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Structure–activity relationships of novel potent MurF inhibitors

Yu Gui Gu,* Alan S. Florjancic, Richard F. Clark, Tianyuan Zhang, Curt S. Cooper, David D. Anderson, Claude G. Lerner, J. Owen McCall, Yingna Cai, Candace L. Black-Schaefer, Geoffrey F. Stamper, Philip J. Hajduk and Bruce A. Beutel

Infectious Disease Research, Global Pharmaceutical Research and Development, Abbott Laboratories, 200 Abbott Park Road, Abbott Park, IL 60064, USA

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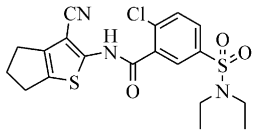
Abstract—A novel class of MurF inhibitors was discovered and structure–activity relationship studies have led to several potent compounds with $IC_{50} = 22 \sim 70$ nM. Unfortunately, none of these potent MurF inhibitors exhibited significant antibacterial activity even in the presence of bacterial cell permeabilizers.

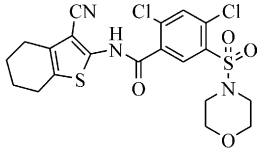
1. Introduction

The emergence of drug resistance poses a major challenge to the antibacterial research community and clinicians worldwide. Among the widely prescribed antibiotics, resistance rates for β-lactams and macrolides have reached ~25%.[1,2] Although resistance rates to fluoroquinolone antibacterial agents have been low in the United States, with increased usage and over-prescription the emergence of resistance to this class of agents is inevitable, and has been reported in several countries.[3,4] Consequently, efforts to discover novel antibacterial agents capable of overcoming drug resistance have been a continuing interest in our laboratories.

Peptidoglycan is an essential and unique building block of the bacterial cell wall and has been the target of many drug classes including β-lactams, cephalosporines and glycopeptides. Murein enzymes are involved in the biosynthesis of bacterial peptidoglycan at various stages.[5] Gene knockout studies have shown that these Murein enzymes are essential for the survival of the bacterial cell and therefore are attractive targets.[6–8] Recently, we discovered two credible MurF inhibitor leads (**1**, **2**) via an affinity selection screening technology developed at Abbott.[9] Here we wish to report our structure–activity relationship study of these novel MurF inhibitors.



1 IC_{50} = 8 μM



2 IC_{50} = 1 μM

2. Synthesis

2-Aminothiophene **3** was readily synthesized from cyclopentanone and malononitrile according to a literature procedure.[10] The reaction of one equivalent of **3** in the presence of a base such as triethylamine with *m*-chlorocarboxbenzenesulfonyl chloride derivatives **5**, which were prepared from the corresponding benzoic acid compounds **4** via chlorosulfonation, produced the sulfonyl chloride derivatives **7** in good yields. Treatment of **7** with either primary or secondary amines gave the various sulfonamides **8** (Scheme 1). The yields varied from 20 to 80% depending on the additional functional groups that the amines bear.

The modification of the central portion of the molecule was accomplished by straightforward functional group manipulations. The cyano group of compounds **8** was hydrolyzed to amide **9** or selectively reduced to primary amine **10** with borane and then further acylated to amide **11**. The ethyl ester analogue **12** was prepared from the commercially available ethyl 2-amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carboxylate and **5** by following the reaction sequences for compounds **8** (b

* Corresponding author. Tel.: + 1-847-937-4792; fax: + 1-847-938-3403; e-mail: yu-gui.y.gu@abbott.com

