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Discovery of the First Antibacterial Small Molecule Inhibitors of MurB

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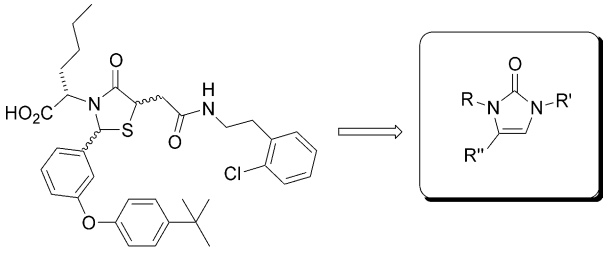
Abstract—A series of imidazolinone analogues was synthesized and shown to possess potent MurB inhibitory as well as good antibacterial activity.

Resistance of pathogenic bacteria to available antibiotics is quickly becoming a major problem in the community and hospital based healthcare settings. For example, the incidence of penicillin resistant *Streptococcus pneumoniae* (PRSP) in the United States has increased from less than 5% in the late 1980s to over 40% today.[1] This dramatic rise in resistance rate is also reflected in other parts of the world and by other strains of bacteria. Consequently, the search for novel agents to combat resistant bacteria has become one of the most important areas of antibacterial research today. The Mur enzymes[2] are integral components in bacterial peptidoglycan biosynthesis and therefore represent attractive new targets for antibacterial design.



1

MurB IC_{50} = 12μM

The MurB enzyme,[3] an NADPH dependant enolpyruvyl reductase, is responsible for the second committed step of bacterial peptidoglycan biosynthesis. Subsequent enzymes in the Mur pathway catalyze cytosolic formation of the UDP-MurNAc pentapeptide which is ultimately incorporated into the nascent bacterial peptidoglycan. We felt that a small molecule inhibitor of MurB would have the potential to be a superior antibacterial agent for a number of reasons. MurB has been shown to be essential for bacterial cell growth,[4] which means that an inhibitor could be expected to be bactericidal. As MurB has no known counterparts in eukaryotes,[5] selectivity and toxicity should not be a problem. Furthermore, as MurB is found in both gram positive and gram negative organisms it would be expected that an inhibitor might possess broad spectrum antibacterial activity. Lastly, since previous studies have elucidated the biochemical,[6] mechanistic,[7] and structural[8] features of the MurB enzyme, we had the potential to use rational drug design techniques in our quest.

It was recently reported[9] that tri-substituted thiazolidinones, exemplified by **1**, inhibit the MurB enzyme at the low micromolar level. These compounds were prepared using a stereorandom parallel synthesis approach, which provided each thiazolidinone analogue as a mixture of all four possible diastereoisomers. We report herein the discovery of a heterocyclic bioisosteric replacement for the thiazolidinone nucleus which eliminates the need to deal with multiple diastereoisomers. This new heterocycle not only retains potent in vitro MurB inhibitory activity, but it demonstrates whole cell antibacterial activity—an attribute not demonstrated by the aforementioned thiazolidinones.

