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Discovery of a Potent and Selective Series of Pyrazole Bacterial Methionyl-tRNA Synthetase Inhibitors

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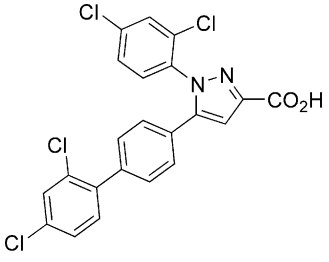
Abstract—Starting with a micromolar lead identified from high-throughput screening, a series of pyrazoles were discovered with significantly improved potency on bacterial methionyl-tRNA synthetase and selectivity over human methionyl-tRNA synthetase.

The emergence of bacterial strains with resistance to currently marketed antibacterial agents has spurred interest in the discovery of new antibacterial agents with novel modes of action.[1,2] One set of potential novel targets are the family of bacterial amino acyl-tRNA synthetases.[3] These enzymes are essential for bacterial growth and have been validated as drug targets by the discovery and development of pseudomonic acid, whose mode of action is inhibition of bacterial isoleucinyl-tRNA synthetase.[4] As part of a broad program to discover bacterial tRNA synthetase inhibitors,[5] pyrazole **1a** was identified as an inhibitor of methionyl-tRNA synthetase (MetRS) by high-throughput screening.[6] This compound is a modest micromolar inhibitor of the bacterial MetRS enzyme from two important Gram-positive pathogens *Staphylococcus aureus* and *Enterococci faecalis* (SaMetRS and EfMetRS) but also inhibits human MetRS (hMetRS) at similar concentrations. In this paper, we report our efforts that resulted in compounds with improved potency on bacterial MetRS and selectivity versus hMetRS.

Pyrazoles with different biphenyl groups at the 5-position were prepared as shown in Scheme 1. Suzuki coupling of different boronic acids to 4-bromoacetophenone provided a set of biphenyl acetophenones. Addition of an acetophenone anion to diethyl oxalate yielded the lithium salt of the diketoester. Treatment of these salts with 2,4-dichlorophenylhydrazine followed by ester hydrolysis provided pyrazoles **1a**–**1f**. Scheme 2 shows the synthesis of additional pyrazoles where different groups are substituted for the dichlorophenyl and/or carboxylic acid. Addition of a variety of hydrazines to diketoester **4a** followed by ester hydrolysis yielded a set of 1-substituted pyrazoles **7**. Pyrazole acids were converted to amides by coupling chemistry. Primary amides were converted to tetrazoles **9** by dehydration to the corresponding nitrile followed by reaction with dibutlytin oxide and trimethylsilylazide. Treatment of **4a** with different pyridylmethyl hydrazines[7] afforded mixtures of ethyl esters of the 1-(pyridylmethyl)-5-biarylpyrazole-3-carboxylic acid and 1-(pyridylmethyl)-3-biarylpyrazole-5-carboxylic acid isomers[8] that were separated by column chromatography. Saponification provided the desired pyrazole acids **10a**–**10c** and **11a**–**11c**.



1a SaMetRS IC_{50} = 4.88 μM
EfMetRS IC_{50} = 8.99 μM
hMetRS IC_{50} = 11.9 μM

Compounds were evaluated for inhibition of bacterial and human MetRS.[9] Table 1 shows the result of profiling a series of pyrazole analogues where the biphenyl

