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Synthesis and structural–activity relationships of 3-hydroxyquinazoline-2,4-dione antibacterial agents

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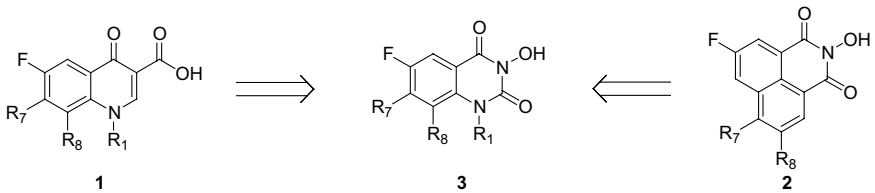
Abstract—A series of 3-hydroxyquinazoline-2,4-diones was synthesized and evaluated for antibacterial activity. This series represents a novel addition to the DNA gyrase inhibitor class of antibacterials. Appropriate substitutions onto the core template yielded compounds with excellent potency against *E. coli* gyrase and significant in vitro Gram-negative and Gram-positive antibacterial activity.

The fluoroquinolones (**1**) constitute a major class of antibacterial agents with both Gram-positive and Gram-negative activity. The antibacterial activity of this series results from selective inhibition against type-2 topoisomerase enzymes essential for DNA replication, namely gyrase and topoisomerase IV.[1–3] Due to their increased use over the past decades as one of the premier classes of antibacterial agents, resistance is developing toward the fluoroquinolones and this is contributing to an emerging world-wide health problem within the infectious diseases therapeutic area.[4] This trend, along with safety issues associated with the fluoroquinolones,[5–10] continues to provide the impetus for the discovery of improved agents within this mechanistic class.

As part of a program to discover new classes of antibacterial agents, we identified the 3-hydroxyisoquinolones (**2**) as possessing moderate in vitro antibacterial activity.[11] Further pursuit in this area led to the discovery of the 3-hydroxyquinazoline-2,4-diones (**3**). This series incorporates structural features imbedded in both the well-known fluoroquinolones and the hydroxyisoquinolones (Scheme 1).



Scheme 1.

Keywords: 3-Hydroxyquinazoline-2,4-diones; Antibacterial agents; Bacterial topoisomerase inhibitors.
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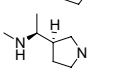
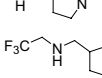
One important difference is the presence of the 3-hydroxamic acid instead of the typical 3-carboxylic acid moiety in the fluoroquinolones. The carboxylic acid has long been considered essential for antibacterial activity in the fluoroquinolone field.[12,13] The acidity of the 3-hydroxyl of the hydroxyquinazoline-2,4-diones ($pK_a = 6.3–7.0$) was found to be similar to that of the 3-carboxylic functionality of the fluoroquinolones ($pK_a = 5.6–6.4$) and thus has turned out to be an appropriate substitution. We also have shown that many of the hydroxyquinazoline-2,4-diones inhibited bacterial DNA gyrase, the very same enzyme targeted by the fluoroquinolones (Table 1). Because of the important features shared by the two series, our strategy was to apply the vast knowledge of the fluoroquinolone field toward optimizing antibacterial activity within the hydroxyquinazoline-2,4-diones, while at the same time attempting to find new structure–activity relationship (SAR) space that might alleviate some of the known toxicities associated with the fluoroquinolones. Toward

Table 1. In vitro antibacterial and gyrase activity

Compd	R_1	R_7	R_8	Minimum inhibitory concentrations (MIC), μg/mL						Gyrase-drug induced cleavage, μM; *E. coli*
				Gram-negative organisms			Gram-positive organisms			
				E. coli WT	*E. coli* LKY	*E. coli* TLC	*E. faec*	*S. aureus*	*S. pyog*	
Ciprofloxacin				0.06	0.06	0.06	0.4	4	2	0.4
10a	H		H	64	32	16	NT	8	32	>100
10b	Me		H	32	16	8	>64	>64	>64	>100
10c	Et		H	>64	2	1	NT	32	32	>100
10d			H	8	0.1	0.13	NT	1	16	7
10e			H	32	0.5	0.5	64	8	8	13
10f			H	>64	32	16	>64	16	32	>100
10g			H	>64	32	32	64	64	32	>100
10h			H	1	0.3	0.25	NT	32	4	2
10i			F	8	2	1	16	32	16	0.28
10j			Cl	2	0.5	1	32	16	4	1
10k			H	8	1	1	NT	16	2	NT
10l			H	8	0.125	0.125	4	1	0.5	6.4
10m			H	8	2	0.5	16	16	32	2.2
10n			H	32	4	2	16	16	2	6.2
10o			H	>64	1	0.5	16	4	16	8
10p			H	1	0.25	0.25	4	4	2	0.7
10q			H	8	2	1	64	32	32	5
10r			H	4	2	1	32	32	32	7.8
10s			H	16	0.25	0.5	NT	>64	>64	18
10t			H	>64	8	2	64	64	64	0.4

