

Structure-Based Design of New DHFR-based Antibacterial Agents (Part 2):
7-(Benzimidazol-1-yl)-2,4-diaminoquinazolines SAR

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ABSTRACT

Background: Based on 7-Aryl-2,4-diaminoquinazolines SAR and crystal structural information, we initiated a program aimed at discovering novel, potent and highly selective DHFR antibiotics against multi-drug resistant *S. aureus* and *S. pneumoniae*.

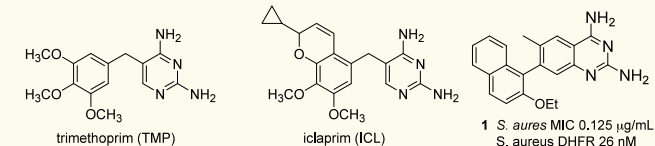
Methods: Compounds were synthesized by means of various chemistry routes (e.g. condensation of 1,2-diamine with proper aldehydes to form benzimidazoles which reacted with difluorobenzonitrile, followed by treatment with guanidine) which allowed introduction of a broad range of substituents. For structure activity relationship (SAR) determination, compounds were tested against TMP-susceptible and resistant DHFR isolated from *S. aureus* and *S. pneumoniae*. MICs were also determined against the same strains according to CLSI guidelines.

Results: Compounds in this series had extremely high enzymatic potency and good antibacterial activity. Antibacterial spectrum includes TMP-susceptible and resistant *S. aureus* as well as *S. pneumoniae*. Superior selectivity was obtained compared to TMP and Iclaprim. Structural information provided a clear SAR for designing highly potent and highly selective compounds. The small differences observed in the active-site between the human and bacterial DHFR provided the base for achieving selectivity.

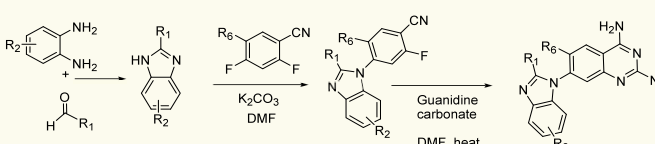
Conclusions: Structure-based drug design yielded compounds with superior selectivity and good antibacterial activity against TMP-susceptible and resistant DHFR *S. aureus* strains.

BACKGROUND

DHFR is a well known antibacterial target and is the site of action of the 5-benzyl-2,4-diaminopyrimidine antibacterial drugs including trimethoprim (TMP) and iclaprim (ICL). In the preceding poster F1-835, we reported on 7-aryl-quinazolines (such as compound 1) that had potent activity on bacterial DHFR and provided details on the SAR of this series. Because this series lacked the desired level of selectivity for desirable antibacterial agents, we examined alternative heterocyclic substituents in the 7-position of the 2,4-diaminoquinazoline core.



METHODS



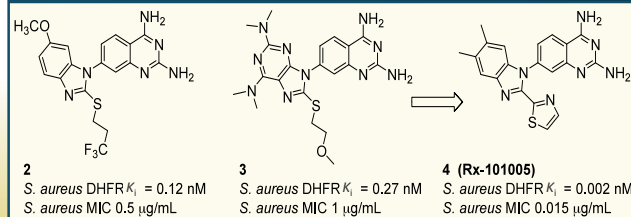
Synthesis: Compounds were prepared by the three step process shown above.

Microbiology: Minimum inhibitory concentrations (MIC) values were determined using CLSI broth microdilution methods. *S. aureus* ATCC 13709 was used.

Enzyme assays: DHFR inhibition was determined by measuring oxidation of NADPH.

Structural biology: Compounds were co-crystallized with the *S. aureus* DHFR enzyme in space group P6₂2 and typically diffracted to 1.7 Å. Structures were solved by molecular replacement.

RESULTS



Building on the knowledge that significant potency could be gained by designing DHFR ligands that occupy the NADP binding pocket, we examined 2,4-diaminoquinazolines with bicyclic ring systems such as benzimidazole 2 and purine 3 in the 7 position. Both of these compounds were designed to contain a substituent that occupies the NADP pocket and an aromatic ring to occupy the same hydrophobic site as the naphthalene of 1. The co-crystal structures of 2 and 3, however, showed that these compounds flip 180° in orientation so that the aryl ring occupies the NADP pocket. Compound 4 was designed to place a small rigid group into the NADP binding pocket. Compound 4 (Rx-101005) is an extremely potent inhibitor of *S. aureus* DHFR and now the benzimidazole ring orients in the expected direction so that the thiazole occupies the NADP pocket. Tables 1 and 2 display the enzymatic and antibacterial potency for this series.

