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Naphthyl Tetronic Acids as Multi-Target Inhibitors of Bacterial Peptidoglycan Biosynthesis

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Since the discovery of penicillin in 1929, many important antibiotic agents have made significant contributions to the prevention and treatment of infections caused by bacteria. Despite these remarkable achievements, infections are still the second-leading cause of death worldwide and remain a major public health problem. Clearly, there is great need for novel antibacterial agents to address resistance problems associated with current antibiotics.[1] Toward this end, three broad strategies have been recently employed in the search for new leads: high-throughput screening of large compound libraries, genomics, and combinatorial biosynthesis. Although some limitations of the former approach to targets in bacterial peptidoglycan biosynthesis have been reported,[2,3] the peptidoglycan biosynthetic pathway remains an attractive target, validated in the clinic with fosfomycin and vancomycin.

Peptidoglycan biosynthesis is a complex process, which involves three main stages: a) cytoplasmic soluble enzymes that include Mur A–F, b) membrane-bound enzymes that include Mra Y and Mur G, and finally c) transglycosylases and transpeptidases, which act external to the cytoplasmic membrane.[4]

The Mur enzymes are unique to bacteria and are involved in essential functions of both Gram-positive and Gram-negative organisms.[5] Another attractive aspect of Mur enzyme inhibitors is the potential to be bactericidal, leading to cell lysis and bacterial death.[6] Inhibitors of peptidoglycan biosynthesis initiate a complex process of gene expression resulting in the induction of Mur A and Mur I in Gram-positive bacteria to compensate for the slower rate of peptidoglycan biosynthesis.[7,8]

Several classes of natural products or their semisynthetic derivatives represented by liposidomycins, amphomycins, and muraymycins are inhibitors of Mra Y, whereas nisin, ramoplanin, and mersacidin are lipid II inhibitors.[4] In the last decade a few small-molecule inhibitors of the Mur enzymes have been reported, including sesquiterpene lactones,[9] 5-sulfonoxyanthranilic acids T6361 and T6362,[10] UDP-MurNAc[11] (Mur A), imidazolinones,[12] 4-thiazolidinones,[13] thienopyrazoles,[14] phosphinates[15] (Mur B), peptidosulfonamides,[16] 3-cyanothiophenes[17] (Mur F), and D-glutamic acid analogues[18] (Mur I). Despite the discovery of small-molecule inhibitors of various Mur enzymes, many limitations have been noted, including poor antibacterial activities in cells.[19,20]

In parallel, a number of new assay formats for the identification of Mur enzyme inhibitors have been described based on different platforms such as ultra-efficient affinity HTS,[21] LC–MS,[22] TLC,[23] HPLC,[24] and solid-support TLC.[25] Our efforts in identifying Mur enzyme inhibitors were based on an initial pathway screen searching for inhibitors of multiple enzymes, Mur A–F. Hits in this assay were evaluated against the individual Mur enzymes for lead optimization. Using this strategy, we identified two classes of inhibitors: 3,5-dioxopyrazolidines[26] and pulvinones,[27] with activities against several of the Mur enzymes. Inhibitors of multiple Mur enzymes are attractive given the essential role of each Mur enzyme in peptidoglycan biosynthesis. This strategy may prevent the development of drug resistance through the multi-target hypothesis.[28] Herein we report on the SAR of the naphthyl tetronic acids and highlight their binding to the *E. coli* enzyme Mur B.

