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Synthesis and antibacterial activity of the C-7 side chain of 3-aminoquinazolinediones

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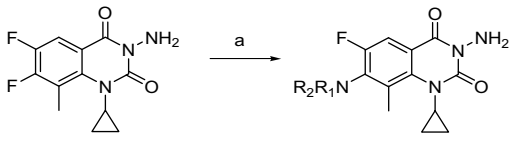
ABSTRACT

A novel series of bacterial topoisomerase (3-aminoquinazolinediones) inhibitors are described. The side-chain SAR against Gram-positive and Gram-negative organisms as well as DNA gyrase activity is reported.

We recently reported a new class of antibacterial agents, the 3-hydroxyquinazolinediones, which have been identified as bacterial type-2 topoisomerase inhibitors.[1] While this class exhibited good potency against *Escherichia coli* gyrase, it possessed no in vivo efficacy. Metabolism studies showed that this was attributed to rapid clearance due to glucuronidation of the 3-hydroxy function. Further investigation showed that replacement of the 3-hydroxy with a 3-amino moiety retained potency and prevented this route of metabolism, thus providing excellent in vivo efficacy in murine infection models.[2,3]

This novel class of agents is similar in structure to the fluoroquinolones (i.e., ciprofloxacin). The acidic functionality of the fluoroquinolones has historically been viewed as being essential for binding to bacterial gyrase and topo IV (pK_a 5.6–6.4).[4,5] Although the 3-hydroxyquinazolinedione series retains acidic functionality at the 3-position (pK_a 6.3–7.0), the 3-aminoquinazolinediones are neither acidic nor basic at the 3-position. Notably, they share the same mechanism of action as the fluoroquinolones. More specifically, the 3-aminoquinazolinediones inhibit the supercoiling of DNA, and, like the fluoroquinolones, provide a linear cleavage complex. Although these two classes share a similar mechanism of action, we have shown that fluoroquinolone-resistant strains are sensitive to the 3-aminoquinazolinediones. Sequencing of resistant mutants grown in *Neisseria gonorrhoeae* identified mutations in or near the quinolone-resistant determining regions (QRDR) in *gyrA* or *gyrB*, suggesting that the binding site is near to or overlapping that of the fluoroquinolones. This lack of cross resistance to quinolone-resistant strains in addition to the 3-aminoquinazolinedione's exceptional activity makes this an attractive new series for further evaluation. Our earlier re-



1 2

Scheme 1. Reagents and conditions: (a) TMG, DMSO, NR^1R^2, 80–100 °C, 30–60%.

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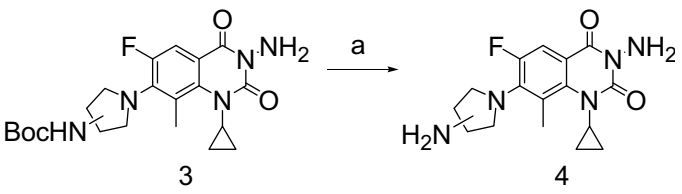


Scheme 2. Reagents and conditions: (a) HCl, CH_2Cl_2 or EtOH, 80–90%.

ports profiled two compounds within the 3-aminoquinazolinedione series.[2,3] In this Letter, a systematic SAR around the C-7 side chain is explored.

The 3-aminoquinazolinediones (3-AQD) were prepared according to Schemes 1 and 2. A substituted heterocyclic side chain was coupled to the 3-AQD core[6] in dimethylsulfoxide using 1,1,3,3-tetramethylguanidine (TMG) providing the desired analogs in moderate yields (unoptimized). In many cases, the functionalized side chains contained Boc-protected nitrogens that were deprotected using anhydrous hydrogen chloride in dichloromethane or ethanol (Scheme 2). In these cases, the compounds were isolated and tested as hydrochloride salts.