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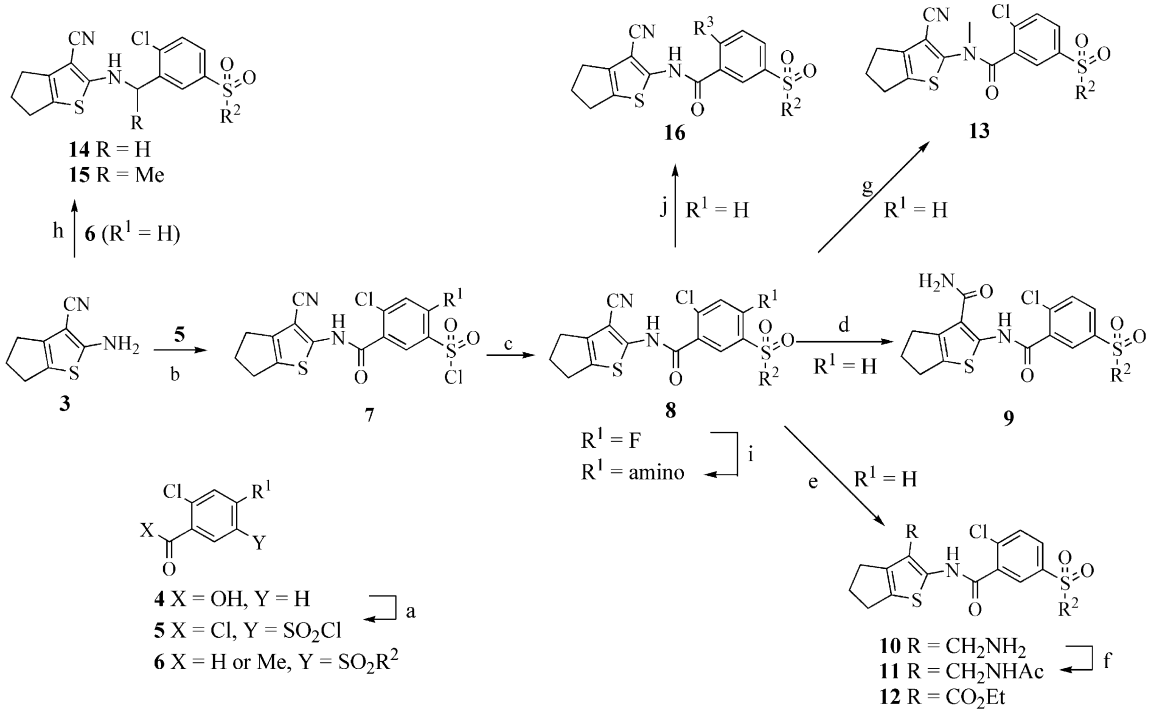
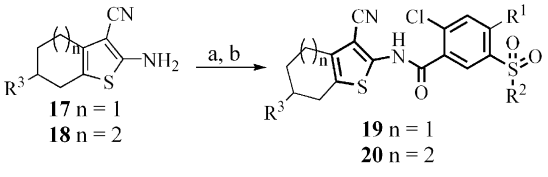


Scheme 1. Reagents and conditions: (a) $ClSO_3H$, 95 °C, 70–88%; (b) toluene, reflux, 20 h, 55–83%; (c) 1′ or 2′ amines, NEt_3, THF, rt, 2–24 h, 20–80%; (d) concd H_2SO_4, 21 h, 55%; (e) BH_3, THF, 65 °C, 2 h, 40%; (f) Ac_2O, CH_2Cl_2, rt, 59%; (g) MeI, K_2CO_3, DMA, 74%; (h) (1) benzene, cat. *p*-TSA, reflux, 30 min; (2) $NaCNBH_3$, 32–40%; (i) amine, NEt_3, THF, rt, 16 h, 40–70%; (j) 1′ or 2′ amines, dioxane, reflux, 1–5 days, 22–79% (R^3 = amines), or 10 equiv alkoxide, DMA, 85 °C, 1–24 h, 35–80% (R^3 = alkoxy).

and c of Scheme 1). Methylation of the amide NH of **8** provided compound **13**. The compounds with an amine linkage (**14** and **15**) were prepared via reductive amination of **3** with an aldehyde or ketone **6**. Selective nucleophilic displacement of **8** (R^1 = F) with amines afforded the various aniline analogues (**8**, R^1 = amino groups) in good yield under mild conditions. On the other hand, displacement of monochlorobenzenesulfonamide **8** (R^1 = H) with amines required harsh conditions (high temperature and long reaction time) to produce desired compounds **16**, while the reaction with alkoxides proceeded more smoothly.