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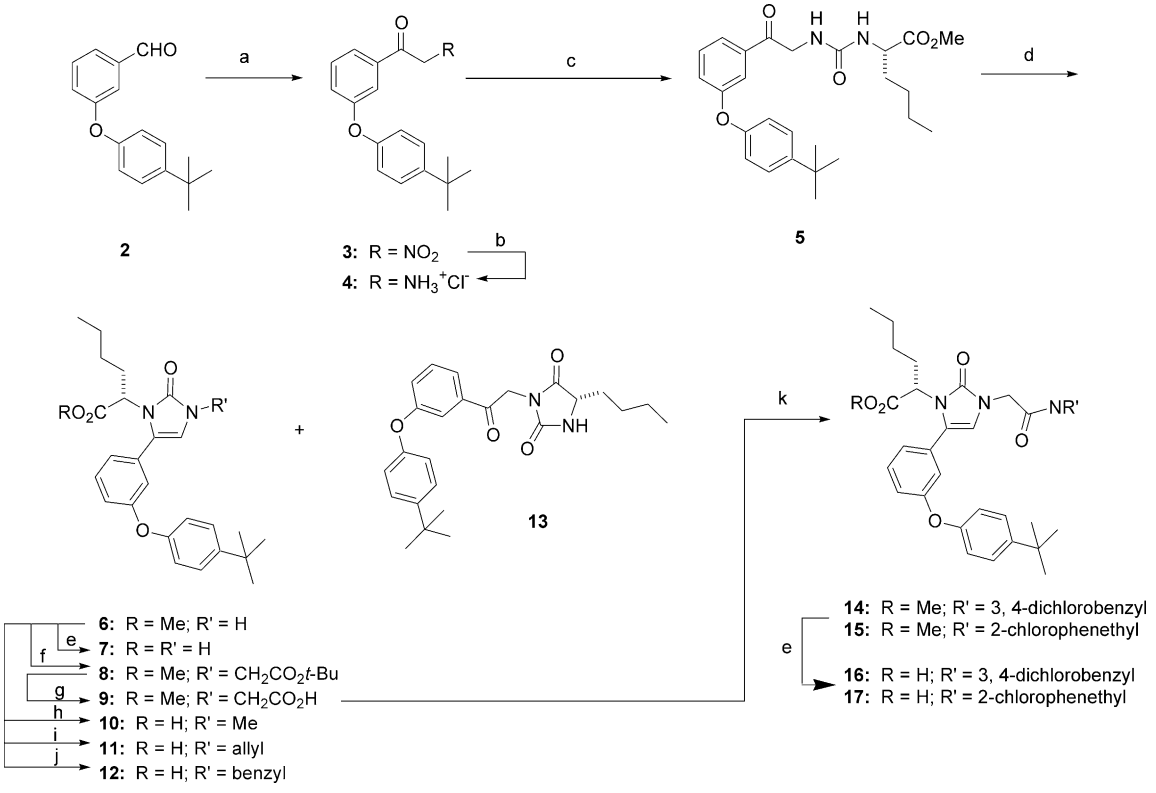
We chose as our initial target the imidazolinone analogue of **1**. Although there are a number of very efficient methods known to produce substituted imidazolinones,[10] there are few which allow for the incorporation of optically active amino acids. We therefore chose to employ a method[11] wherein an α-amino ketone is condensed with an appropriately substituted isocyanate. The requisite aminoketone was prepared in a straightforward manner starting from the commercially available biphenyl ether **2** (Scheme 1). Henry reaction followed by oxidation afforded the α-nitroketone **3** which was subsequently reduced to provide **4**. Condensation with the isocyanate derived from norleucine[12] provided urea **5**, which was in turn cyclodehydrated to yield imidazolinone **6** along with minor amounts of the corresponding hydantoin **13**. Alkylation of the remaining unsubstituted nitrogen atom was accomplished by allowing **6** to react with potassium carbonate in the presence of *t*-butyl bromoacetate and tetrabutylammonium iodide at 100 °C followed by liberation of the free carboxylic acid **9** by the action of trifluoroacetic acid.

Although amidation could in principal be accomplished with any number of traditional reagents, we have found that the acyl fluoride, generated in situ, functions remarkably well as an activated carboxylic acid synthon. Acyl fluorides have long been known to be competent partners in amidation reactions,[13] however, they are typically generated with cyanuric fluoride or DAST and *isolated* prior to amine addition. A recent report[14] has delineated the usage of tetramethylfluoroformamidinium hexafluorophosphate (TFFH) to convert carboxylic acids to their respective acyl fluorides which are then reacted with amines *without* prior isolation and/or purification of the intermediate acyl fluoride. We have found that commercially available DAST can also function in this regard. In the event, treatment of **9** with DAST in dichloromethane followed by addition of amine provided good yields of amides **14** and **15** which were subsequently saponified to provide **16** and **17**, respectively.[15] In order to investigate the role of the amide side chain in conferring MurB activity, we prepared the NH imidazolinone **6** as well as the alkyl substituted analogues **10**–**12**. It is worth mentioning that this synthetic sequence should in principal be amenable to a solid supported parallel synthesis approach by attaching the starting amino acid to a solid support. This would result in the production of a library of imidazolinones with three points of diversity.

