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Novel inhibitors of bacterial protein synthesis: structure–activity relationships for 1,8-naphthyridine derivatives incorporating position 3 and 4 variants

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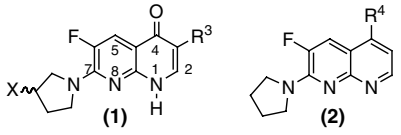
Abstract—Structure–activity relationships for a recently discovered novel ribosome inhibitor (NRI) class of antibacterials were investigated. Preliminary efforts to optimize protein synthesis inhibitory activity of the series through modification of positions 3 and 4 of the naphthyridone lead template resulted in the identification of several biochemically potent analogues. A lack of corresponding whole cell antibacterial activity is thought to be a consequence of poor cellular penetration as evidenced by the enhancement of activity observed for a lead analogue tested in the presence of a cell permeabilizing agent.

The clinical effectiveness of currently available antibiotics is threatened by an increasing prevalence of resistant pathogenic bacteria. Multidrug resistance among Gram-positive organisms, including *Streptococcus pneumoniae*, the most common respiratory pathogen, is of particular concern. A recent worldwide surveillance study found that resistance rates among *S. pneumoniae* isolates have reached alarming levels for frontline therapies such as penicillin (36%) and macrolides (31%).[1] Emerging resistance to fluoroquinolones has also been documented.[2] To address this pressing healthcare issue, new antibacterial drug classes unaffected by existing resistance mechanisms are needed.[3,4]

Recently, we described the discovery and characterization of A-72310 (**1a**, X = NH_2, R^3 = COOH), the parent member of a novel ribosome inhibitor (NRI) class that exhibits selective and broad-spectrum antibacterial activity.[5] Despite a close structural relationship to quinolone antibacterials that inhibit type II topoisomerases, NRIs function in a mechanistically distinct manner. Detailed mechanistic studies revealed that these agents exert their antibacterial effects by inhibiting bacterial protein synthesis at the translation level through a direct interaction with the bacterial ribosome.[5]



(1) (2)

Here we report our preliminary investigation of the structure–activity relationships (SAR) at positions 3 and 4 of the NRI series (**1**, **2**) in the context of bacterial protein synthesis inhibition. Position 3 in A-72310 was viewed as an attractive starting place for our study given the general requirement for a carboxylic acid moiety at this position in classical quinolone SAR.[6] In addition to providing a point of attachment for a potentially superior ribosomal binding element, the productive incorporation of a non-quinolone substituent at this site would further differentiate the NRI class. In the course of these studies we found that removal of the terminal