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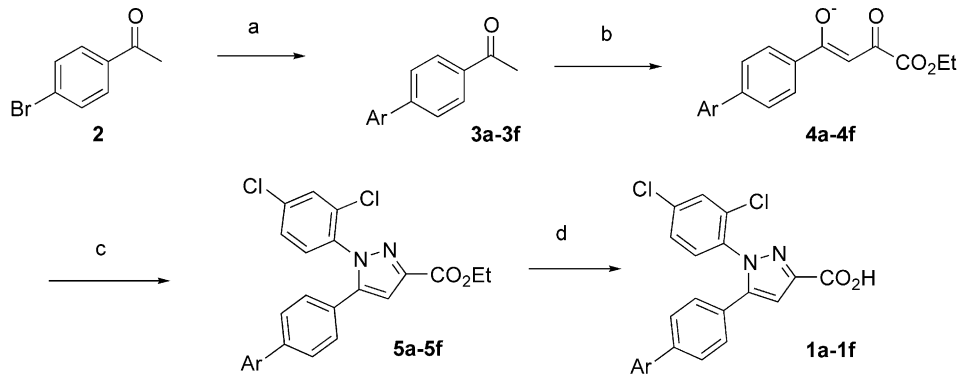
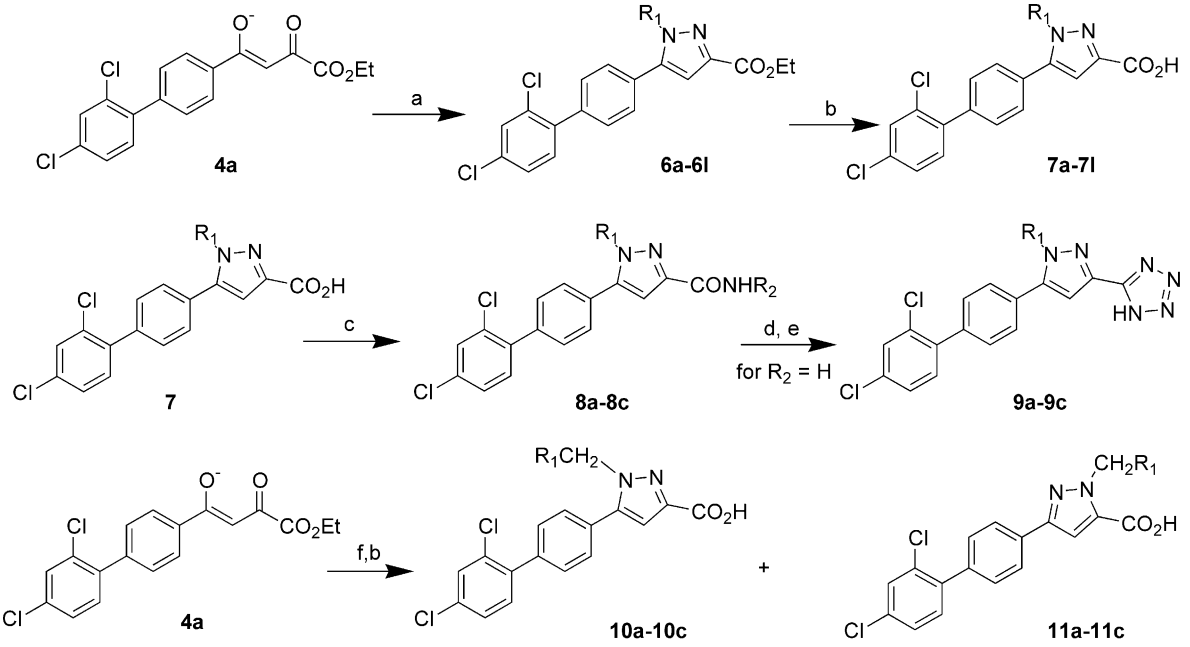


Scheme 1. Synthesis of 1-(2,4-dichlorophenyl)5-biarylpyrazoles-3-carboxylic acids: (a) $Pd(PPh_3)_4$, $ArB(OH)_2$, K_2CO_3; (b) $LiN(TMS)_2$, diethyl oxalate; (c) 2,4-dichlorophenylhydrazine, EtOH, H^+; (d) NaOH.

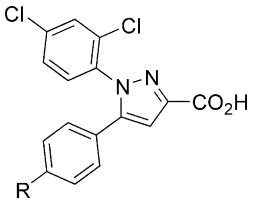


Scheme 2. Synthesis of pyrazole analogues: (a) R_1NHNH_2, EtOH, H^+; (b) NaOH; (c) EDC, NH_2R_2; (d) $POCl_3$; (e) Bu_2SnO, $TMSN_3$; (f) $R_1CH_2NHNH_2$, EtOH, H^+; separate isomers.