Biological Results

Both **16** and **17** exhibited good potency against the isolated MurB enzyme[6,9,16] (Table 1). Furthermore, as anticipated, they were equipotent to the previously reported stereochemical mixture of thiazolidinones[9]



Scheme 1. Reagents and conditions: (a) CH_3NO_2, EtOH, then PCC, CH_2Cl_2; (b) $SnCl_2$, EtOH then HCl/Et_2O; (c) Norleucine methyl ester, DMAP, $(BOC)_2O$, CH_2Cl_2 (72%); (d) H_2SO_4, MeOH, reflux (50%, along with ~10% of **13**); (e) NaOH, MeOH; (f) K_2CO_3, $Bu_4N^+I^-$, *t*-butyl bromoacetate, DMF, 100 °C (84%); (g) TFA, CH_2Cl_2 (87%); (h) K_2CO_3, MeI then NaOH, MeOH (43%); (i) K_2CO_3, allyl bromide then NaOH, MeOH (56%); (j) K_2CO_3, benzyl bromide then NaOH, MeOH (38%); (k) (i) DAST, CH_2Cl_2; (ii) For compound **14**: 3,4-dichlorobenzyl amine; For compound **15**: 2-chlorophenethyl amine.

Table 1. Antibacterial and MurB inhibitory activity of imidazolinones

Compd	In vitro inhibition of MurB IC_{50} (μM)	Antibacterial activity[a] MIC (μg/mL)
7	>118	—
9	>101	—
10	>115	—
11	40	4
12	16	2
16	15	4
17	25	4
1	12	—

[a]*Staphylococcus aureus* A9537.

From the data shown in Table 1 it is apparent that a lipophilic substituent on the nitrogen distal to the biphenyl ether moiety (N-1) is necessary for MurB inhibitory activity. Compounds **16** and **17** both meet this requirement and effectively inhibit the isolated enzyme. Additionally, it is not necessary to have an amide linkage as exemplified by **11** and **12**. It is not sufficient, however, to merely 'cap' the NH with a methyl group as this leads to complete loss of activity (**10**). The requirement for lipophilicity of the N-1 substituent is again demonstrated by the inactivity of **7** and **9**. Of equal importance, these novel imidazolinone analogues possess whole cell antibacterial activity which tracks with MurB inhibitory activity. It remains, however, to be shown that the antibacterial activity observed in *S. aureus* is due to inhibition of the MurB enzyme.

In conclusion, the imidazolinone analogues described in this communication are the first reported stereochemically discrete MurB inhibitors possessing antibacterial activity, and as such, represent a promising chemotype in the search for novel antibacterial agents.

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15. Experimental procedure for the conversion of **9** to **15**: to 50 mg (0.10 mmol) carboxylic acid **9** in 5 mL dichloromethane under nitrogen was added 0.12 mL (1 mM in dichloromethane) of DAST. After 10 min, 0.15 mL (1 mM in dichloromethane) of 3, 4-dichlorobenzylamine was added. After an additional 10 min, the reaction was filtered through a preconditioned SAX cartridge followed by filtration through a preconditioned SCX cartridge. Concentration provided 36 mg (55%) of analytically pure ester **14**. ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.40–7.30 (m, 5H), 7.18–6.91 (m, 6H), 6.31 (s, 1H), 4.52–4.38 (m, 5H), 3.62 (s, 3H), 2.33–2.19 (m, 1H), 2.09–1.95 (m, 1H), 1.32 (s, 9H), 1.20–1.08 (m, 4H), 0.79 (app t, 6.9 Hz, 3H). To 12.5 mg (0.019 mmol) ester **14** in 0.5 mL methanol was added 57 μL (0.057 mmol) 1 N NaOH. After 24 h the reaction mixture was acidified with 1 N HCl, extracted with Et_2O, washed with brine and dried over $MgSO_4$ to provide 10mg (82%) of **16**. ^{1}H NMR (300 MHz, CD_3OD) δ 7.49–7.39 (m, 5H), 7.27–7.23 (m, 1H), 7.12–7.03 (m, 2H), 6.98–6.93 (m, 3H), 6.60 (s, 1H), 4.54 (dd, 4.79 and 10.8 Hz, 1H), 4.43–4.40 (m, 4H), 2.17–2.10 (m, 1H), 2.03–1.99 (m, 1H), 1.34 (s, 9H), 1.20–0.90 (m, 4H), 0.797 (t, 6.9 Hz, 3H).
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