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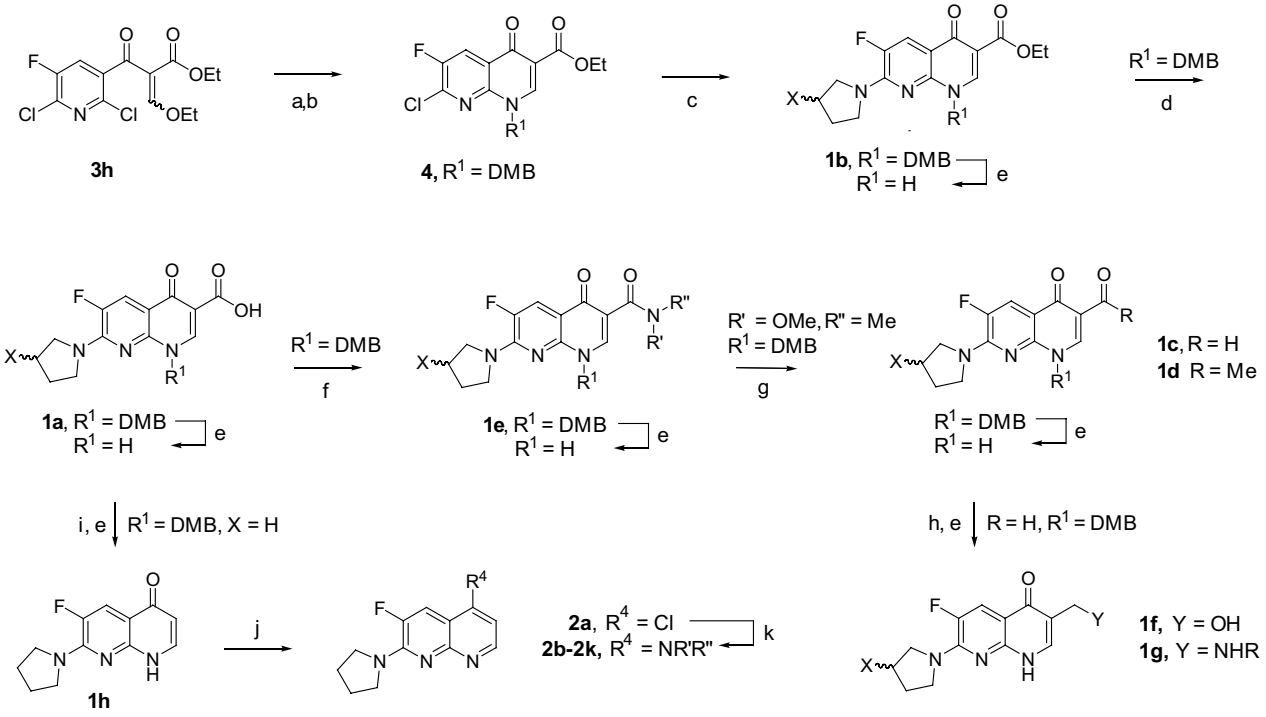
amino group from the C-7 pyrrolidine in A-72310 resulted in a compound (**1a**, X = H, R^3 = COOH) with retained protein synthesis inhibitory activity and improved antibacterial potency. We therefore explored the SAR at position 3 on this modified parent template. Additionally, to complement our acid-replacement studies, we synthesized a series of analogues wherein the carboxylic acid at position 3 was removed from the naphthyridone nucleus. This modification enabled us to assess position 4 as an alternative site for optimization on the simplified 1,8-naphthyridine core **2**.

The synthetic targets were accessed through modifications of the C-3 carboxylic acid group in common intermediates **1a** as depicted in Scheme 1. Compounds **1a** were prepared by adapting known quinolone chemistry.[7] Treatment of enol ether **3**[8] with 2,4-dimethoxybenzylamine followed by base-promoted cyclization of the resulting enamine afforded precursor **4**. Subsequent nucleophilic aromatic substitution of the 7-chloro substituent with either 3-Boc-aminopyrrolidine or pyrrolidine provided esters **1b**, which, upon saponification, gave the desired naphthyridone intermediates (**1a**). The 2,4-dimethoxybenzyl (DMB) and Boc protecting groups were removed in the presence of trifluoroacetic acid to unmask all final products including **1a** (R^1 = H). Elaboration of **1a** (R^1 = DMB) to corresponding C-3 amides (**1e**) was achieved using various standard coupling methods. In turn, Weinreb amides **1e** (R′ = OMe, R″ = Me) were readily transformed to C-3 aldehyde (**1c**, R = H) and ketone (**1d**, R = Me) derivatives by reduction with DIBAL and treatment with MeMgBr, respectively. Subsequent conversion of aldehyde intermediates (**1c**) to alcohol (**1f**) and amine (**1g**) targets proceeded smoothly using conventional methodologies as outlined. Finally, position 4 variants (**2b**–**2l**) were prepared from reactive intermediate **2a** by displacement of the 4-chloro substituent with nitrogen nucleophiles. Accordingly, decarboxylation of **1a** (R^1 = DMB, X = H) with quinoline and copper (I) oxide at elevated temperatures and ensuing N-1 deprotection yielded naphthyridone **1h** which, when heated with phosphoryl chloride, provided the requisite precursor **2a**.