Similarly, analogues **19** and **20** with different cycloalkyl ring sizes were synthesized from compounds **17** and **18**[10] (Scheme 2).



Scheme 2. Reagents and conditions: (a) **5**, toluene, relux, 20 h, 60–80%; (b) 1′ or 2′ amines, NEt_3, THF, rt, 2–24 h, 40–75%.

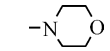
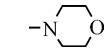
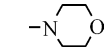
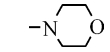
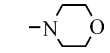
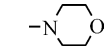
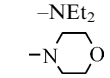
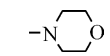
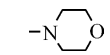
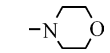
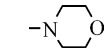
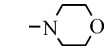
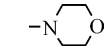
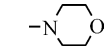
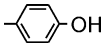
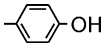
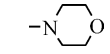
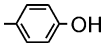
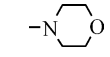
3. Results and discussion

The MurF enzyme is responsible for incorporation of a D-alanyl D-alanyl moiety during peptidoglycan synthesis.[5] The D-alanyl D-alanine adding activity of MurF was monitored by measuring the concomitant release of radiolabeled inorganic phosphate from ATP. In a modification of the ATPase end-point assay of Seals et al.,[11] purified recombinant MurF from *Streptococcus pneumoniae* (preparation of which will be described elsewhere[9]) was combined with UDP-*N*-acetylmuramyl-L-Ala-γ-D-Glu-Lys (purified from *Staphylococcus aureus*[12]), D-Ala-D-Ala (Sigma, St. Louis, MO, USA) and adenosine 5′-[γ-^{33}P]-triphosphate (Amersham, Piscataway, NJ, USA). Progress of the reaction was determined by measuring the number of counts released as free phosphate. Compound concentrations resulting in 50% inhibition of enzyme activity (IC_{50}) were graphically determined from four-point plots of concentration ranges spanning this value. Each plotted value was the mean of at least two determinations, and each IC_{50} value was the mean derived from at least two independent plots.

Various sulfonamides are well tolerated exhibiting MurF inhibitory activity comparable to **1** (**8**, entries 1–4, Table 1). Interestingly, the compound with a basic amine group attached to the sulfonamide is poorly tolerated (**8**, entry 5) while the corresponding hydroxyl analogue (**8**, entry 4) follows the general trend. A halogen atom *ortho* to the sulfonamide improves activity, especially for the *ortho*-chloro morpholino sulfonamide (**8**, entry 8), which is 30 times more potent than the corresponding parent compound (**8**, entry 1). However, replacement of the halogen with an amine group significantly lowers potency especially in those analogues containing a large amine (**8**, entries 9–11).

It is interesting that the cyano group of compound **1** is essential for MurF inhibitory potency. Conversion of

Table 1. MurF activities from the radiolabeled phosphate release assay

Entry	Compd	R^1	R^2	R^3	MurF IC_{50} (μM)
1	**8**	H			9
2	**8**	H	–NHMe		15
3	**8**	H	–NHPh		26
4	**8**	H	$–NH(CH_2)_3OH$		8
5	**8**	H	$–NH(CH_2)_3NMe_2$		>100
6	**8**	F			6.4
7	**8**	Cl	$–NEt_2$		5.2
8	**8**	Cl			0.3
9	**8**	–NHMe			62
10	**8**	$–NH(CH_2)_3OH$			>100
11	**8**	$–NH(CH_2)_3NMe_2$			>100
12	**9**		$–NEt_2$		66
13	**10**		$–NEt_2$		>100
14	**11**		$–NEt_2$		>100
15	**12**		$–NEt_2$		>100
16	**13**		$–NEt_2$		>100
17	**14**		$–NEt_2$		>100
18	**15**		$–NEt_2$		>100
19	**16**			$–NH(CH_2)_3NMe_2$	74
20	**16**			$–NMe_2$	>100
21	**16**			–OEt	>100
22	**16**			$–OCH_2CHMePh$	>100
23	**16**			H	>100
24	**16**			Br	4.2
25	**19**	H	$–NEt_2$	H	1.4
26	**19**	H		H	1.7
27	**19**	Cl		Ph	0.07
28	**19**	H	$–NEt_2$		0.054
29	**19**	H	$–NEt_2$	$–CO_2Et$	6
30	**19**	Cl	$–NEt_2$		0.067
31	**19**	Cl			0.022
32	**20**	H	$–NEt_2$	H	3.4
33	**20**	H		H	4.2

