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Bioorganic & Medicinal Chemistry Letters 17 (2007) 2718–2722
Bioorganic & Medicinal Chemistry Letters

Tetrahydroindazole inhibitors of bacterial type II topoisomerases. Part 2: SAR development and potency against multidrug-resistant strains

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Received 14 December 2006; revised 28 February 2007; accepted 1 March 2007
Available online 6 March 2007

Abstract—We have previously reported a novel class of tetrahydroindazoles that display potency against a variety of Gram-positive and Gram-negative bacteria, potentially via interaction with type II bacterial topoisomerases. Herein are reported SAR investigations of this new series. Several compounds possessing broad-spectrum potency were prepared. Further, these compounds exhibit activity against multidrug-resistant Gram-positive microorganisms equivalent to that against susceptible strains.

Bacterial type II topoisomerases are essential enzymes with roles in DNA replication, chromosome segregation, and DNA compaction. Among the type II topoisomerases are DNA gyrase and topoisomerase IV. DNA gyrase is a tetramer composed of two GyrA and two GyrB subunits that catalyzes the ATP-dependent introduction of negative supercoils into bacterial DNA as well as the decatenation and unknotting of DNA. Topoisomerase IV has a similar structure and is composed of two ParC and two ParE subunits. Topoisomerase IV relaxes supercoiled DNA and has a primary role in decatenation of daughter chromosomes following DNA replications.[1] The quinolone class of antibacterial agents, including such marketed drugs as levofloxacin and ciprofloxacin (Fig. 1), is widely used for the treatment of bacterial infectious diseases and targets these two distinct topoisomerases.[2,3] Quinolones inhibit the DNA breakage-reunion cycle by binding to the gyrase subunit A and by locking the gyrase–DNA complex.

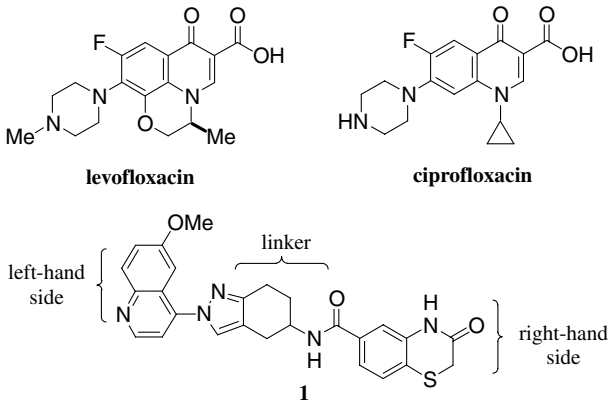

Figure 1. Selected inhibitors of bacterial type II topoisomerases.

Naturally occurring agents such as coumarins, though generally plagued by toxicity limitations, are also known to achieve good antibacterial activity through inhibition of DNA gyrase.[3–6] Specifically, coumarins inhibit ATPase activity of DNA gyrase by competing with ATP for binding to subunit B of the enzyme.[7,8] A significant obstacle to antibacterial therapy is posed by multidrug-resistant Gram-positive bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA),

Keywords: Gyrase; Topoisomerase; Bacteria; Bacterial resistance; MIC; Broad-spectrum; Antibacterial; *Escherichia coli*; *Staphylococcus aureus*; *Streptococcus pneumoniae*; Quinolone; Tetrahydroindazole; SAR.
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doi:10.1016/j.bmcl.2007.03.004

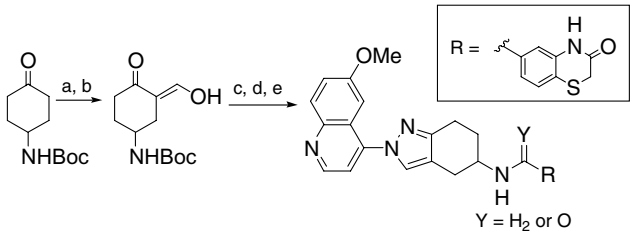
penicillin-resistant *Streptococcus pneumoniae* (PRSA), and vancomycin-resistant enterococci (VRE). These species are typically resistant, as well, to quinolone agents such as norfloxacin, sparfloxacin, and ciprofloxacin. Thus, interest in developing a new class of DNA gyrase/topoisomerase IV inhibitors, preferably with broad-spectrum (Gram-positive and Gram-negative) antibacterial activity, has grown.