As previously described,[5,9] analogues were evaluated for protein synthesis inhibitory activity in a panel of cell-free translation assays. Reported here are the IC_{50} values measured in ribosome-containing *S. pneumoniae* cell extracts translating a luciferase mRNA. Inhibitory activity was quantified using a luminescence assay to measure luciferase production in the presence of compound. All observed inhibition (*S. pneumoniae* translation $IC_{50} < 100\,\mu M$) was confirmed by conducting a parallel assessment of protein synthesis inhibitory activity using an *Escherichia coli* transcription/translation assay[9] employing a β-galactosidase rather than luciferase reporter to ensure that the observed activity was not a consequence of direct luciferase inhibition. Inhibitory activities obtained for the corresponding



Scheme 1. Reagents and conditions: (a) 2,4-dimethoxybenzylamine, CH_2Cl_2, 25 °C, 3 h; (b) K_2CO_3, CH_3CN, reflux, 16 h, 81%; (c) 3-(*t*-Butoxycarbonylamino)pyrrolidine or pyrrolidine, K_2CO_3, CH_2Cl_2, 25 °C, 16 h, 93–97%; (d) LiOH, EtOH–H_2O, 60 °C, 91–94%; (e) TFA, 25–80 °C, 63–92%; (f) Method A (Table 1, entry 4): (i) isobutyl chloroformate, NEt_3, DMAP (cat.), CH_2Cl_2, 0 °C; (ii) NH_3, 25 °C, 83%. Method B (Table 1, entries 5–7): HOAT, HATU, Hunig's base, DMA, 25 °C, 27–49%. Method C (Table 1, entry 15): (i) CDI, DMF, 100 °C, 2 h; (ii) NH_2OH HCl, pyridine, 34%. Method D (**1e**, R′ = OMe, R″ = Me): HNMeOMe HCl, TBTU, Hunig's base, $CHCl_3$, 25 °C, 79–88%; (g) **1c**: DIBAL, CH_2Cl_2, −78 °C, 54–61%; **1d** (X = H): MeMgBr, THF–CH_2Cl_2, 0 °C, 84%; (h) **1f** (X = H): $NaBH_4$, EtOH–CH_2Cl_2, 0 °C, 72%; **1g**: RNH_2, $NaBH_3CN$, AcOH, MeOH–CH_2Cl_2, 25 °C, 43–76%; (i) Cu_2O, quinoline, 180 °C, 20 h, 88%; (j) $POCl_3$, 100 °C, 1 h, 68%; (k) 1° or 2° amine or hydrazine, DMA, 115 °C, 37–91%.

Table 1. Bacterial protein synthesis inhibitory activity for compounds **1**

Entry	Compd	X	R^3	*S. pneumoniae* translation IC_{50}, μM
1	**1a**	NH_2	COOH	5
2	**1b**	NH_2	COOEt	20
3	**1d**	NH_2	COMe	50
4	**1e**	NH_2	$CONH_2$	23
5	**1e**	NH_2	$CONHCHMe_2$	>100
6	**1e**	NH_2	$CONH(CH_2)_2CHMe_2$	25
7	**1e**	NH_2	$CONMe(CH_2)_2CHMe_2$	>100
8	**1g**	NH_2	CH_2NHPh	54
9	**1g**	NH_2	CH_2NHCH_2Ph	25
10	**1g**	NH_2	$CH_2NH(CH_2)_3Ph$	10
11	**1g**	NH_2	$CH_2NH(CH_2)_3NEt_2$	10
12	**1a**	H	COOH	10
13	**1b**	H	COOEt	>100
14	**1c**	H	CHO	>100
15	**1e**	H	CONHOH	>100
16	**1f**	H	CH_2OH	>100
17	**1g**	H	CH_2NHPh	>100
18	**1g**	H	CH_2NHCH_2Ph	>100
19	**1g**	H	$CH_2NH(CH_2)_2NMe_2$	>100
20	**1h**	H	H	>100

assays were found to be in good agreement. Representative quinolone antibacterials including ciprofloxacin, levofloxacin, and norfloxacin did not inhibit bacterial protein synthesis in our assays.

In general C-7 aminopyrrolidinyl derivatives (Table 1, entries 1–11) displayed a high tolerance for structural variation at position 3 with regard to both nature and size of substituents. Replacement of the carboxylic acid group with ester (entry 2), amide (entries 4 and 6) or amine-linked substitutions (entries 8–11) produced potent inhibitors. Steric parameters of C-3 variants had greater effects on inhibitory potency than did the type of functional group incorporated. Although sterically demanding substitution near the naphthyridone nucleus was detrimental (entries 5 and 7), small adducts more closely approximating the spatial environment of the parent carboxyl were better tolerated as exemplified by entries 2–4. Similarly, elongated tethers lacking branch points proximal to the core resulted in good potency for amide- or amine-linked derivatives projecting a variety of functionality including aliphatic (entry 6), aryl (entries 9 and 10), and amino groups (entry 11). Significantly, the best inhibitors (entries 10 and 11) within this series exhibited potency comparable to the parent acid (entry 1) despite pronounced structural deviation at position 3.

