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Synthesis and antibacterial activity of 5-methoxy- and 5-hydroxy-6-fluoro-1,8-naphthyridone-3-carboxylic acid derivatives

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Abstract—A series of 5-methoxy- and 5-hydroxy-6-fluoro-1,8-naphthyridone-3-carboxylic acid derivatives were prepared and evaluated for cell-free bacterial protein synthesis inhibition and whole cell antibacterial activity. When compared to the analogous 5-hydrogen compounds, the presence of the 5-OH group negatively affects biochemical potency. However, a tolerance of the 5-methoxy group is indicated. Only moderate whole cell antibacterial activity is seen, but this could be due to poor cellular penetration. Because only a few 7-position variants were made for this study, further investigation into this novel series combining a broader range of 7-amino derivatives with these 5-position modifications is warranted.

Antibacterial resistance is a major public health issue. *Streptococcus pneumoniae* is the most common pathogen responsible for community-acquired pneumonia and its resistance to penicillin and macrolide antibiotics is on the rise.[1] Studies have shown that 80% of methicillin-resistant *Staphylococcus aureus* (MRSA) in the United States are now resistant to ciprofloxacin,[2a–f] and about 40% of hospital-acquired staphylococci are resistant to all forms of therapy except vancomycin.[2e,f] Nearly two million patients in the United States get an infection in the hospital each year, of those patients about 90,000 die as a result of their infection. This number is up from 13,300 patient deaths in 1992.[2g] The overall global emergence of antimicrobial resistance is severely limiting the effectiveness of current drugs. Along with a need for societal restraint in antibiotic usage, the development of novel antibiotics is one way to maintain lower levels of resistance and are urgently needed.[1]

We recently discovered a novel class of ribosome inhibitors by screening a compound library to identify inhibitors of cell-free bacterial transcription or translation.[3] As a result of this screening, A-72310 (**1**) emerged as the lead compound representing a novel ribosome inhibitor (NRI) class which showed selective (prokaryotic vs eukaryotic) and broad-spectrum antibacterial activity. Despite being structurally similar to the quinolone class of antibiotics, which inhibit DNA gyrase/topoisomerase IV, NRIs undoubtedly provide antibiotic action via a different mechanism. A complete description of the discovery of these novel antibacterials and the corresponding mechanism of action studies have recently been reported.[3]

Through the course of an extensive medicinal chemistry effort at Abbott, it was demonstrated that the antibacterial activity of this class of compounds is profoundly influenced by the combination of substituents at the C_3–C_7 and N_1 positions.[4a–c] Despite intense interest in 1,8-naphthyridone-3-carboxylic acids in antibacterial research, a survey of the literature produced no examples of 5-hydroxy- or 5-methoxy-6-fluoro-1,8-naphthyridones which had been evaluated as antibacterial agents. There have been, however, a few examples of 5-hydroxy-, 5-methoxy- and 5-amino-quinolones reported.[5] Consistent with our efforts to improve the overall potency of our NRI series, we wanted to evaluate the potential of introducing a hydrogen bonding group at C_5 and gaining a valuable synthetic handle to further probe the SAR at the 5-position. We therefore undertook the synthesis of a few NRIs substituted with R^1 = OMe (**2**) or R^1 = OH (**3**) combined with three different C_7 amino substituents (R^2 = azetidine, pyrrolidine and aminopyrrolidine).

Keywords: Novel ribosome inhibitors; 1,8-Naphthyridone antibiotics.
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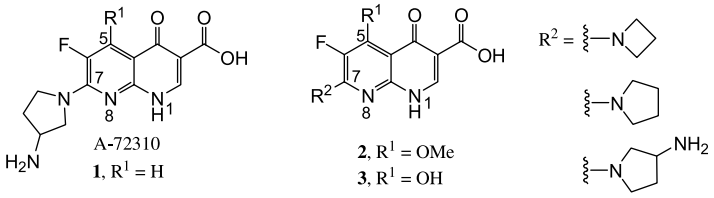
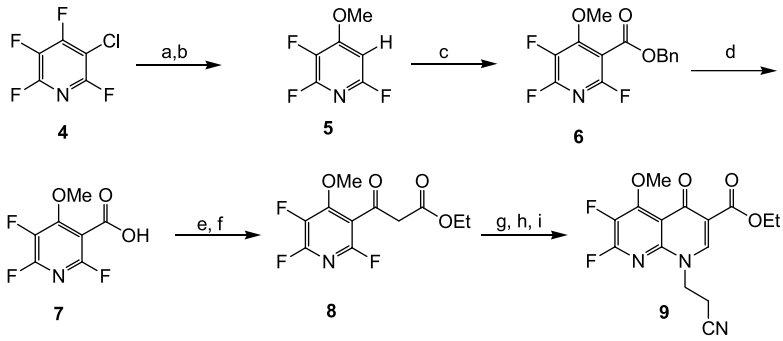


Our strategy for the construction of the 5-methoxy- and 5-hydroxy-1,8-naphthyridone core was to install the 5-methoxy group early in the synthesis and then use well-known quinolone chemistry to complete the construction of a common intermediate which would serve as a point of departure for C_7 modification. Late-stage cleavage of the methyl ether would be a key transformation to provide the 5-hydroxy-1,8-naphthyridone derivatives.

