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Bioorganic & Medicinal Chemistry Letters 15 (2005) 2527–2531
Bioorganic & Medicinal Chemistry Letters

4-Alkyl and 4,4′-dialkyl 1,2-bis(4-chlorophenyl)pyrazolidine-3,5-dione derivatives as new inhibitors of bacterial cell wall biosynthesis

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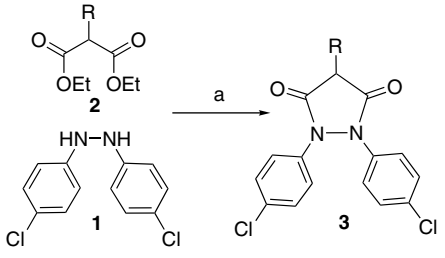
Received 29 December 2004; revised 14 March 2005; accepted 16 March 2005
Available online 12 April 2005

Abstract—Over 195 4-alkyl and 4,4-dialkyl 1,2-bis(4-chlorophenyl)pyrazolidine-3,5-dione derivatives were synthesized, utilizing microwave accelerated synthesis, for evaluation as new inhibitors of bacterial cell wall biosynthesis. Many of them demonstrated good activity against MurB in vitro and low MIC values against Gram-positive bacteria, particularly penicillin-resistant *Streptococcus pneumoniae* (PRSP). Derivative **7l** demonstrated antibacterial activity against both Gram-positive and Gram-negative bacteria. Derivatives **7f** and **10a** also demonstrated potent nanomolar K_d values in their binding to MurB.

Scientific effort for the design and synthesis of novel antibacterials has focused mainly on modifications within the same class of antibiotics already on the market. Due to the ease with which bacteria can exchange genetic material and develop resistance to the preexisting antibiotic classes, our focus has been to develop antibacterials with a novel mode of action. As peptidoglycan is an essential cell wall component of most bacteria, cytosolic enzymes MurA-F at early stages of peptidoglycan biosynthesis are unique and selective target for antibiotic action.[1] Furthermore, the MurA-F cascade is ubiquitous to both Gram-positive and Gram-negative bacteria, but have no mammalian homologue.[1] Inhibition of any of these essential enzymes leads to loss of cell shape and integrity followed by bacterial death.[2,3] From screening of a focused set of the corporate compound collection, the pyrazolidinedione scaffold was identified as a potential inhibitor of MurB. Here we report the synthesis and antimicrobial activity of 4-alkyl and 4,4-bis-alkyl pyrazolidinedione derivatives as new inhibitors of MurA and MurB.

Pyrazolidinediones are conventionally synthesized through the condensation of diethylmalonates with hydrazine using in situ generated sodium ethoxide as the base and heating at elevated temperatures for extended time periods.[4] In order to generate large numbers of compounds, we developed a simple, fast, high yielding, one step method for the synthesis of 4-alkyl-pyrazolidinediones **3** via microwave heating of alkyl-diethylmalonates **2** with diarylhydrazine **1** using commercially available sodium ethoxide (Scheme 1).[5]

Diarylhydrazine derivative **1** was prepared in ~50% overall yield by oxidative dimerization of 2 equiv of 4-chloroaniline (**4**) in the presence of manganese dioxide,



Scheme 1. Reagents and conditions: (a) NaOEt, microwave, 4 min.

Keywords: Microwave-assisted synthesis; Pyrazolidinediones; MurB; MurA; Antibacterial activity.
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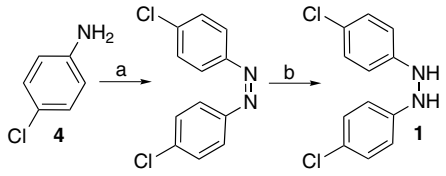
doi:10.1016/j.bmcl.2005.03.058

followed by reduction in the presence of zinc dust and ammonium chloride[6] (Scheme 2).

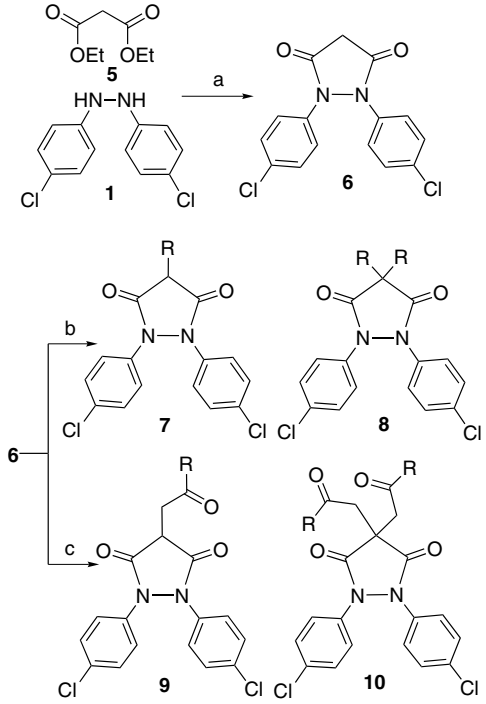
Since limited numbers of diethyl alkylmalonates are available, techniques for the alkylation of pyrazolidinedione itself were also developed utilizing microwave heating (Scheme 3). First unsubstituted pyrazolidinedione **6** was prepared in 95% yield by the microwave-assisted condensation of 1,2-di(4-chlorophenyl)hydrazine **1** with diethylmalonate **5**. Next **6** was reacted with alkyl bromides or alkyl chlorides and lithium carbonate under microwave heating to produce a mixture of 4-alkyated (**7**) and 4,4′-dialkylated products (**8**).[7] Of other bases attempted, potassium carbonate produced somewhat more dialkylated product and no alkylation resulted when triethylamine, pyridine or *N*,*N*-diisopropylethylamine were used. Higher boiling *N*,*N*-dimethylformamide was used as the solvent rather than acetone to prevent evaporation and subsequent charring of reactions in open vessels. For the addition of more reactive 2-bromoacetophenones to unsubstituted pyrazolidinedione **6** *N*,*N*-diisopropylethylamine was utilized as a base to yield a mixture of mono (**9**) and bis (**10**) products.



