_3$	64	2	>64	>64	>64	10

NT, not tested.

best compounds of this series are potent inhibitors of bacterial DNA gyrase and they display outstanding antibacterial activity, especially against the Gram-positive organisms evaluated. Investigation of the SAR to explore optimal substitutions on the quinazolinedione core has been described. Aside from R_3, where substitution is not tolerated, appropriate substitutions at all other sites can lead to enhanced antibacterial and DNA gyrase activity. Combining the optimal substitutions for all sites, the best compounds, **2r** and **2s**, display Gram-positive activity and inhibition of DNA gyrase that are superior to that of ciprofloxacin.

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