In the preceding paper,[9] the synthesis and biological evaluation of a novel class of pyrazoles led to the identification of a series of tetrahydroindazoles (e.g., **1**, Fig. 1) with activity against type II topoisomerases and good Gram-positive and Gram-negative anti-microbial potency.[10]

In an effort to improve potency, investigations into the SAR of this template were undertaken. Variation of the left-hand side biaryl architecture was explored in accord with the racemic synthetic route previously reported,[9] represented in Scheme 1, employing commercially available biaryl hydrazines or biaryl bromides, which were converted to the corresponding hydrazines using standard procedures.[10] All bicyclic right-hand side fragments were prepared according to literature procedures.[10]

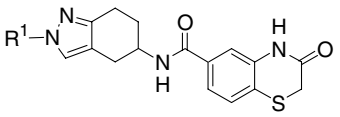
Generally, a variety of biaryls display good enzymatic activity against topoisomerase IV and acceptable activity against DNA gyrase (Table 1). This trend does not translate well to activity against Gram-positive and, especially, Gram-negative organisms; whereas Gram-positive activity is retained with various changes to the left-hand side, only the methoxy-substituted quinoline **2** shows any activity against a Gram-negative strain. Subsequent investigations employed exclusively the quinoline and naphthyridine moieties of compounds **1** and **2**, respectively, given the combination of their good enzymatic activities and MIC values.

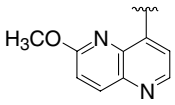
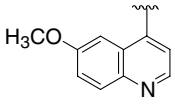
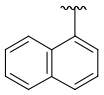
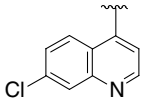
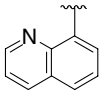
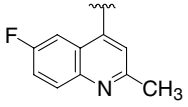
Initial variation of the bicyclic right-hand side identified a number of molecules with potency against DNA gyrase and topoisomerase IV as well as substantial antibacterial activity (Table 2). The benzodioxanes **7** and **8** are not as potent as the benzothiazinones **1** and **2**, yet connection of the benzodioxane to the indazole core via an amine linker increases both enzymatic and antimicrobial activity (**9** and **10**). Increasing or decreasing the ring size of the benzodioxane analogs (compounds **11** and **12**) brings no significant change to enzymatic activities and MIC values. The amine-linker variants of lead compounds **1** and **2**, benzothiazinones **13** and **14**, display the same in vitro enzymatic activity, but have increased potency against *Escherichia coli*. Removal of the thiazinone oxygen, as in the thiazine analogs **15** and **16**, is tolerated, albeit with degradation in Gram-negative activity. The pyridooxazinone analogs **17** and **18**, in which the sulfur is replaced by oxygen and a nitrogen atom is incorporated into the aromatic ring, behave similarly to the benzothiazinones **13** and **14**. Compared to the amide-linked benzothiazinone lead compounds **1** and **2**, the benzooxazones **19** and **20** show similar enzymatic activity, and, again, increased Gram-negative potency. Fully aromatic bicyclic architectures typically show loss of antimicrobial activity, as represented by the indole analog **21**. With these dramatic structural changes to the right-hand side of compounds **1** and **2** affording no significant improvements, it was decided next to pursue finer alterations to the amide-linked thiazinones.



Scheme 1. Reagents and condition: (a) $[(CH_3)_2N]_2CH(OtBu)$, THF, 65 °C; (b) 1 N HCl; (c) 6-methoxyquinoline-hydrazine, THF then *p*-TsOH; (d) 4 M HCl, dioxane; (e) RCHO, $NaBH_3CN$, CH_3CN or RCOOH, EDC, HOBt, DMF.

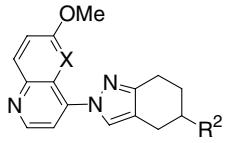
Table 1. Biaryl left-hand side SAR

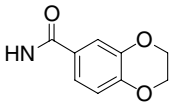
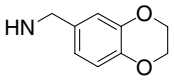
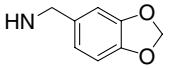
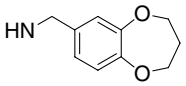
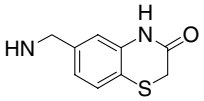
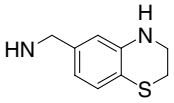
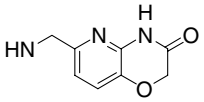
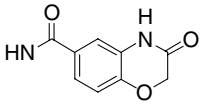
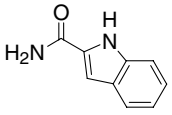


Compound	R^1	DNA gyrase/ Topo IV IC_{50} (μg/mL)	*E.c.*[a]/*S.a.*[b]/ *S.p.*[c] MIC (μg/mL)
1		0.25/0.25	>128/0.06/4
2		0.12/0.125	4/0.25/2
3		2/0.125	>128/0.5/8
4		16/ > 0.5	>128/2/ > 128
5		2/0.125	16/0.25/4
6		4/0.5	>128/8/ > 128
Norfloxacin		ND/ND	ND/l/ND
Sparfloxacin		ND/ND	ND/0.062/ND
Ciprofloxacin		ND/ND	ND/0.25-0.5/ND

[a] *E.c.*,*E.coli* KL-16 (GSC strain 4245).
[b] *S.a.*, *S. aureus* ATCC13709.
[c] *S.p.*, *S. pneumoniae* ATCC49619.