the cyano group to amides **9**, **11**, amine **10**, or ethyl ester **12**, all resulted in the significant or complete loss of activity. The amide linker in the center of the molecule is also essential for potency. A total loss of activity was observed when the amide of compound **1** was methylated (**13**) or converted to amines (**14**, **15**). When the chlorine atom *ortho* to the amide linker was displaced with amino or alkoxy groups, the resulting analogues **16** (entries 19–22) were inactive compared with the parent compound (**8**, entry 1), regardless of the size of the substituents (entry 19 vs 20, and entry 21 vs 22). A cyano group at the C-3 position of thiophene, a NH amide linker, and a chlorine *ortho* to the amide linker are all required for MurF activity. These strict requirements suggest that both the cyano and an amide NH may be involved in hydrogen bonding interactions with

the enzyme. The chlorine atom may provide proper orientation for such interactions by forcing the phenyl ring into a non-coplanar position with the amide linker. Indeed, removal of the chlorine results in loss of activity (entry 23), whereas the corresponding bromide[13] analogue (entry 24) slightly increases potency. Itai and coworkers reported that *N*-methylbenzanilide exists in a *cis*-amide conformation while the unsubstituted NH benzanilide exists in a *trans*-amide conformation.[14] Therefore, it is also possible that the lack of activity of **13** is due to the change of amide conformation.[15]

Cyclohexyl analogues **19** exhibit better potency than the corresponding cycloheptyl counterparts **20**, which in turn seem to be more potent than cyclopentyl analogues. This is demonstrated by the decreasing potency from **19** (entry 25, $IC_{50} = 1.4\,\mu M$) to **20** (entry 32, $IC_{50} = 3.4\,\mu M$) to **1** ($IC_{50} = 8\,\mu M$) and from **19** (entry 26, $IC_{50} = 1.7\,\mu M$) to **20** (entry 33, $IC_{50} = 4.2\,\mu M$) to **8** (entry 1, $IC_{50} = 9\,\mu M$). A major potency boost was achieved when an aryl group was appended to the cyclohexyl group. A phenyl group increases potency 14-fold (**2**, $IC_{50} = 1\,\mu M$ vs **19**, entry 27, $IC_{50} = 70\,nM$) and 4-hydroxyphenyl boosts potency by 45-fold (**19**, entry 31, $IC_{50} = 22\,nM$). The corresponding diethyl sulfonamide analogues **19** (entries 28 and 30) also show high potency with $IC_{50} = 54\,nM$ and $IC_{50} = 67\,nM$, respectively. Introduction of an ester at the same position of the cyclohexyl group reduces potency more than 4-fold (**19**, entry 25 vs 29), indicating that a π–π interaction or stacking of the aryl group with the enzyme may be responsible for the potency boost.

In conclusion, we have discovered a novel class of potent MurF inhibitors. Unfortunately, even the most potent compounds do not show significant antibacterial activity. There could be many reasons for the lack of antibacterial activity, including poor cellular permeability, efflux, non-selective intracellular binding of our compounds to other proteins or other unknown reasons. To address the permeability and efflux issues, we measured antibacterial activity of our MurF inhibitors in the presence of some well-known permeabilizers (*Escherichia coli* with 1 mg/mL ethylenediaminetetraacetic acid, *S. aureus* with 64 μg/mL nisin[16]) as well as *E. coli* AcrAB efflux pump mutants.[17] Unfortunately, no antibacterial activities were observed. Since the gene encoding MurF is essential for bacterial survival, the lack of antibacterial activity of this series could be due to MurF not catalyzing a rate limiting step of the biosynthetic pathway. More research is needed to understand why the inhibition of MurF does not result in whole cell antibacterial activity.

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