group is varied. Table 2 shows the results from testing analogues where the dichlorobiphenyl group was kept constant and the other two positions varied. None of the alternative biphenyl groups examined in Table 1 gave an improvement in either potency or selectivity. Significant improvements in potency and selectivity were found by variation of the 1-dichlorophenyl group. Pyrazoles with small substituents in this position have potency equal or better than the initial lead **1a** on SaMetRS (**7a** IC_{50} 3.3 μM, **7h** IC_{50} 1.0 μM, **7i** IC_{50} 4.5 μM). These results demonstrated that the hydrophobic dichlorophenyl group in the 1-position was not needed for enzymatic potency. Improvements in SaMetRS potency were found in several of the 1-aryl substituted pyrazoles as demonstrated by 1-(3-benzoic acid)-pyrazole **7d** (IC_{50} 1.2 μM) and 1-(2-methoxyphenyl)-pyrazole **7e** (IC_{50} 1.5 μM). The position of phenyl substitution is important for SaMetRS potency as shown by 1-(4-methoxyphenyl)-pyrazole **7f** (IC_{50} 10.5 μM). These results indicate that a hydrogen bond acceptor group may be beneficial for enzymatic inhibition.

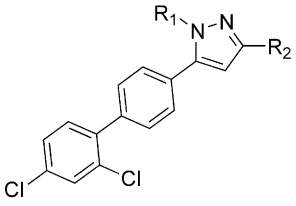
Introduction of pyridyl groups in the 1-position provided the most potent and selective compounds in the series. With a carboxylic acid in the 3-position, both the 1-(2-pyridyl) **7k** and the 1-(3-pyridyl) **7l** pyrazoles have submicromolar activity on the SaMetRS enzyme (**7k** IC_{50} 0.64 μM; **7l** IC_{50} 0.30 μM). In addition, some indications of Gram-positive enzymatic spectrum were observed for 1-(pyridyl)-pyrazoles, as shown by their ability to inhibit EfMetRS (e.g., **7k** EfMetRS IC_{50} 1.93 μM.) Multiple compounds also have good selectivity versus the human MetRS enzyme (>150× for **7k** and 150× for **7l**). As in the 1-phenyl series, substitution of a tetrazole in the 3-position of the pyridyl seies provided compounds with improved SaMetRS potency, for example: 1-(2-pyridyl)-pyrazole **9b** (SaMetRS IC_{50} 0.15 μM) and 1-(3-pyridyl)-pyrazole **9c** (SaMetRS IC_{50} 0.13 μM). Extension of the acidic functionality by a glycine linking group yielded pyrazoles **8b** and **8c**, which were as potent on SaMetRS as the corresponding 3-carboxylic acids **7l** and **9c** indicating a flexible nature to this enzymatic interaction.

Table 1. Inhibition of MetRS by 5-biphenyl pyrazole analogues

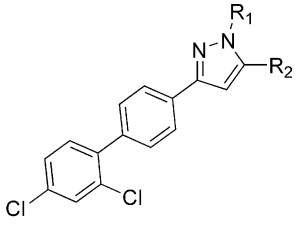


Compd	R	SaMetRS	IC_{50} (μM)		hMetRS/SaMetRS ratio selectivity
			EfMetRS	hMetRS	
1a	2,4-$Cl_2C_6H_3$	4.88	8.99	11.9	2
1b	3-Cl,4-FC_6H_3	17.2		20.9	1
1c	3-$(CO_2H)C_6H_4$	41.3		40.9	1
1d	4-$CF_3C_6H_4$	33.9		33.5	1
1e	4-FC_6H_4	43.2		41.3	1
1f	C_6H_5	40.9		44.3	1

Table 2. Inhibition of MetRS by 5-(2′,4′-dichlorobiphenyl)pyrazole-3carboxylic acid (i) and 3-(2′,4′-dichlorobiphenyl)pyrazole-5-carboxylic acid (ii) analogues