Table 2. Bicyclic right-hand side SAR



Compound	R^2	DNA gyrase/ Topo IV IC50 (μg/mL)	*E c.*[c]/*S.a.*[d]/ *S.p.*[e] MIC (μg/mL)
7[a]		>32/>1	>128/0.5/8
8[b]		4/ND	>128/2/8
9[a]		1/0.25	32/0.03/0.5
10[b]		2/0.06	128/0.5/1
11[a]		0.25/0.25	32/0.5/2
12[a]		4/1	>128/l/4
13[a]		0.125 /0.03	1/0.25/1
14[b]		0.06/0.16	2/0.5/2
15[a]		0.5/0.03	8/<0.125/0.25
16[b]		0.5/0.0625	8/0.5/0.5
17[a]		<0.125/0.016	2/0.25/2
18[b]		0.06/0.016 0	2/1/2
19[a]		1/0.25	4/0.125/8
20[b]		0.25/0.5	2/0.125/4
21[b]		>16/1	>128/l/8

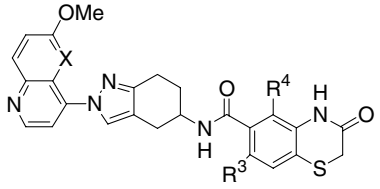
[a] X = N.
[b] X = CH.
[c] *E. c., E. coli* KL-16 (GSC strain 4245).
[d] *S.a., S. aureus* ATCC13709.
[e] *S.p., S. pneumoniae* ATCC49619.

As such, substitutions on this right-hand side thiazinone bicyclic framework were explored (Table 3). Comparing analogs **1** and **2** to analogs with fluorine substitution (**22**–**25**) revealed similarities in MIC values, with the quinoline analog of each showing improved activity against Gram-negative species relative to the naphthyridines. Significantly, the bromine and chlorine substituted analogs **26**–**29** (and, to a lesser degree, the trifluoromethyl-substituted analogs **30** and **31**) show broad-spectrum antimicrobial activity for both the quinoline and the naphthyridine analogs. Larger electron withdrawing functional groups such as esters and acids (**35**–**38**) lead to retention of activity against *S. aureus*, though also to a general decline in Gram-negative antibacterial activity; a similar trend is observed with simple methyl substitution (**33** and **34**). Introduction of an electron-donating methoxy substituent (**32**) is deleterious to MIC values.

Unconstrained, monocyclic variants of the bicyclic benzothiazinone right-hand side analogs were prepared using commercially available carboxylic acids and aldehydes (Table 4). Notably, these compounds possess similar potency against *S. aureus* and *S. pneumoniae* to their bicylic counterparts, though they exhibit a loss of activity against Gram-negative organisms (e.g., compound **39,** MIC > 128 μg/mL against *E. coli*). These preliminary results indicate that the bicyclic functionality may not be crucial in achieving DNA gyrase/topoisomerase IV inhibition as well as reasonable MICs; rather, some flexibility with regard to the right-hand side ring positioning and substitution is allowed and warrants further investigation.

Importantly, several of these tetrahydroindazole-based antibacterial agents display potency against various quinolone resistant strains (Table 5). MIC values of ciprofloxacin, norfloxacin, and sparfloxacin against methicillin-susceptible *S. aureus* (MSSA) are 0.5, 1.0, and 0.062 μg/mL, respectively. Against several quinolone-resistant isolates, though, these agents prove wholly

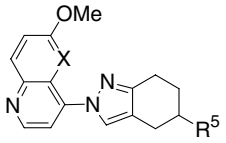
Table 3. Thiazinone right-hand side SAR

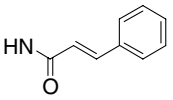
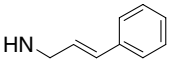
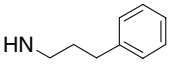
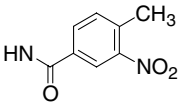
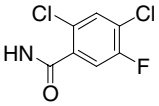


