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A Series of Quinoline Analogues as Potent Inhibitors of *C. albicans* Prolyl tRNA Synthetase

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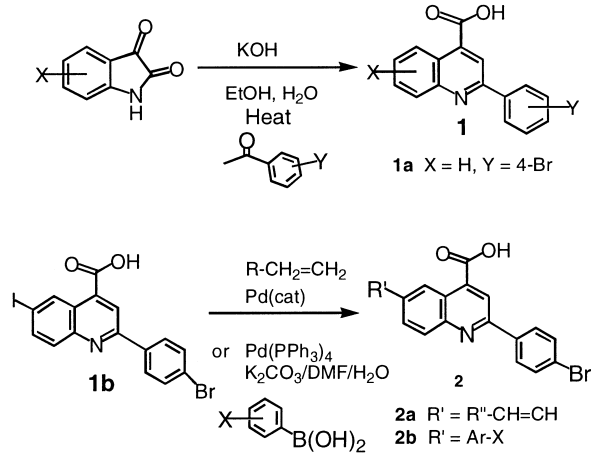
Abstract—A series of quinoline inhibitors of *C. albicans* prolyl tRNA synthetase was identified. The most potent analogue, 2-(4-bromo-phenyl)-6-chloro-8-methyl-4-quinolinecarboxylic acid, showed $IC_{50} = 5\,nM$ (Ca. ProRS) with high selectivity over the human enzyme.

The incidence of serious fungal infection continues to grow and there is an urgent need for novel antifungal therapies.[1,2] Aminoacyl-tRNA synthetases (aaRSs) are essential enzymes for biological cell growth.[3–7] Because there are significant structural difference between fungal and human enzymes, selective inhibition of aaRSs offers a potential pathway for discovery of novel antifungal agents. Previously, we have described novel antibacterials targeting aminoacyl-tRNA synthetases.[8,9] Herein, we report novel quinoline inhibitors of *C. albicans* prolyl tRNA synthetase. The quinoline lead, 2-(4-bromo-phenyl)-4-quinoline-carboxylic acid **1a**, was identified from high-throughput screening of library compounds. Compound **1a** inhibits *C. albicans* prolyl-tRNA synthetase ($IC_{50} = 0.5\,\mu M$) with high selectivity over the human enzyme ($IC_{50} > 100\,\mu M$). To examine the structure–activity relationships, we have prepared and evaluated a series of quinoline analogues.



Scheme 1.

Our synthetic approach for the preparation of quinoline analogues is shown in Scheme 1. Reaction of a substituted isatin with a ketone gave 4-quinoline carboxylic acid derivative **1**.[10] The 6-alkenyl group (**2a**) was introduced from coupling of iodoquinoline **1b** with alkene under a typical Heck condition.[11] The 6-aryl group (**2b**) was introduced from a palladium-catalyzed (Suzuki) coupling of arylboronic acid and iodoquinoline **1b**.[12]

Quinoline analogues with modification at the 8-methyl position were prepared as shown in Scheme 2. The quinoline **1c** was esterified and converted to the bromide quinoline **3**.[13] Treatment of **3** with aqueous silver nitrate followed by hydrolysis gave the corresponding alcohol **4**.[14] Dibromination of **1c** followed by a similar treatment with aqueous silver nitrate, depending upon the conditions, yielded benzaldehyde quinoline **6** or benzoic acid quinoline **7**.

Derivatives of the 4-quinolinecarboxylic acid were prepared as shown in Scheme 3. Reduction of **1c** gave the hydroxyl derivative **8**, which was converted to the sulfamoyl derivative **9**.[8] Treatment of the primary amide with thionyl chloride yielded the nitrile derivative **12**,[15] which