NT = Not tested.

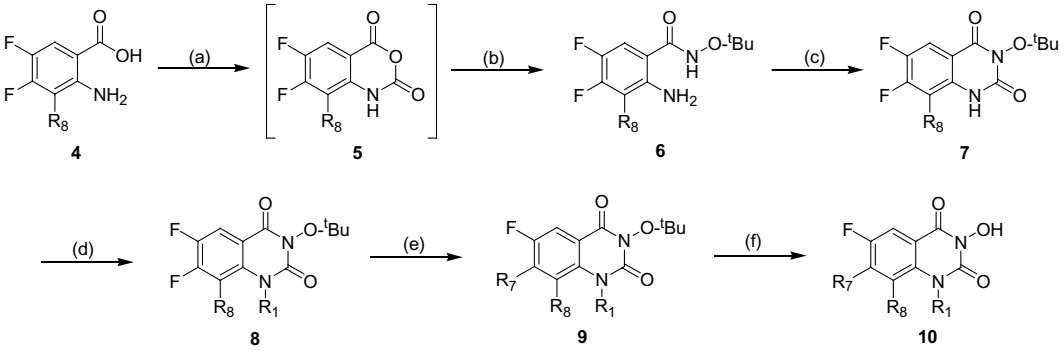
realizing the first part of this goal, this paper describes the synthesis and in vitro antibacterial activity of the 3-hydroxyquinazoline-2,4-dione series.

The 3-hydroxyquinazoline-2,4-diones were prepared according to Schemes 2–4. In most cases, target molecules were derived from appropriately substituted anthranilic acid precursors. Typically, compounds with nonaryl R_1 substitution were synthesized according to Scheme 2. Acid **4** was treated with carbonyldiimidazole in tetrahydrofuran at reflux to form the isatoic anhydride **5**.[14] The anhydride intermediate was reacted with *O*-*tert*-butyl hydroxylamine hydrochloride in the presence of triethylamine to afford hydroxamate **6**, which was then cyclized with phosgene to provide the quinazoline-2,4-dione nucleus **7**. Alkylation of **7** gave compound **8** with the desired *N*-1 substitution (R_1). The next step involved the installation of R_7 side chains, which were typically pyrrolidines with amino functionality bearing an acid labile protecting group. These heterocyclic amine side chains were prepared according to established literature procedures.[15–18] Aromatic nucleophilic displacement of the *C*-7 fluorine by R_7 heterocyclic amine in *N*,*N*-dimethylacetamide at elevated temperature gave penultimate **9**, which was treated then with trifluoroacetic acid to remove the *tert*-butyl group from the 3-hydroxyl moiety as well as the protecting group of the R_7 side chain to provide target **10**.

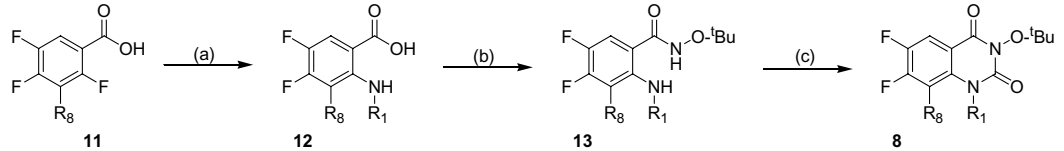
Scheme 3 shows the synthetic route of targets with aryl R_1 functional groups. Acid **11** and aniline were treated with lithium diisopropylamide separately, then the two species were combined to form **12**. Treatment of acid **12** with a carbodiimide and *O*-*tert*-butyl hydroxylamine hydrochloride gave hydroxamate **13**, which was then cyclized with phosgene to provide the quinazoline-2,4-dione nucleus **8**. Displacement of the 7-fluorine with heterocyclic amino side chain and then deprotection with trifluoroacetic acid, as according to Scheme 2, afforded targets **10** with aryl R_1 substitutions.