Compound	R^3	R^4	DNA gyrase/ Topo IV IC_{50} (μg/mL)	*E. c.*[c]/*S. a.*[d]/ *S. p.*[e] MIC (μg/mL)
22[a]	F	H	0.12/0.125	128/0.25/2
23[b]	F	H	8/0.25	4/0.03/1
24[a]	F	F	1/ND	>128/0.125/4
25[b]	F	F	1/ND	8/0.125/1
26[a]	Cl	H	0.12 /ND	2/<0.125/4
27[b]	Cl	H	0.5/ND	2/<0.125/2
28[a]	Br	H	0.25/0.25	2/0.125 /2
29[b]	Br	H	0.5/0.125	2/0.125/2
30[a]	CF_3	H	0.25/ND	4/0.125/8
31[b]	CF_3	H	1/0.25	8/0.125/8
32[a]	OCH_3	H	ND/ND	>16/2/16
33[a]	CH_3	H	ND/ND	>16/0.25/>16
34[b]	CH_3	H	ND/ND	8/0.5/16
35[a]	CO_2Et	H	ND/ND	>16/0.5/>16
36[b]	CO_2Et	H	ND/ND	>16/2/>16
37[a]	CO_2H	H	ND/ND	>16/0.5/16
38[b]	CO_2H	H	ND/ND	>16/1/>16

[a] X = N.
[b] X = CH.
[c] *E. c., E. coli* KL-16 (GSC strain 4245).
[d] *S.a., S. aureus* ATCC13709.
[e] *S.p., S. pneumoniae* ATCC49619.

Table 4. Monocyclic right-hand side SAR



Compound	R^5	DNA gyrase/ Topo IV IC_{50} (μg/mL)	*S. a.*[c]/ *S.p.*[d] MIC (μg/mL)
39[a]		8/ND	0.25/2
40[b]		16/0.5	1/4
41[a]		0.12/<0.015	0.06/<0.125
42[b]		4/ND	0.5/1
43[a]		0.8/ND	0.25/<0.125
44[a]		ND/ND	0.25/8
45[b]		>16/ND	0.25/4
46[a]		ND/ND	0.25/8

[a] X = N.
[b] X = CH.
[c] *S.a.*, *S. aureus* ATCC13709.
[d] *S.p.*, *S. pneumoniae* ATCC49619.

ineffective. In contrast, a variety of the tetrahydroindazole-based compounds demonstrate equal potency against susceptible and resistant strains. Compounds **22** and **23**, for example, are unaffected by the resistance mutations; on average, these compounds are 3360-fold more active than ciprofloxacin against the resistant panel. That these novel compounds interact with the same type II topoisomerase enzymes as the quinolones yet display such markedly different antibacterial profiles suggests that they may in fact operate via a different mechanism of action. This is further supported by the demonstration that while these compounds do not form cleavage complexes with DNA gyrase, they do block the formation of such complexes by ciprofloxacin (unpublished results). Such activity against quinolone-resistant species is of special importance given the proclivity of bacterial pathogens rapidly to develop resistance, and testifies to the significance of this novel class of antimicrobial agents.

In summary, the SAR of a novel class of tetrahydroindazole-based inhibitors of type II bacterial topoisomerases with broad-spectrum antimicrobial activity has been described. Compounds containing halogen-substituted thiazinone right-hand side moieties (**22**–**29**) offer the best combination of potency against both susceptible and multidrug-resistant Gram-positive strains, with additional activity against Gram-negative organisms. These promising results warrant further investigations into this new antimicrobial class.

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Table 5. MICs (μg/mL) against quinolone-susceptible and -resistant strains

Compound	Susceptible MIC (μg/mL) MRSA OC2878	Resistant MIC (μg/mL)			
		CipR MRSA OC3946	CipR MRSA OC4159	CipR MRSA OC4222	CipR MRSA OC5273
Ciprofloxacin	0.5	16	128	64	128
Norfloxacin	1	128	>128	>128	>128
Sparfloxacin	0.062	4	16	8	16
1	0.031	0.007	0.5	0.5	0.062
22	0.031	0.007	0.007	0.015	0.031
2	0.125	0.015	0.031	0.062	0.25
23	0.062	0.015	0.031	0.031	0.062
5	0.25	0.125	4	4	0.5
14	0.5	0.25	0.25	0.25	0.5
41	0.125	0.062	0.062	0.062	0.125
10	0.5	0.5	16	16	1
13	0.5	0.125	0.25	0.125	0.5
42	0.5	0.5	0.5	0.25	1

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