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Novel anti-infection agents: Small-molecule inhibitors of bacterial transcription factors

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Abstract—Structure-based drug design was utilized to identify potent small-molecule inhibitors of proteins within the AraC family of bacterial transcription factors, which control virulence in medically important microbes. These agents represent a novel approach to fight infectious disease and may be less likely to promote resistance development. These compounds lack intrinsic antibacterial activity in vitro and were able to limit a bacterial infection in a mouse model of urinary tract infection.

The emergence of multidrug-resistant bacteria has challenged researchers to develop novel therapies for the prevention and treatment of infectious diseases. Traditional antibiotics exert strong selective pressure for resistance development, since they target bacterial processes essential for the growth of the organism. Alternative approaches have involved the modulation of virulence factors, elements that contribute to infection in vivo but are not essential for growth of the organism in vitro. Most attempts along this path have targeted individual virulence factors, such as pili or toxins, but these approaches have suffered from a limited spectrum of activity. The targeting of regulatory mechanisms that could affect multiple virulence factors simultaneously may offer an alternative approach. Toward this end, the AraC family of bacterial transcription factors represents a novel target for this unique therapeutic approach.

The AraC family of proteins is a large group of transcription factors found in a variety of medically important bacteria, particularly the *Enterobacteriaceae*.[1] These proteins regulate virulence by controlling antibiotic and oxidative stress resistance, expression of type III secretion, toxin production, and other processes that are important during infection.[2–6] Previous studies have demonstrated that the inactivation of genes encoding AraC proteins attenuates virulence in human[7] and animal models of infection.[3,4,6,8–10,12] Since all of the AraC proteins contain a highly conserved DNA-binding domain, it is envisioned that a small-molecule inhibitor directed at or otherwise affecting this domain could interfere with protein function and act as an effective agent for preventing infection.

Using a structure-based drug design strategy, we chose to focus on a subset of AraC proteins including those related to MarA and other multiple antibiotic resistance (Mar) proteins.[11] Previous work in our laboratory demonstrated that Mar proteins (MarA, SoxS, and Rob) regulate virulence of *Escherichia coli* in a murine model of ascending pyelonephritis.[12] Structural information from crystallographic studies[13,14] of Mar proteins and supportive data from NMR[13–15] and mutational experiments[16] were used to construct a computer model of the conserved DNA-binding domain. A set of combinatorial chemistry scaffolds was docked to this active-site template to identify potential small-molecule inhibitors. High scoring hits were used to search the ACX-SC database (CambridgeSoft Corp., Cambridge, MA) for commercially available compounds with structural similarity. From this database, approximately 2000

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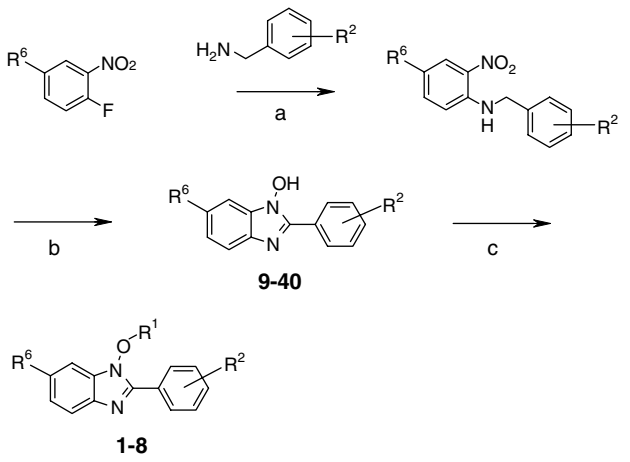


Scheme 1. Reagents and conditions: (a) Na_2CO_3, DMF, rt; (b) NaOH (aq), rt or NaOMe, MeOH, 25–60 °C or NaH, THF, 40–60 °C; (c) R^1-X, (X = Cl or Br), $NaHCO_3$, DMF, rt.

compounds were purchased and screened for inhibitory activity with an in vitro protein–DNA-binding assay using MarA, SoxS, and Rob.[17] Five unique structural classes of inhibitors were identified that negatively affected the binding of Mar proteins to their cognate DNA. Based on the potential for chemical diversity, we focused our initial drug discovery effort on the 1-hydroxybenzimidazole class of Mar inhibitors. Analogs of the hit compound **1** were prepared as previously described[18,19] to systematically explore the effects of the 1, 2, and 6-position on activity (Scheme 1). The new derivatives were screened for their ability to inhibit the binding of SoxS, a representative Mar protein, to its DNA target. Screening of the 1-position derivatives **2**–**9** identified the 1-hydroxy analog **9** as the most active inhibitor of SoxS (Table 1). This compound demonstrated improved activity over the original hit compound **1** (38% versus 14% inhibition, respectively) and was used as the core structure for further exploration of the 6 and 2-positions.