i



ii

Compd	R_1	R_2		IC_{50} (μM)			hMetRS/SaMetRS ratio selectivity
				SaMetRS	EfMetRS	hMetRS	
1a	2,4-$Cl_2C_6H_4$	CO_2H	i	4.88	8.99	11.9	2
7a	H	CO_2H	i	3.28			
7b	C_6H_5	CO_2H	i	5.47			
7c	4-$CF_3OC_6H_4$	CO_2H	i	47.4	>100	>100	>2
7d	3-$(CO_2H)C_6H_4$	CO_2H	i	1.19	6.34	>100	>84
7e	2-CH_3O–C_6H_5	CO_2H	i	1.51	17.8		
7f	4-CH_3O–C_6H_5	CO_2H	i	10.5		>100	>10
7g	$C_6H_5CH_2$	CO_2H	i	3.94	29.9		
7h	Et	CO_2H	i	1.02		46.4	45
7i	$HOCH_2CH_2$	CO_2H	i	4.46	11.4	>100	>22
7j	CH_2CO_2H	CO_2H	i	3.42		100	29
7k	2-Pyridyl	CO_2H	i	0.63	1.93	>100	>159
7l	3-Pyridyl	CO_2H	i	0.30		47.0	157
8a	2-Pyridyl	$CONH_2$	i	2.95		>100	>34
8b	3-Pyridyl	$CONHCH_2CO_2H$	i	0.30		20.0	67
8c	3-Pyridyl	$CONHCH_2CN_4H$	i	0.13	3.7	13.8	106
9a	2-CH_3O–C_6H_5	Tetrazole	i	0.53		20.0	38
9b	2-Pyridyl	Tetrazole	i	0.15	1.79	25.0	167
9c	3-Pyridyl	Tetrazole	i	0.13	7.0	10.6	82
10a	2-Pyridyl-CH_2	CO_2H	i	1.34		42.5	32
10b	3-Pyridyl-CH_2	CO_2H	i	0.66		>100	>152
10c	4-Pyridyl-CH_2	CO_2H	i	0.50		26.2	52
11a	2-Pyridyl-CH_2	CO_2H	ii	2.68		22.3	8
11b	3-Pyridyl-CH_2	CO_2H	ii	1.09		19.9	18
11c	4-Pyridyl-CH_2	CO_2H	ii	0.91		17.3	19

The 3-position of the pyrazole requires an acidic functionality for enzymatic activity as all of the ester derivatives **6a–6l** are inactive in SaMetRS assays at 100 μM. Replacement of the 3-carboxylic acid with a tetrazole for the 1-(2-methoxyphenyl)-pyrazole provided a compound, pyrazole **9a** (SaMetRS IC_{50} 0.53 μM), with improved potency relative to the carboxylic acid but with a reduction in selectivity (38×) over hMetRS. The only non-acidic functionality that displayed activity in the 3-position was the primary amide **8a** (SaMetRS IC_{50} 2.95 μM) but this potency was significantly less (5×) than the corresponding acid **7k**.

Preparation of 1-(pyridylmethyl)-pyrazole analogues provided an opportunity to compare the 1,5-disubstituted-pyrazole-3-carboxylic acid analogues with 1,3-disubstituted-pyrazole-5-carboxylic acid analogues Using a series of pyridylmethylhydazines, six different 1-pyridylmethyl-pyrazoles were prepared. In every case, the 1,5 disubstituted-pyrazole-3-carboxylic acid analogue was preferred for SaMetRS potency. These 1-(pyridylmethyl)-pyrazoles have similar potency to the 1-(pyridyl)-pyrazoles.

In summary, optimization of a micromolar pyrazole lead that lacked selectivity provided a set of submicromolar 1-pyridyl-pyrazoles. These compounds have significant increased selectivity for the bacterial MetRS enzyme over the human MetRS enzyme. Although multiple pyrazole analogues displayed antibacterial activity versus *S. aureus*, the mechanism of action could not be tied soley to inhibition of MetRS. The advances in potency and selectivity for the pyrazole series suggests that MetRS may be a useful target for discovering other series of inhibitors with potency and selectivity.

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8. Treatment of lithium aroylpyruvates with aryl hydrazines is known to provide the 1,5-diarylpyrazole-3-carboxylate isomer with high regioselectivity. See: Murray, W.; Wachter, M. *J. Hetero. Chem.* **1989**, *26*, 1389. When different pyridylmethylhydrazines were used the 1,3-disubstituted-pyrazole-5-carboxylate isomers were also isolated as the minor products with the 1,5-disubstituted-pyrazole-3-carboxylate isomer with ratios ranging between 3:2 and 3:1. The isomers are readily differentiated by the NMR signal of the methylene group with the methylene signal for the 5-carboxylate isomer is 0.5 ppm downfield of that for the 3-carboxylate isomer (e.g., 5.60 ppm for **10c** and 6.10 ppm for **11c**).
9. Enzymatic inhibition was determined by measuring the amount of radiolabeled methionine incorporated into the product, the charged methionyl–tRNA complex. The enzymatic reaction was run at K_m concentrations for ATP and methionine and a saturating level of 90 μM for tRNA. A compound's IC_{50} was determined by fitting the results from a 10-point dose/response curve. All results are the average of at least two measurements.