Many of the side chains included in this study are either available commercially (R^1 for compounds **5**–**11**), or prepared as reported in the literature (R^1 for compounds **12**–**15**, **17**–**22**).[7–9] The side chain for compound **16** was prepared in six steps from commercially available methyl *N*-benzyl-3-pyrrolidinecarboxylate.[10]

The prepared 3-aminoquinazolinediones were tested according to CLSI guidelines (formerly NCCLS)[11,12] against representative Gram-negative and Gram-positive strains using standard microbroth dilution methodology.[13] The Gram-negative strains reported here include *E. coli* MC4100, a wild-type strain, as well as *E. coli* TolC, an efflux pump knock-out strain. The Gram-positive strains represented here include *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Enterococcus faecalis*. The minimum inhibitory concentrations are summarized in Tables 1–4 and are compared to that of ciprofloxacin.

The compounds were also tested for their inhibition of DNA gyrase.[14]

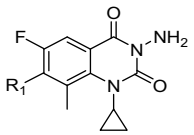
We have previously published on the SAR around the 3-hydroxyquinazolinedione core.[1] The work described here focuses on the SAR of the C-7 position of the 3-aminoquinazolinediones. All compounds reported in this Letter utilize the core with optimal substitution at the C-1, C-6, and C-8 positions (Fig. 1). The results of this effort are shown in Table 1. In general, the MICs against wild-type *E. coli* are poor, although the MICs versus the TolC strain are significantly improved, suggesting that these compounds are substrates for efflux. Both the pyrrolidine (**6**) and the piperidine (**7**) analogs show good MICs against Gram-positive organisms, with pyrrolidine **6** exhibiting the best MICs overall. Similar to the fluoroquinolones, the 5- and 6-membered heterocycles demonstrate the best activity in Gram-positive organisms and the piperazine side chain has the best activity in wild-type *E. coli*.[4]

Historically, fluoroquinolones containing amino-substituted pyrrolidines give improved Gram-positive activity.[4] Therefore, substitution of the pyrrolidine with an additional amino group was explored and found to give significantly improved potency (Table 2). The optimal positioning of an amino substituent was determined by varying the distance of the terminal amino group to the pyrrolidine. Although pyrrolidine **9** has excellent enzyme activity, the aminomethyl-substituted pyrrolidine analog (**10**) displays the best overall activity with significantly better Gram-positive activity relative to its comparators.

Having identified the methyl amino pyrrolidine side chain as having the best activity, we then looked to further explore the SAR around this template. Several analogs are shown in Table 3. Many of these targets with substitution of the exocyclic methylene showed improved activity over the unsubstituted analog **10**. Most notably, the larger ethyl and phenyl substituents (**15** and **16**) demonstrate superior enzyme activities. Disubstitution of the exocyclicmethylene was also examined. While an additional methyl group (**13** vs **12**) gave a modest decrease in antibacterial activity, incorporating the cyclopropyl group resulted in one of the most active of the 3-aminoquinazolinediones (compound **14**).

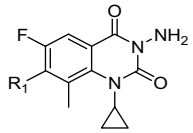
Having determined that substitution at the methylene provided enhanced activity, the role of stereochemistry was also explored examining the isomers of **12**. A comparison of the enzyme activity of each of the four stereoisomers shows that the stereocenter adjoining the pyrrolidine ring is the most important for enhanced activity. Compounds **17** and **18** with the R

Table 1
Structure–activity relationships of several heterocycles at the C-7 position



Compound	R^1	MICs (μg/mL)					IC_{50} (μM)
		E. coli MC4100	*E. coli* Tol C	*E. faecalis* MGH-2	*S. aureus* 29213	*S. pyogenes* C-203	*E. coli* gyrase
Ciprofloxacin		0.06	0.06	0.4	0.5	2	0.4
5		>64	2	64	32	64	12.5
6		16	0.13	2	1	1	1.9
7		>64	0.25	4	4	4	6.3
8		4	1	32	32	4	3.1

Table 2
Structure–activity relationships of aminopyrrolidines at the R-7 position