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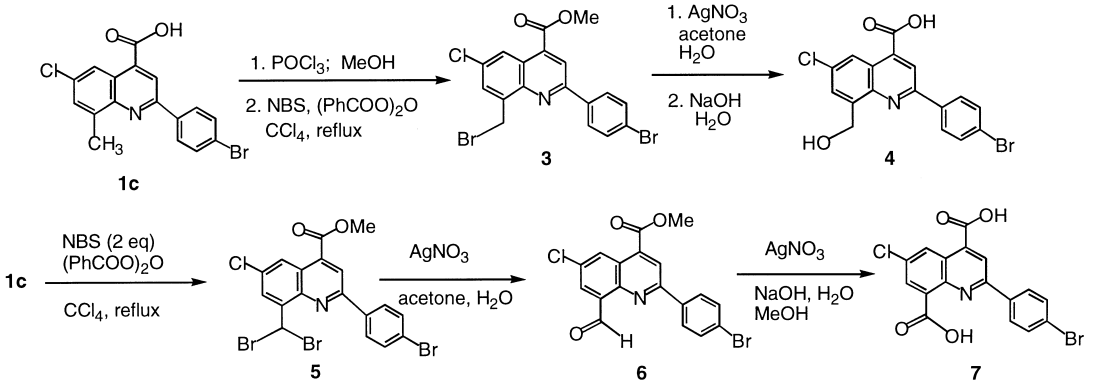
was reacted with trimethylsilyl azide in the presence of dibutyltin oxide to afford the tetrazole derivative **13**.[16]

2-Aryl analogues were evaluated in assays of the aminoacylation activity of *C. albicans* prolyl-tRNA synthetase and the results are shown in Table 1. The bromophenyl compound **1a** was significantly more potent than the simple phenyl analogue **1d**. Analogue **1e** with an electron-donating -OMe group and analogue **1f** with an electron-withdrawing $-CF_3$ group showed a significant loss in potency. Replacement of the bromophenyl group by a heterocyclic group such as the 2-Cl-thiophene group (**1i**) decreased the activity. The furyl analogue **1j** and the pyridyl analogue **1k** were inactive.

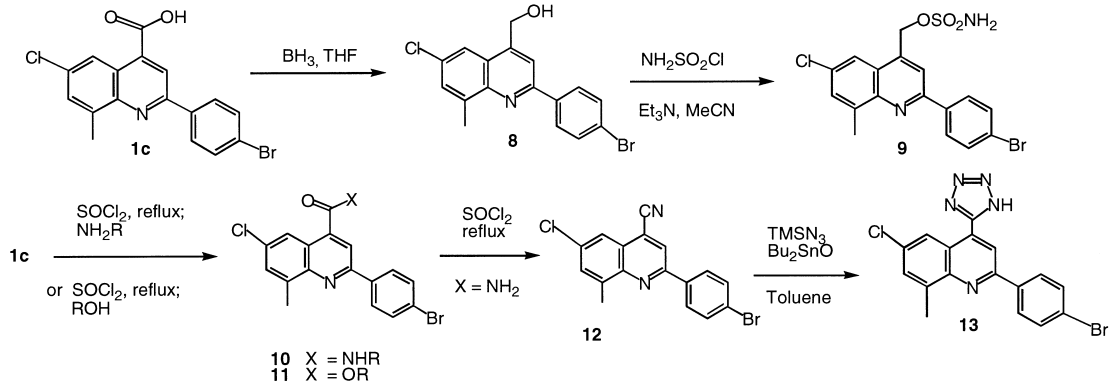
Substitution in the benzene portion of quinoline can dramatically improve the activity (Table 2). 6-Halogen substituted analogues (**1b**, **1l** and **1m**) were all very active. However, incorporation of hydrophilic moieties at the 6-position (**1o** and **1p**) decreased the potency. The 6-chloro-8-methyl analogue **1c** was the most potent compound ($IC_{50} = 5$ nM). The 6-chloro-8-hydroxylmethlene analogue **4** ($IC_{50} = 1500$ nM), however, was considerably less potent. This observation implied the importance of lipophilicity of the substitution in the 8-position for good inhibition. A comparison of analogue **1c** and analogue **1r** reveals that changing the relative position of the chloro group and the methyl group resulted in a 5-fold weaker inhibition. The 6,8-dimethyl analogue **1q** was 5000 times less potent than the 6-chloro-8-methyl analogue **1c**. Substitution with a halogen at the 7-position and a methyl at the 8-position (**1v**) and (**1w**) decreased the potency. All of these compounds have high selectivity over the human enzyme.

To explore other functional groups at the 4-position, we replaced the carboxylic acid with sulfamoyl amide, amide, ester, and tetrazole (Table 3). The replacement of the carboxylic acid with the methylene sulfomyl amide (**9**), the amide (**10a**) and the phenylbenzyl ester (**11a**) decreased the potency. The 3,4-dichlorobenzyl ester (**11b**), the furfuryl ester (**11c**), and the tetrazole derivative **13** had a moderate inhibition. The results suggest that a carboxylic acid functionality at this position is critical for good enzyme inhibition.