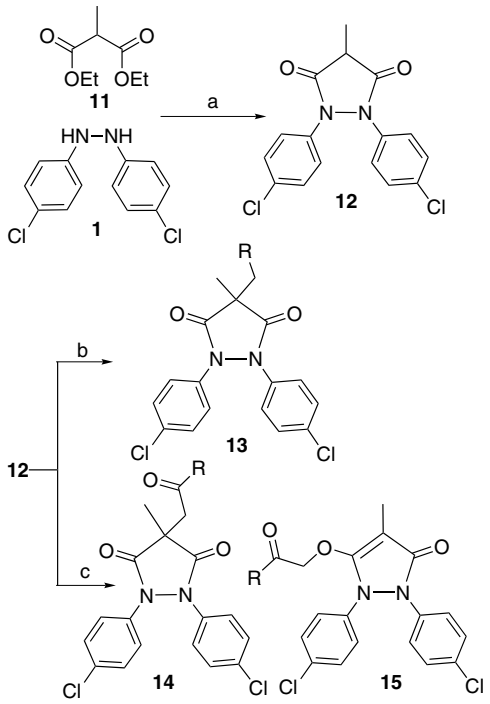
Scheme 2. Reagents and conditions: (a) MnO_2, benzene, reflux, 1 h; (b) Zn dust, NH_4Cl, acetone, 1 h.

4,4′-Differentially alkylated products, Scheme 4, were obtained in a manner similar to Scheme 3. 4-Methyl 1,2-bis(4-chlorophenyl)pyrazolidine-3,5-dione **12** was prepared in 85% yield by the microwave-assisted condensation of diarylhydrazine **1** with methyl diethylmalonate **11**. Next **12** was reacted with alkyl bromides or alkyl chlorides and lithium carbonate under microwave heating (900 W) to produce 4′alkyl-4-methyl-pyrazolidine-3,5-dione products (**13**). Similarly, 2-bromoacetophenones were also added to yield a mixture of 4′-alkyl-4-methyl-pyrazolidine-3,5-dione (**14**) and 4-methyl-5-alkyloxy-pyrazol-3-one (**15**) products.

According to molecular modeling studies, the 4-position of the pyrazolidinedione ring points into a long and large lipophilic pocket. Hence, using Schemes 1, 3 and 4 over 195 4-alkyl or 4,4′-alkyl derivatives were synthesized to probe the lipophilic pocket and submitted for evaluation[8–10] as new inhibitors of bacterial cell wall biosynthesis. SAR (structure activity relationship) trends were established and some are highlighted in Tables 1–3. In terms of activity against MurB and Gram-positive bacteria, particularly *Streptococcus pneumoniae* (PRSP), lipophilic 4-alkyl groups were preferred over somewhat more hydrophilic groups (**3b** vs **3f**, **7i** vs **3e**). Potency was improved against MurB by incorporating an aromatic ring in the lipophilic chain (Table 1, **3a**, **b** compared to **7a**–**d**). Longer chain length (Table 1, **7a**–**d** vs **3c**, **d**) was also a factor in improving IC_{50} values against MurB ($n = 1$ $IC_{50} > 50$ μM, $n = 6$ $IC_{50} \sim 9$ μM) and MIC (minimum inhibitory concentration) values for *Streptococcus pneumoniae* bacteria strains ($n = 1$, 200 μM and $n = 6$, 6.25 μM). Derivative **7d** (chain length



Scheme 3. Reagents and conditions: (a) NaOEt, microwave, 2 min; (b) R-Br, Li_2CO_3, DMF, microwave, 2–4 min; (c) R-C(O)–CH_2–Br, DIPEA, toluene, microwave, 1–3 min.



Scheme 4. Reagents and conditions: (a) NaOEt, microwave, 4 min; (b) R-Br, Li_2CO_3, DMF, microwave, 2–4 min; (c) R-C(O)–CH_2–Br, DIPEA, toluene, microwave, 1–3 min.