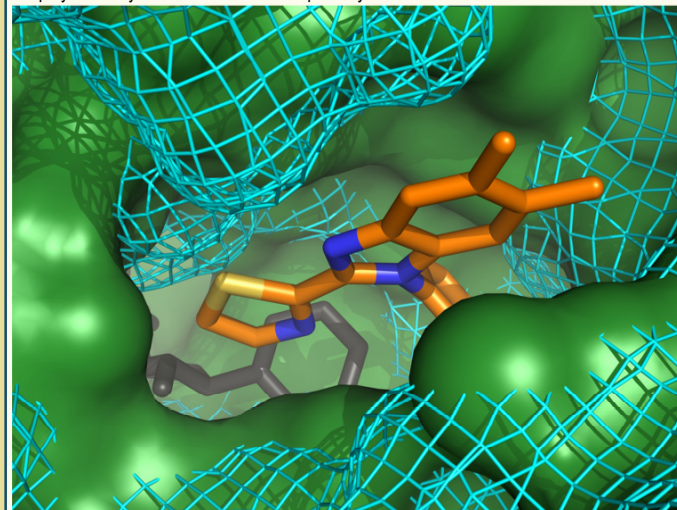
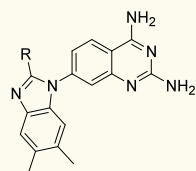

















Fig. 1: Structure of 4 (Rx-101005) bound to *S. aureus* DHFR (solid). An overlay of the human DHFR enzyme is shown in mesh. The thiazole ring fully occupies the NADP⁺ pocket accounting for the extremely high potency. The lack of space in this pocket for the human DHFR enzyme explains the high selectivity. This selectivity is increased by the addition of a proton onto the 5-membered rings for the furan and thiophene rings. The methyl group also contributes to the selectivity.

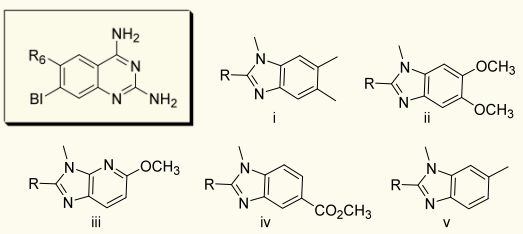
TABLE 1: Enzymatic and Antibacterial Data for 2,4-Diamino-7-(5,6-dimethylbenzimidazol-1-yl)-quinazolines















Cmpd	R	DHFR K_i (nM)			MIC (µg/mL)		
		<i>S. aureus</i>	human	Ratio ^a	<i>S. aureus</i>	<i>S. aureus</i> + 20% serum	<i>S. aureus</i> F99Y
TMP	-	1.24	19,070	15,400	1	1	32
ICL	-	0.081	775	9,600	0.125	0.125	2
5	H	1.7	1,607	950	2	32	>64
4		0.002	93.5	46,700	0.015	0.125	0.25
6		0.096	1,101	11,500	2	2	16
7		0.007	943	135,000	0.015	0.125	1
8		0.008	723	90,000	0.06	1	2
9		0.005	1,026	205,000	0.015	0.5	0.25
10		0.012	663	55,000	0.03	0.25	2
11		1.1	2,922	2,600	4	32	64
12		4	>5,000	>1,250	8	32	64
13		0.022	572	26,000	0.125	0.25	4
14		0.26	1,564	>1,250	0.5	2	32
15		0.026	437	17,000	0.125	0.5	8
16		0.34	2,299	6,800	2	8	>64
17		0.14	1,164	8,300	0.5	1	8
18		0.6	1,086	1,800	1	4	>64
19		4.2	>5,000	>1,000	8	64	>64

Ratio = Human DHFR K_i / *S. aureus* DHFR K_i

TABLE 2: Enzymatic and Antibacterial Data for 6-Substituent Quinazolines and Substituents on the Benzimidazole ring



Cmpd	Bl	R	R6	DHFR K_i (nM)			MIC (µg/mL)		
				<i>S. aureus</i>	human	Ratio	<i>S. aureus</i>	<i>S. aureus</i> + 20% serum	<i>S. aureus</i> F99Y
4	i		H	0.002	93	46,500	0.015	0.125	0.25
20	i		Me	0.030	2.9	97	0.03	0.06	0.125
21	i		Cl	0.011	7.6	690	0.125	1	0.25
22	i		Cl	0.010	>3,000	>100,000	0.03	1	4
23	ii		H	0.16	854	5,300	0.25	0.25	16
24	ii		Me	0.019	170	8,950	0.5	0.5	8
25	ii		Cl	0.011	320	29,000	0.125	0.5	2
26	ii		Cl	0.048	>3,000	>20,000	0.5	0.5	4
27	ii		Cl	0.024	610	25,000	0.5	0.5	8
28	iii		H	0.039	797	20,000	0.25	0.5	4
29	iv		H	0.021	53	2,500	0.25	2	2
30	v		H	0.005	314	62,800	1	1	1

SUMMARY

7-(Benzimidazole-2-yl)-2,4-diaminoquinazolines are a new class of DHFR antibacterial agents with excellent antibacterial activity versus *S. aureus* including TMP resistant strains. Further, multiple members of this series have selectivity ratios greater than TMP versus the human DHFR enzyme.

Due to their excellent potency and selectivity, multiple members of this class were advanced into efficacy and detailed microbiology studies to evaluate their potential as a new antibacterial class.