Intermediate **8** was also synthesized via an alternate route (Scheme 4). Lithiation of trifluorobenzoic acid (**14**) followed by an electrophilic quench installed the appropriate *C*-8 substituent (R_8) for certain core templates. Treatment of acid **15** with a carbodiimide and *O*-*tert*-butyl hydroxylamine hydrochloride gave hydroxamate **16**. Hydroxamate **16** was deprotonated with sodium hydride and the resulting anion was reacted with an alkyl or aryl isocyanate to provide urea **17**, which was cyclized with sodium hydride to form the quinazolinedione core **8**.

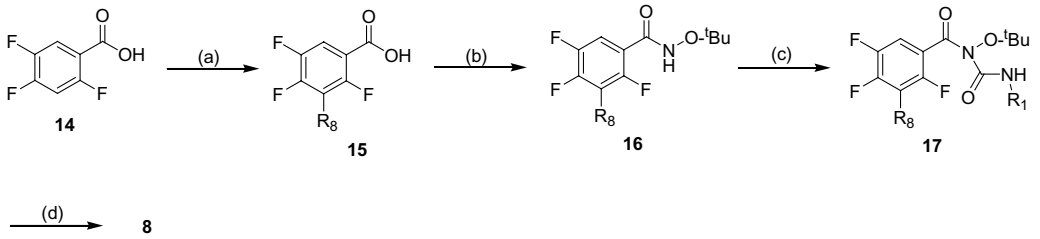
All synthesized 3-hydroxyquinazoline-2,4-diones were tested against representative Gram-negative and Gram-positive pathogens using standard microtitration techniques.[19] The strains reported in this paper are *E. coli* MC4100 (wild type), *E. coli* LKY (cell wall deficient, semi-permeable), *E. coli* TLC (membrane efflux pump deficient), *Enterococcus faecalis*, *Staph. aureus*, and *Strep. pyogenes*. *E. coli* LKY and *E. coli* TLC, laboratory-constructed strains, were used to determine the intrinsic activity for analogues in the absence of physiochemical factors that influence permeability and transport, respectively. All compounds were less active against the Gram-negative wild-type strain than both the mutant strains. These results suggest that against native *E. coli*, the hydroxyquinazoline-2,4-diones have



Scheme 2. Reagents and conditions: (a) CDI (1.5 equiv), THF, reflux, overnight; (b) *t*-BuONH$_3$Cl (1.5 equiv), TEA (2 equiv), THF, room temp to reflux, 16 h, 52–84% (two steps); (c) $COCl_2$ (1.9 solution in toluene, 1.2 equiv), THF, reflux, 2–5 h, 67–88%; (d) R_1–X (1.3 equiv), NaH (1.5 equiv), DMF, 0–50 °C, 6 h, 50–87%; (e) R_7 (amino side chain) (2 equiv), TEA (2–5 equiv), DMA, 90 °C, overnight, 22–70%; (f) TFA, CH_2Cl_2, overnight, 80–90%.



Scheme 3. Reagents and conditions: (a) LDA (3 equiv), aniline (1.5 equiv), −78–0 °C, 4 h, 55–84%; (b) *t*-BuONH$_3$Cl (1.3 equiv), TEA (2 equiv), EDCI (1.5 equiv), CH_2Cl_2, overnight, 73–90%; (c) $COCl_2$ (1.9 solution in toluene, 1.2 equiv), THF, reflux, 2–5 h, 68–85%.



Scheme 4. Reagents and conditions: (a) LiHMDS (2.2 equiv), THF, R_8X (1.5 equiv), −78–0 °C, 2–4 h, 48–93%; (b) *t*-$BuONH_3Cl$ (1.3 equiv), TEA (2 equiv), EDCI (1.5 equiv), CH_2Cl_2, overnight, 81–94%; (c) NaH (1.5 equiv), RNCO (2–3 equiv), CH_2Cl_2, 0 °C to reflux, 1–6 h, 67–85%; (d) NaH (1.5 equiv), DMF, 0 °C to reflux, 6 h, 63–96%.

limited permeability across the cell membrane and once inside the cell they are actively pumped out. This is a problem also encountered by a number of antibacterial agents.[20] The minimum inhibitory concentrations (MICs) of this series are summarized in Table 1, and compared to ciprofloxacin. Overall, this series shows lower antibacterial activity than ciprofloxacin.