Several analogs of **9** modified at the 6-position (**10**–**21**) were prepared to explore the replacement of the nitro group. The removal of the nitro group (**10**) eliminated activity against SoxS. However, comparable activity to **9** was observed for the 6-acetyl (**17**), 6-ethanoneoxime (**18**), and 6-methanesulfonyl (**19**) analogs. Less, but detectable, inhibition was observed for electron-withdrawing substitutions (**11**–**13**) and the dimethylamino analog **14**.

The most dramatic improvement in activity was obtained through substitution of the 2-position phenyl group of **9** (**22**–**35**). Screening of these derivatives identified the *p*-amino-substituted analogs (**25**, **29**, **31**, and **34**) as the most potent inhibitors of SoxS. In particular, the *p*-aminoacyl analogs (**31**, **34**) demonstrated complete inhibition of SoxS in the screening assay. Compound **34** also demonstrated complete inhibition of SoxS when diluted to 25 μg/mL (67 μM). Based on these results, **34** was chosen for further testing.

An IC_{50} of 17 μM was determined for compound **34** in the SoxS DNA-binding inhibition assay (Table 2). This value is at least 15-fold lower than the IC_{50} of >250 μM estimated for compound **9** (exact IC_{50} determination of **9** was limited by solubility of the compound). A number of exploratory analogs of **34** substituted on the benzamide side chain were prepared as before and tested in the SoxS assay (**36**–**40**, Table 2). All of the analogs demonstrated 100% inhibition at 25 μg/mL in the screening assay (data not shown) and low micromolar IC_{50} values against SoxS. In particular, an IC_{50} of 820 nM was observed for the *p*-dimethylamino derivative **40**. This IC_{50} represents a more than 300-fold increase in potency over **9**.

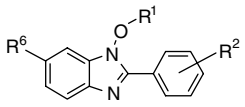
The above 1-hydroxybenzamidazoles were designed to specifically target proteins controlling the expression of virulence factors which are not essential for growth of the organism in vitro. Therefore, it was necessary to confirm that these compounds (e.g., **34**, **36**–**40** for illustrative purposes) were devoid of intrinsic antibacterial activity. No inhibition of bacterial growth in vitro was observed when these derivatives were screened against antibiotic susceptible strains of *Staphylococcus aureus* and *E. coli* (Table 2).

Since AraC proteins have a highly conserved DNA-binding domain, representative compounds **38** and **40** were screened for activity against other AraC family members from *E. coli* (MarA, Rob), *Pseudomonas aeruginosa* (ExsA), *Salmonella typhimurium* (Rma), and *Proteus mirabilis* (PqrA). As shown in Table 3, compounds **38** and **40** demonstrated comparable activity in vitro for all AraC family members tested.

Using an animal model of ascending pyelonephritis caused by *E. coli*,[20,12] compounds **37** and **40** were judged for the ability to affect kidney infection. Previous studies using this urinary tract infection model have shown that *E. coli* mutants with a *soxS* gene deletion colonize the mouse kidney in numbers approximately 1-log fewer than the wild type strain. When administered at 10 mg/kg by way of an intraperitoneal route, compound **37** showed a statistically significant 1-log decrease in the number of *E. coli* colony forming units relative to the untreated control (Table 4). Compound **40** also reduced the colony number relative to the untreated control, but the effect did not reach statistical significance.