The target naphthylfuran-2-ones **5a–k** were prepared by a three-step process starting from 3-bromo-4-methoxy-5*H*-furan-2-one (**1**) and the appropriately substituted aldehydes **2** (Scheme 1). Bromofuranone **1** was acquired by bromination of the commercially available 4-methoxy-5*H*-furan-2-one with *N*-bromosuccinimide in carbon tetrachloride at reflux.[29] Deprotonation of **2** at C5 with lithium isopropylcyclohexylamide (LICA)[30] followed by an aldol reaction with substituted aldehydes **2** mediated by anhydrous $ZnCl_2$ afforded diastereomeric alcohols, which were converted into their mesylate or chloride derivatives in situ followed by elimination to generate the exocyclic double bond of **3** in the thermodynamically more stable *Z* configuration.[31] The key step involved a Suzuki cross-coupling of **3** with aryl boronic acids catalyzed by either [Pd(PPh_3)$_4$] or [$PdCl_2$(dppf)$_2$] to afford the methoxyfuranones **4a–k**. Demethylation of methoxyfuranones **4a–k** with lithium bromide[32] in the final step afforded the desired naphthylfuran-2-ones **5a–k**. Purification by silica gel column chromatography was followed by an acid wash of the collected fractions to restore the acidic functionality.

A panel of nine enzymes was used to assess the abilities of the naphthylfuranones to inhibit the Mur enzymes, and thus to define SAR trends for multiple enzyme inhibition (Table 1). The four isomeric bis-naphthyl compounds (Entries 1–4) were evaluated to determine whether there is a preference for α- or β-linked naphthyl groups at either C3 or C5. The trend seems to favor C3 β and C5 α substitution. Replacement of naphthyl with *p*-chlorophenyl (Entries 5–8) gave compounds **5e–h** with good broad-spectrum activity against the Mur enzymes, thus confirming the desired SAR trend. Further optimization of the C5 α methylidene naphthyl derivative **5h** by changing the *p*-

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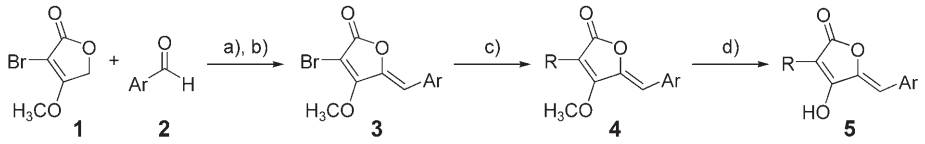

Scheme 1. Synthesis of naphthyl tetronic acids: a) LICA, THF, −78 °C; b) MsCl, TEA, CH_2Cl_2, 0 °C; or NCS, PPh_3, THF, 0 °C→RT; c) $[PdCl_2(dppf)_2]$ or $[Pd(PPh_3)_4]K_3PO_4$, dioxane, 90–100 °C; d) LiBr, DMF, 150 °C. LICA = lithium isopropylcyclohexylamide, TEA = triethylamine, NCS = *N*-chlorosuccinimide, dppf = 1,1′-bis(diphenylphosphanyl)ferrocene.