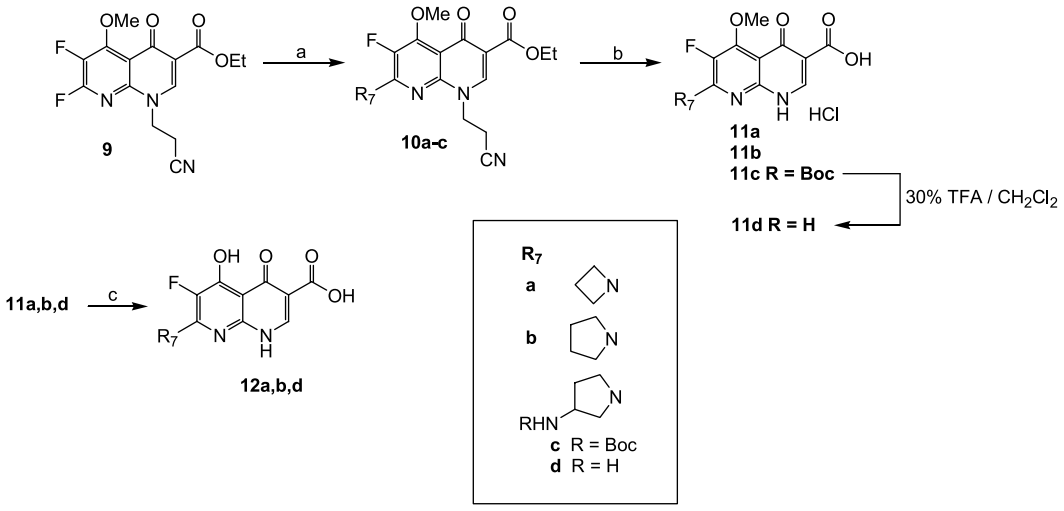
We chose commercially available 3-chloro-2,4,5,6-tetrafluoropyridine (**4**) as a convenient starting material. Illustrated in Scheme 1 is the selective displacement of the 4-position fluoride with NaOMe to provide the corresponding methoxy derivative. This material was subsequently dechlorinated by hydrogenation to furnish compound **5**.[6] The lithium anion of **5** reacted smoothly with benzyl cyanoformate to provide benzyl ester **6** in high yield. Exposure of **6** to hydrogenolysis afforded carboxylic acid **7** in near-quantitative yield. With **7** in hand, we were able to use quinolone chemistry to construct our novel 5-methoxy-1,8-naphthyridone core. Accordingly, the carboxylic acid was converted to the acyl chloride using standard oxalyl chloride/DMF conditions. The crude acyl chloride intermediate was then acylated using the magnesium malonate complex derived from potassium ethyl malonate to provide β-oxo ester **8**.[7] Combining **8** with Ac_2O and $(EtO)_3CH$ formed the corresponding ethoxymethylene derivative which was used directly in the next step without further purification. Treatment of the crude ethoxymethylene with 3-aminopropionitrile and subsequent cyclization of the enamine by treatment with NaH gave the desired 5-methoxy-6-fluoro-1,8-naphthyridone core (**9**).[8a,b]

Because diversification of C_7 has been thoroughly investigated within the context of our NRI research,[4a–c] we were able to choose three of the better variants for this preliminary study. The 7-position azetidine (**a**), pyrrolidine (**b**) and aminopyrrolidine (**c**) were selected and synthesized from our common core (**9**) shown in Scheme 2. Displacement of the 7-fluorine by the requisite amine (**a**, **b** or **c**) proceeded smoothly in the presence of Hunig's base in CH_3CN to provide **10a–c** in high yield. Concomitant deprotection of the cyanoethyl group and saponification followed by acidification provided the hydrochloride salts of **11a–c**. In the case of **11c**, the Boc group was removed using 30% trifluoroacetic acid (TFA) in CH_2Cl_2 to give TFA salt **11d**. The C_5-hydroxyl was liberated by treating compounds **11a**,**b** and **d** with an excess of pyridine hydrochloride (ca. 140 °C, 10 min). Both the 5-methoxy- and 5-hydroxy-compounds were submitted for biological evaluation (Table 1) and each structure was confirmed by ^1H NMR and mass spectroscopy.