The compounds were also tested for their inhibition of DNA gyrase, which was isolated and purified from *E. coli* H560,[21] to determine the effects of these compounds at the target enzyme level. An assay was employed that measures the lowest concentration of drug (μg/mL), which will produce a detectable level of cleavage from relaxed bacterial Col E1 plasmid DNA, as visualized by agarose gel elecrophoresis and staining with ethidium bromide.[21] The in vitro results of the hydroxyquinzoline-2,4-diones showed an important correlation between the ability to inhibit the DNA gyrase to antibacterial activity. It was evident from the data presented in Table 1 that all compounds with poor gyrase activity also displayed poor or no antibacterial activity. But it was also notable that the best gyrase activity did not always translate to the best in vitro activity (**10i** vs **10j**, **10t**). This trend was also observed with the fluoroquinolones.[22]

The effect of substitution at the *N*-1, *C*-7, and *C*-8 positions (R_1, R_7, and R_8, respectively) was examined. For R_1 = alkyl or aryl, the data shown in Table 1 indicate that substituent size is important for both antibacterial and gyrase activity. Both small (**10a**, **10b**, **10c**) and large (**10f**, **10g**) substituents did not impart good activity. The cyclopropyl and cyclopropylmethyl substitutions (**10d**, **10e**) showed the most promising results. They both displayed notably improved Gram-negative activity and drastically better enzymatic activity. In particular, the cyclopropyl moiety (**10e**) provided the broadest spectrum antibacterial activity and good potency toward gyrase. These data demonstrate both similarities and differences between the hydroxyquinazoline-2,4-diones and the fluoroquinolones. Whereas the bulky *N*-1 aromatic substitutions maintain potency in the fluoroquinolones, they are not tolerated in these hydroxyquinazoline-2,4-diones.[23] Meanwhile, the *N*-1 cyclopropyl group, a prominent presence in the fluoroquinolone antibiotics, is an essential substitution for this series.

Substitution at *C*-8 was explored and MICs and gyrase activity are given for R_8 = H, F, Cl (**10h**, **10i**, **10j**, respectively). The fluoro substituent (**10i**) gave the best enzyme potency (0.28 μg/mL), but this did not translate to improved in vitro antibacterial activity versus the unsubstituted and the 8-Cl analogues (**10h** and **10j**). The unsubstituted analogue (**10h**) had the best Gram-negative activity, with MICs two- to fourfold lower than substituted analogues **10i** and **10j**.

The SAR of the *C*-7 position was examined more extensively. For comparison purposes, keeping R_1 and R_8 constant, many different R_7 groups were synthesized and their SAR evaluated. Based on the fluoroquinolone literature, we chose to append many nitrogen heterocycles, with functionalized pyrrolidines being the dominant side chain. Two nonpyrrolidinyl substitutions were examined. The azetidinylamine (**10s**) displayed good Gram-negative activity, but not Gram-positive, and had poor enzymatic activity. While the imidazole analogue (**10t**) was an effective inhibitor of the gyrase enzyme, this did not translate to antibacterial activity. In general, an amino substituent appended to the 3-position of the pyrrolidine side chain enhanced antibacterial activity, with primary amino analogues (**10k**, **10l**, **10m**) more active than secondary or tertiary amino compounds (**10n**, **10o**, **10q**). Branched alkyl substitutions alpha to the distal amine gave some of the most active compounds in this series. The mono-methyl (**10l**) and the [3.1.0] bicycle (**10p**) substitutions both imparted good broad-spectrum antibacterial activity and enzymatic activity.

In summary, we have described the discovery of the 3-hydroxyquinazoline-2,4-dione series that possesses in vitro antibacterial activity and good potency toward the gyrase enzyme. The two remarkable compounds of this series (**10l**, **10p**) have significant broad-spectrum antibacterial properties. While compounds of this class display a structural similarity to the fluoroquinolone antibacterials and inhibit the same enzymatic target, they exhibit some markedly different SAR. Pursuing and exploring these differences will enable us to expand on the already well-studied SAR of the fluoroquinolones, and these findings can be exploited toward the exploration of new antibacterial agents within this mechanistic class.

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