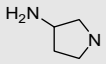
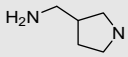
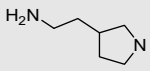
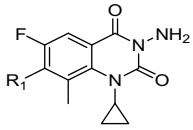
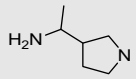
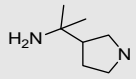
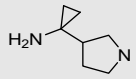
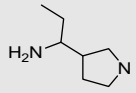
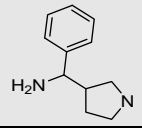
Compound	R^1	*E. coli* MC4100	MICs (μg/mL)				IC_{50} (μM)
			E. coli Tol C	*E. faecalis* MGH-2	*S. aureus* 29213	*S. pyogenes* C-203	*E. coli* gyrase
6		16	0.13	2	1	1	1.9
9		1	0.25	8	8	1	0.2
10		2	0.13	0.5	0.5	0.06	0.4
11		16	0.5	4	4	0.125	1.2

Table 3
Effect of substitution on the exomethylene of the pyrrolidine **10**



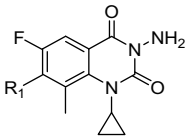
Compound	R^1	MICs (μg/mL)					IC_{50} (μM)
		E. coli MC4100	*E. coli* Tol C	*E. faecalis* MGH-2	*S. aureus* 29213	*S. pyogenes* C-203	*E. coli* gyrase
12		4	0.25	1	0.5	0.03	0.6
13		16	0.25	2	1	0.125	1.6
14		2	0.03	0.13	0.25	0.03	0.8
15		8	0.13	0.25	0.5	0.03	0.4
16		4	0.06	0.25	0.13	0.03	0.26

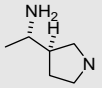
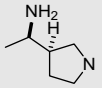
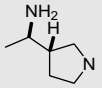
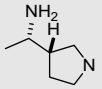
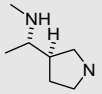
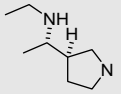
configuration at the pyrrolidine ring showed increased antibacterial activity in most strains versus their comparators **20** and **19**. Of these two compounds, **17** has superior activity with minimally 4x better MICs in all organisms. Historically, the stereocenter on the pyrrolidine ring had a profound effect on activity in the fluoroquinolones as well, and as in the AQD series, the R configuration is favored.[4,15]

Finally, we explored the effect of substitution on the primary amine of analog **17**. Although methyl substitution on the nitrogen showed similar activity to compound **17**, larger groups showed a decrease in activity, especially noticeable in *Strep. pyogenes* in compound **22**.

In conclusion, we have reported on the SAR of the C-7 position of 3-aminoquinazolinediones, which has a remarkable similarity to the fluoroquinolone SAR. A number of analogs were prepared exhibiting good antibacterial activity, especially in Gram-positive organisms. Further exploration in this series will be reported in due course.

Table 4
Effect of stereochemistry and substitution on the distal nitrogen



Compound	R^1	MICs (μg/mL)					IC_{50} (μM)
		E. coli MC4100	*E. coli* TolC	*E. faecalis* MGH-2	*S. aureus* 29213	*S. pyogenes* C-203	*E. coli* gyrase
17		0.5	0.03	0.06	0.13	0.015	0.2
18		2	0.13	0.5	0.5	0.06	0.3
19		2	0.13	0.25	1	0.25	0.8
20		4	0.13	2	2	0.5	0.6
21		4	0.03	0.13	0.5	0.015	0.2
22		8	0.13	0.25	0.5	0.13	0.9

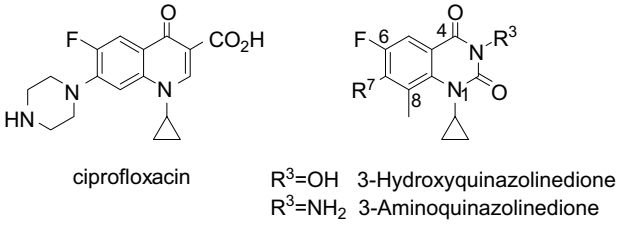


Figure 1. Structural comparison.