In contrast, any modification at position 3 in the corresponding C-7 pyrrolidinyl series (entries 12–20, Table 1) abolished inhibitory activity. These results were surprising given the potency of the parent acid (entry 12) and in light of the tolerance for variation observed in the corresponding C-7 aminopyrrolidine series. Interestingly, however, by installing a compensatory substituent at position 4 on related core **2**, potent inhibition was achieved in the absence of the C-3 carboxylic acid moiety (Table 2). Nitrogen-linked C-4 derivatives projecting amine (**2g**,**h**) or alkyl (**2f**) side chains displayed the best activity across a range of functional groups examined. Furthermore, amine **2g** was among the most potent NRIs identified in the current study. C-4 adducts containing acid (**2b**), amide (**2c**), alcohol (**2e**) or hydrazine (**2k**) groups were less active. The relative inactivity of piperazinyl analogue **2i** compared to noncyclized amine entries **2g** and **2h** reveals a preference for side chain flexibility within this series.

Table 2. Bacterial protein synthesis inhibitory activity for compounds **2**

Entry	Compd	R^4	*S. pneumoniae* translation IC_{50}, μM
1	**2b**	$NHCH_2COOH$	>100
2	**2c**	$NHCH_2CONH_2$	82
3	**2d**	$NH(CH_2)_2OMe$	39
4	**2e**	$NH(CH_2)_2OH$	91
5	**2f**	$NH(CH_2)_2Me$	19
6	**2g**	$NH(CH_2)_2NH_2$	11
7	**2h**	$NHMe(CH_2)_2NMe_2$	18
8	**2i**	1-Piperazinyl	>100
9	**2j**	NHCH(Me)Ph	>100
10	**2k**	$NHNH_2$	89

The data presented demonstrate that the position 3 carboxylic acid in lead compounds **1a** is not required for potent inhibition of bacterial protein synthesis. A variety of alternative side chain functionality extending from positions 3 or 4 resulted in good biochemical potency. However, while not required for biochemical potency, the C-3 carboxyl appears to be important for whole cell antibacterial activity. Although parent acids **1a** exhibited good antibacterial activity against *S. pneumoniae* 7257, a quinolone-resistant strain,[10] and *Haemophilus influenzae* GYR 1435, a representative Gram-negative pathogen (Table 3), all position 3 and 4 variants were devoid of activity against these strains.[11,12] To preliminarily assess whether this lack of activity might be associated with poor cellular penetration relative to the C-3 acids, **2g** was tested for antibacterial activity in the presence of ethylenediaminetetraacetic acid (EDTA, 1.1 mg/ml), a permeabilizer of the bacterial cell envelope. Interestingly, a greater than 4-fold improvement in

Table 3. Antibacterial activity[a] for NRI parent acids **1a**

Compd	X	R^3	*S. pneumoniae* 7257[b]	*H. influenzae* GYR 1435
1a	NH_2	COOH	32	4
1a	H	COOH	4	1
Levofloxacin			16	0.015

[a] MIC (minimum inhibitory concentration), μg/mL.
[b] Quinolone-resistant.

Table 4. Effect of permeabilizer[a] on the antibacterial activity[b] of NRI **2g**

Compd	R^4	*E. coli* 7703	*E. coli* 7703 + EDTA
2g	$NH(CH_2)_2NH_2$	>128	32

[a] Ethylenetetraacetic acid (EDTA).
[b] MIC, μg/mL.

antibacterial activity was observed in the permeabilized cells (Table 4). These findings suggest that the contributions made by the C-3 carboxylic acid in NRI lead structures **1a** are physical rather than biochemical. It is hoped that a more systematic study of NRI cores **1** and **2** aimed at identifying structural features that both facilitate penetration into the bacterial cell and enhance ribosomal inhibition will yield NRIs from the current series with more desirable antibacterial profiles.

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