The biochemical assays used for this study have previously been described.[3,9] Compounds were subjected to a panel of cell-free translation assays. Reported here are the concentrations causing 50% inhibition of bacterial protein synthesis (IC_{50} values) measured in ribosome-containing *S. pneumoniae* cell extracts translating a luciferase mRNA. In order to confirm bacterial protein synthesis inhibition in extracts from a Gram-negative bacterium and rule out direct luciferase inhibition, a second assay was implemented using cell-free extracts from *Escherichia coli* charged with mRNA encoding β-galactosidase. In this case, the two assays were found to be in agreement, confirming that these compounds are inhibitors of bacterial protein synthesis. It should also be pointed out that quinolone antibiotics, including ciprofloxacin and levofloxacin, did not inhibit bacterial protein synthesis in these assays (data not shown). Compounds were also evaluated for antibacterial activity



Scheme 1. Reagents and conditions: (a) NaOMe, MeOH–THF (1:1), 0 °C to rt, 20 h; (b) H_2, 10% Pd–C, Et_3N, MeOH, 3 atm, 24 h, 54% over two steps; (c) *n*-BuLi (2.5 M hexane), THF, −78 °C, 1 h, then benzyl cyanoformate −78 °C to rt, 1 h, 74%; (d) H_2, 10% Pd–C, EtOH, rt, 1.5 h, 98%; (e) oxalyl chloride, DMF (cat.), CH_2Cl_2, 0 °C to rt, 1.5 h; (f) potassium ethyl malonate, $MgCl_2$, Et_3N, CH_3CN, 10 °C to rt, 2.5 h, 78% two steps; (g) $(EtO)_3CH$, Ac_2O, 130 °C, 15 h; (h) 3-aminopropionitrile, CH_2Cl_2, rt, 1 h; (i) NaH (60% oil dispersion), THF, 0 °C to rt, 1 h, 51% over three steps.

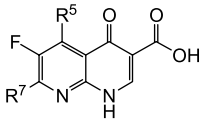


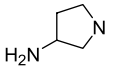
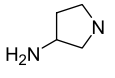
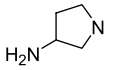
Scheme 2. Reagents and conditions: (a) amine (**a**, **b** or **c**), DIPEA, CH_3CN, 60 °C, 90–100%, (b) 1.0 N NaOH, EtOH, 80 °C, 1.5 h, then acidification with $HCl_{(aq)}$, 70–80%, (c) pyridine hydrochloride, 140 °C, 5–10 min, 80–90%.

against a variety of quinolone-resistant and susceptible strains of bacteria. Minimum inhibitory concentrations (MICs) for a representative quinolone-resistant strain and a Gram-negative strain are reported.

As shown in Table 1, the 5-methoxy derivatives (entries 1–3) are more potent protein synthesis inhibitors than the corresponding 5-hydroxy analogs (entries 4–6). More importantly, when entries 1–3 are compared to

Table 1. Cell-free bacterial protein synthesis inhibition and antibacterial activity



Entry	Compound	R^5	R^7	*S. pneumoniae* translation IC_{50} (μM)	*S. pneumoniae* 7257[a] MIC (μg/ml)	*H. influenzas* GYR 1435[b] MIC (μg/ml)
1	**11a**	OMe		14	8	32
2	**11b**	OMe		32	32	64
3	**11d**	OMe		4	64	64
4	**12a**	OH		200	64	64
5	**12b**	OH		51	4	4
6	**12d**	OH		17	64	32
7	**13**	H		5	4	1
8	**14**	H		10	4	1
9	**1**	H		7	32	4
Levofloxacin				>100	16	0.015

[a] Quinolone-resistant strain.
[b] Gram-negative.

the previously described 5-hydrogen compounds (entries 7–9),[3,4a–c] similar in vitro potencies are seen. In particular, compound **11d** (IC_{50} = 4 μM) is roughly equipotent to the parent compound **1** (IC_{50} = 7 μM). Compound **11a** (IC_{50} = 14 μM) where R^7 = azetidine, is only 2–3 times less potent than the analogous 5-hydrogen compound (**13**, IC_{50} = 5 μM). These data indicate a tolerance for this type of substitution at the 5-position.

Also shown in Table 1 is the antibacterial activity of each compound. Data for levofloxacin, a second-generation quinolone antibiotic, are included as a reference. Moderate to good antibacterial activity is seen for compounds **11a**,**b**, **12b** and **d** against *S. pneumoniae* 7257, a strain resistant to the fluoroquinolones due to mutations in the DNA gyrase and topoisomerases IV,[10] and *Haemophilus influenzae* GYR 1435, a representative Gram-negative pathogen. These MICs, taken together with the biochemical assay results, provide solid evidence that these compounds exert their antimicrobial effects through a non-quinolone mechanism of action. A possible explanation for the lack of antibacterial activity for **11d** and **12d** in either of these strains could be poor cellular penetration. No permeabilizer studies were done with this group of compounds.[4c]

In summary, we have developed a flexible synthetic entry to novel 5-methoxy- and 5-hydroxy-6-fluoro-1,8-naphthyridone-3-carboxylic acid antibiotics. Members of this class of 1,8-naphthyridones had not previously been evaluated as antibacterial agents. The data indicate incorporation of a 5-methoxy group does not have a deleterious effect on in vitro potency and given the profound influence the 7-position has in combination with the 5-position,[11] further exploration of this class of NRIs should be undertaken. The 5-hydroxyl also constitutes a new synthetic handle for this class of compounds, making it possible to thoroughly investigate the advantages of hydrophilic substitution at the 5-position of 1,8-naphthyridone antibacterials. Extension of this work will include a comprehensive survey of 5-hydroxy, 5-alkoxy, 5-amino derivatives in combination with a wide variety of C_7 amino substitution, allowing further development and differentiation of this novel class. Detailed accounts of continuing studies will be reported in due course.

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