Acknowledgments

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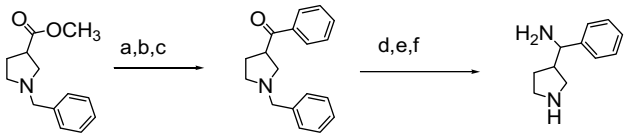
References and notes

1. Tran, T. P.; Ellsworth, E. L.; Stier, M. A.; Domagala, J. M.; Showalter, H. D. H.; Grachek, S. J.; Shapiro, M. A.; Joannides, T. E.; Singh, R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4405.
2. Ellsworth, E. L.; Tran, T. P.; Showalter, H. D. H.; Sanchez, J. P.; Watson, B. M.; Stier, M. A.; Domagala, J. M.; Grachek, S. J.; Joannides, T. E.; Shapiro, M. A.; Dunham, S.; Hanna, D.; Huband, M.; Gage, J. W.; Nguyen, D. Q.; Singh, R. *J. Med. Chem.* **2006**, *49*, 6435.
3. Huband, M. D.; Cohen, M. A.; Zurack, M.; Hanna, D. L.; Skerlos, L. A.; Sulavik, M. C.; Gibson, G. W.; Gage, J. W.; Ellsworth, E. L.; Stier, M. A.; Gracheck, S. J. *Antimicrob. Agents Chemother.* **2007**, *51*, 1191.
4. Gootz, T. D.; Brighty, K. E. *Med. Res. Rev.* **1996**, *16*(5), 433.
5. Wentland, M. P. Structure–activity relationships of fluoroquinolones. In *The New Generation of Quinolones*; Siporin, C., Heifetz, C. L., Domagala, J. M., Eds.; Marcel Dekker: New York, 1990; p 1.
6. Tran, T. P.; Ellsworth, E. L.; Watson, B. M.; Sanchez, J. P.; Showalter, H. D. H.; Rubin, R. R.; Stier, M. A.; Yip, J.; Nguyen, D. Q.; Bird, P.; Sing *J. Heterocycl. Chem.* **2005**, *42*, 669.
7. Kimura, Y.; Atarashi, S.; Takahashi, M.; Hayakawa, I. *Chem. Pharm. Bull.* **1994**, *42*, 1442.
8. Fleck, T. J.; McWhorter, W. W., Jr.; DeKam, R. N.; Pearlman, B. A. *J. Org. Chem.* **2003**, *6*(8), 961.
9. Schroeder, M. C.; Kiely, J. S.; Laborde, E.; Johnson, D. R.; Szoteck, D. L.; Domagala, J. M.; Stickney, T. M.; Michel, A.; Kampf, J. W. *J. Heterocycl. Chem.* **1992**, 1481.
10. The side chain for compound **16** was prepared from methyl *N*-benzyl-3-pyrrolidinecarboxylate. The ester was converted to the Weinreb amide via the carboxylic acid using standard conditions. Addition of phenyl Grignard provided the phenyl ketone, which was converted to the oxime. Reduction to the amine followed by deprotection of the benzyl group gave the desired side chain.



Reagents and conditions: (a) NaOH, dioxane, 60 °C; (b) $MeON(Me)H_2Cl$, EDCI, CH_2Cl_2, 68% (2 steps); (c) PhMgBr, THF 0 °C to reflux, 95%; (d) $MeONH_2.HCl$, EtOH; (e) BH_3.THF, reflux, 87%; (f) 10% Pd/C, H_2, MeOH, 94%.

11. National Committee for Clinical Laboratory Standards. January 2003. Methods for dilution antimocrobial tests for bacteria that grow aerobically, Approved Standard (6th ed). M7-A6, NCCLS, Wayne, PA.
12. National Committee for Clinical Laboratory Standards. January 2003. MIC testing supplemental tables. M100-S13 (for use with M7-A6), NCCLS, Wayne, PA.
13. Cohen, M. A.; Griffin, T. J.; Bien, P. A.; Heifetz, C. L.; Domagala, J. M. *Antimicrob. Agents Chemother.* **1985**, *28*, 766.
14. Domagala, J. M.; Hanna, L. D.; Heifetz, C. L.; Hutt, M. P.; Mich, T. F.; Sanchez, J. P.; Solomon, M. *J. Med. Chem.* **1986**, *29*, 394.
15. Domagala, J. M.; Hagen, S. E.; Joannides, T.; Kiely, J. S.; Laborde, E.; Schroeder, M. C.; Sesnie, J. A.; Shapiro, M. A.; Suto, M. J.; Vanderroest, S. *J. Med. Chem.* **1993**, *36*, 871.