In summary, we have developed a series of potent and selective *C. albicans* prolyl-tRNA synthetase inhibitors. The enzymatic potency observed with several of these analogues is significantly superior to that of lead compound **1a** ($IC_{50} = 500$ nM, *C. albicans* ProRS). SAR studies suggest that the potency is greatly influenced by the substitution pattern on the quinoline. Analogue **1c** possesses excellent activity against *C. albicans* ProRS ($IC_{50} = 5$ nM) with high selectivity over the human enzyme (IC_{50} >100 μM). Enzymology studies indicate that compound **1c** is a non-competitive inhibitor of the *C. albicans* ProRS with respect to proline and a competitive inhibitor with respect to ATP. The K_i's were 15 and 5.1 nM versus proline and ATP, respectively. However, the

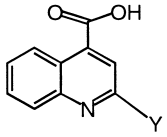


Scheme 2.



Scheme 3.

Table 1. Inhibition of *C. albicans* prolyl-tRNA synthetase



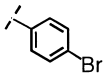
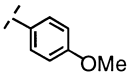
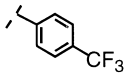
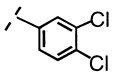
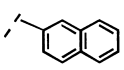
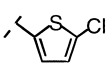
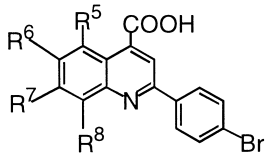
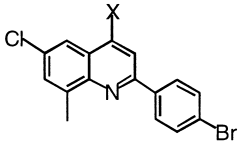
Y Compd									
	1a	**1d**	**1e**	**1f**	**1g**	**1h**	**1i**	**1j**	**1k**
IC_{50} (μM) (Ca. ProRS)	0.5	>100	>100	>100	19	3.6	32	>100	>100
IC_{50} (μM) (human ProRS)	>100	>100	>100	>100	>100	>100	>100	>100	>100

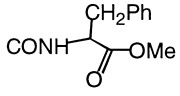
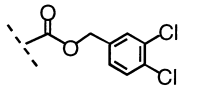
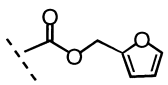
Table 2. Inhibition of *C. albicans* prolyl-tRNA synthetase



Compd	R^5	R^6	R^7	R^8	IC_{50} (μM) (Ca. ProRS)	IC_{50} (μM) (human ProRS)
1a	H	H	H	H	0.500	>100
1b	H	I	H	H	0.019	>100
1l	H	Cl	H	H	0.026	>20
1m	H	Br	H	H	0.025	>20
1n	H	CF_3O	H	H	0.053	>100
1o	H	HOS_2O	H	H	15	>100
1p	H	NH_2	H	H	3.3	95
2a	H	$HO_2CCH{=}CH_2$	H	H	1.3	80
2b	H	4′-OH-PH	H	H	0.350	35
1c	**H**	**Cl**	**H**	**Me**	**0.005**	**>20**
1q	H	Me	H	Me	25	>100
1r	H	Me	H	Cl	0.025	>20
4	H	Cl	H	CH_2OH	1.5	>100
1s	Me	H	Cl	H	1.5	>100
1t	Cl	H	Cl	H	0.140	>100
1u	H	Br	H	CF_3	0.220	56
1v	H	H	Br	Me	0.150	70
1w	H	H	F	Me	0.120	60

Table 3. Inhibition of *C. albicans* prolyl-tRNA synthetase



X Compd	CO_2H	$CH_2OSO_2NH_2$		CO_2CH_2PhPh			
	1c	**9**	**10a**	**11a**	**11b**	**11c**	**13**
IC_{50} (Ca. ProRS, μM)	0.005	2.4	5.7	8.8	0.02	0.07	0.03
IC_{50} (human ProRS, μM)	>100	—	>100	>100	>100	>100	>100

most active analogue **1c** has no whole cell activity (MIC >100 μg/mL, *C. albicans*). Analogue **1v** has cellular activity (MIC = 25 μg/mL, *C. albicans*). Further studies indicate that the activity is due to a lytic mechanism.

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