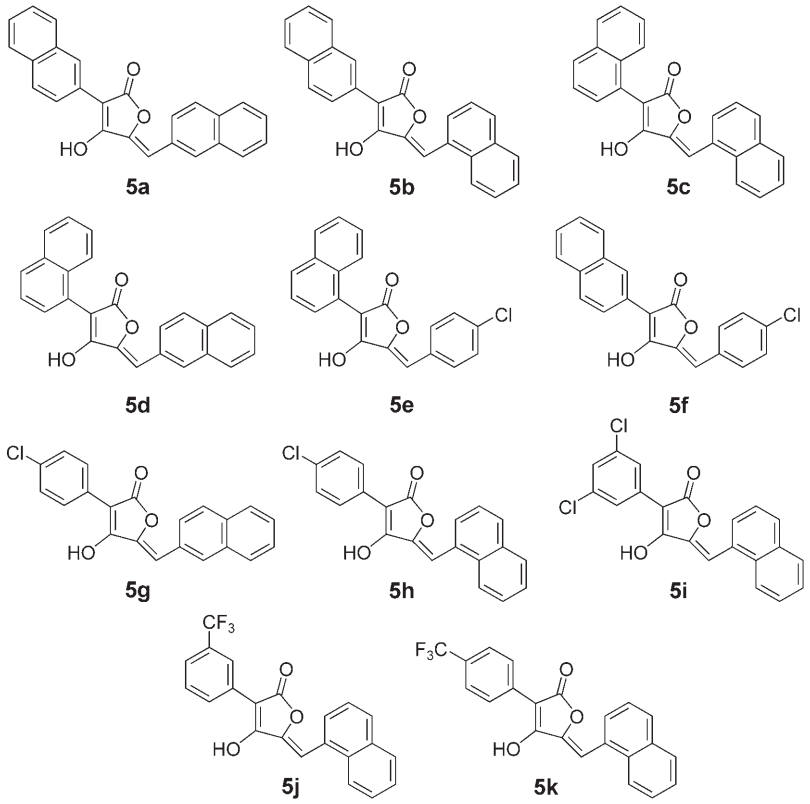

Table 1. Inhibition of a panel of Mur enzymes.[a]

Entry	Compd	IC_{50} [μM]								
		E. coli Mur A	*E. coli* Mur B	*S. aureus* Mur B	*E. coli* Mur C	*S. aureus* Mur C	*E. coli* Mur D	*S. aureus* Mur D	*E. coli* Mur E	*S. aureus* Mur E
1	**5a**	>69	>69	NT	47	>69	63	55	NT	NT
2	**5b**	49	19	NT	16	NT	>69	NT	NT	NT
3	**5c**	>69	63	27	30	NT	>69	NT	>69	>69
4	**5d**	>69	>69	NT	14	30	>69	52	NT	NT
5	**5e**	>72	>72	NT	46	NT	>72	NT	NT	NT
6	**5f**	51	15	13	10	54	>64	>64	>64	>64
7	**5g**	>72	23	NT	14	32	>72	>72	NT	NT
8	**5h**	>72	14	29	20	46	>72	69	>72	NT
9	**5i**	39	18	13	13	21	44	13	16	13
10	**5j**	>65	21	16	16	NT	>65	NT	NT	NT
11	**5k**	>65	26	26	29	18	>65	44	65	55

[a] NT = not tested.

chloro substituent led to compounds **5i–k** (Entries 9–11) with broad-spectrum activity, particularly compound **5j**, which inhibited all nine Mur enzymes studied.

While the level of Mur enzyme inhibition is in the micromolar range, it was necessary to determine the K_d values toward Mur B with this series (Table 2). Importantly, these compounds demonstrate excellent K_d values against *E. coli* Mur B, in the range of 43–800 nM. Correlation between the IC_{50} and K_d values for this enzyme were consistent despite the narrow range displayed by the IC_{50} values. It is reasonable to assume that the apparent large difference in the respective IC_{50} and K_d values is due to the presence of high substrate concentrations in the enzyme assay (NADPH: 100 μM and EP-UNAG: 50 μM), which are 10- and 5-fold higher than their K_M values, respectively.[26] Additionally, binding of the inhibitors in the substrate binding site competitively decreases the potency of inhibitors. Our results clearly demonstrate the need to rely on both IC_{50} and K_d values to drive the SAR for the inhibition of Mur enzymes.

The cellular activities of inhibitors **5a–k** were evaluated against a set of *Staphylococcus aureus* isolates (Gram-positive) and an *Escherichia coli* isolate (Gram-negative) by measuring the lowest concentration that completely inhibited the growth of the organism (MIC).[33] For the Gram-negative organism, an outer-membrane-permeable *E. coli* strain was used to eliminate potential issues of permeability.

The data in Table 3 indicate activity against both bacterial species, with **5c** and **5e** being the least active, and **5i** and **5b** showing the highest activity. This trend is consistent with the corresponding K_d values reported for these inhibitors in Table 2. It would have been difficult to correlate the MIC values with enzyme activity without establishing the correlation to the K_d values first for these inhibitors.

Table 2. Inhibition of *E. coli* MurB.