The results of this preliminary study identify the 1-hydroxybenzimidazoles as effective "broad spectrum" small-molecule inhibitors of proteins within the AraC family. In addition, select derivatives were able to attenuate an *E. coli* infection in a murine model of urinary tract infection. Since these compounds are devoid of intrinsic antibacterial activity in vitro, the risk of rapid resistance development is expected to be less than that observed with traditional antibiotics. The potent activity in vitro and efficacy in vivo highlight the potential for these derivatives as a novel treatment for infectious diseases. Efforts are ongoing to establish a detailed SAR of the 1-hydroxybenzimidazoles against several AraC proteins with the hope

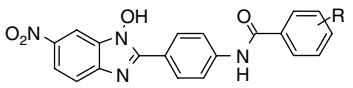
Table 1. Inhibition of SoxS binding to its cognate DNA by 1-hydroxybenzimidazoles



Compound	R^6	R^1	R^2	% Inhibition[a]
1	NO_2	CH_2CO_2H	H	14
2	NO_2	CH_2CO_2Et	H	0
3	NO_2	$CH_2CH_2CO_2H$	H	0
4	NO_2	CH_2CH_2OH	H	0
5	NO_2	CH_2CN	H	12
6	NO_2	$CH_2CH_2CH_3$	H	0
7	NO_2	CH_3	H	17
8	NO_2	$CH_2CH_2NH_2$	H	0
9	NO_2	H	H	38
10	H	H	H	0
11	F	H	H	26
12	CN	H	H	30
13	F_3C	H	H	26
14	$(H_3C)_2N$	H	H	28
15	*tert*-Butyl	H	H	0
16	H_3CO	H	H	0
17	$H_3CC(O)$	H	H	37
18	$H_3CC(NOH)$	H	H	38
19	H_3CSO_2	H	H	35
20	HOC(O)	H	H	12
21	$H_2NC(O)$	H	H	14
22	NO_2	H	*p*-Br	26
23	NO_2	H	*p*-OH	35
24	NO_2	H	*p*-OCH_3	12
25	NO_2	H	*p*-NH_2	80
26	NO_2	H	*m*-NH_2	60
27	NO_2	H	*o*-NH_2	2
28	NO_2	H	*p*-OC_6H_5	14
29	NO_2	H	*p*-$N(CH_3)_2$	75
30	NO_2	H	*m*-$N(CH_3)_2$	30
31	NO_2	H	*p*-$NHC(O)CH_3$	100, 58[b]
32	NO_2	H	*m*-$NHC(O)CH_3$	32
33	NO_2	H	*o*-$NHC(O)CH_3$	31
34	NO_2	H	*p*-$NHC(O)C_6H_5$	100, 100[b]
35	NO_2	H	*p*-$CH_2NHC(O)C_6H_5$	44

[a] Values are means of three experiments, standard deviation is less than 15%. Compounds screened at 50 μg/mL.
[b] % Inhibition at 25 μg/mL.

Table 2. In vitro activity of SoxS inhibitors



34, 36-40

Compound	R	SoxS IC_{50}[a] (μM)	Median MIC[b] (μg/mL)	
			S. aureus[c]	*E. coli*[d]
34	H	17	>64	>64
36	*p*-CH_3	10	>64	>64
37	*p*-OCH_3	2.5	>64	>64
38	*p*-F	2.8	>64	>64
39	*m*-Cl	3.4	>64	>64
40	*p*-$N(CH_3)_2$	0.82	>64	>64

[a] Standard error <15% for all values.
[b] Minimum inhibitory concentration (MIC) for bacterial growth.
[c] Antibiotic susceptible *S. aureus* RN450.
[d] Antibiotic susceptible *E. coli* ML308-225.

Table 3. In vitro IC_{50} (μM) values for **37** and **39** against several AraC family members: MarA, SoxS, and Rob (*E. coli*), ExsA (*P. aeruginosa*), Rma (*S. typhimurium*), and PqrA (*P. mirabilis*)

Compound	IC_{50}[a] (μM)					
	MarA	SoxS	Rob	ExsA	Rma	PqrA
38	nd[b]	2.8	4.7	4.0	4.9	nd[b]
40	1.2	0.82	1.3	1.9	1.8	1.4

[a] Standard error <15% for all values.
[b] Not determined.

Table 4. Efficacy in vivo for compounds **36** and **39** in a mouse model of urinary tract infection

Compound	Log decrease CFU/g kidney[a]
37	1.0 ($p < 0.05$[b])
40	0.5

[a] CFU, colony forming units.
[b] Statistically significant by ANOVA with normal distribution of data.

of identifying potent compounds with useful activity against a range of pathogens.

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