Entry	Compd	K_d [μM]	IC_{50} [μM]
1	**5b**	0.19	19
2	**5c**	0.80	63
3	**5f**	0.33	19
4	**5h**	0.39	22
5	**5i**	0.04	18
6	**5j**	0.37	21
7	**5k**	0.25	26

Table 3. Minimum inhibitory concentration values.

Entry	Compd	MIC [μg mL^{-1}]	
		S. aureus	*E. coli*
1	**5a**	4–16	8
2	**5b**	4–8	8
3	**5c**	16–32	8
4	**5d**	8–32	8
5	**5e**	32–64	16
6	**5f**	8	16
7	**5g**	8–16	8
8	**5h**	8–16	16
9	**5i**	1–2	2
10	**5j**	8	8
11	**5k**	8	8

The co-crystal structure of naphthyl compound **5h** with *E. coli* MurB was determined at a resolution of 2.5 Å (PDB code: 2Q85, RCSB 043269). The structure shows important interactions between the three portions of the compound, the furanone core, the naphthyl moiety, and the chlorophenyl side chain (Figure 1). There is a hydrogen bond between the furanone carbonyl group and side chain nitrogen atom of Gln 288. The hydroxy substituent on the furanone also appears to form a hydrogen bond with a high-occupancy water molecule that is coordinated by Asn 233 and Tyr 158. The chlorophenyl group projects into a pocket formed by residues Leu 218, Ser 229, Gln 288, Val 291, and the flavin moiety of FAD. The side chain hydroxy group of Ser 229 is within 3 Å of the face of the chlorophenyl group of **5h**, suggesting a dipole–π interaction. The naphthyl moiety of **5h** occupies a second, more hydrophobic pocket away from the cofactor binding site formed by residues Pro 252, Tyr 254, Lys 262, and Ala 264.

In summary, a strategy to develop inhibitors of multiple Mur enzymes has led to the identification of a set of naphthyl tetronic acids with excellent inhibitory activity against Mur enzymes as exhibited by their K_d values. Compound **5i** is the most potent, with a K_d value of 40 nM and MIC values of 1–2 μg mL^{-1} against multiple *S. aureus* and *E. coli* strains. The co-crystal structure of **5h** bound to *E. coli* MurB indicated binding in the substrate site with multiple hydrogen bonds to the hydroxy and carbonyl moieties of the core structure, while the substituents at C3 and C5 maintain hydrophobic interactions in two distinct pockets, one of which projects into the cofactor binding site.[34]

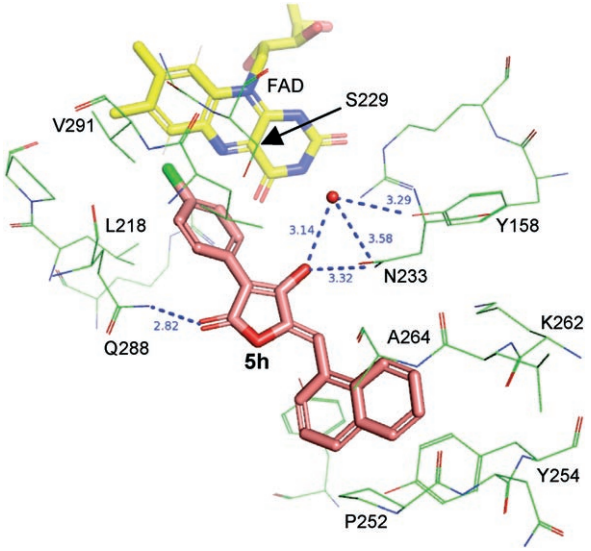

Figure 1. Co-crystal structure of **5h** (salmon-colored carbon skeleton) with *E. coli* MurB: The FAD cofactor is shown in yellow, and the MurB binding site is shown with green carbon atoms and thin bonds. A coordinated water molecule is shown as a red sphere; dashed blue lines represent electrostatic interactions and are labeled with the interaction distance (Å). Residues discussed in the text are indicated.

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