N-Chlorooxazolidin-2-ones as Antimicrobial Agents at Neutral pH

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ACS National Meeting August 29-September 1, 2011 (Denver, CO)

Introduction

Bacteria are becoming resistant to currently available antibiotics, driving the need for new antimicrobial agents with novel mechanisms of action and low potential for the development of resistance. N-Chlorotaurine 1 and N,N-Dichlorotaurine 2 are endogenous antimicrobials that are generated in the oxidative burst of activated neutrophils and macrophages. They are utilized by the innate immune system to destroy invading pathogens and have shown minimal potential for developing resistant bacteria.1,2 Sulfonyl acids 3 and 4 have been designed to possess improved aqueous stability over the parent analogs, 1 and 2. Compound 3 is currently being evaluated as a clinical candidate for several topical applications.3 Nonetheless, compounds with increased activity over a range of pH's would have utility for therapeutic indications that necessitate application to physiologically buffered environments.4

Microbial Activity of Chloramines

Mono- and di-chloramine analogs of endogenous chlorinated taurines, 3 and 4, display pH dependent anti-microbial activity with increased killing at lower pH. In part, the changes in biological activity between pH 4 and 7 are attributed to increasing reaction rates of these compounds with nucleophiles under the more acidic conditions, as has been reported with 1.5 In order to broaden the utility of this class of antimicrobials, we endeavored to design compounds with improved activity at neutral pH. N-Chloroacetamide heterocycles that contain electron withdrawing groups adjacent to the chlorinated nitrogen were investigated. By reducing the electrophilicity of the nitrogen, we envisioned minimizing potential protonation at rate limiting steps to mitigate the pH dependence typically observed in aliphatic chloramines.

Initial investigations into heterocyclic chloramines showed that sulfonic acid functionalized 2-imidazolidinones 5, pyrrolidones 10, and oxazolidinones 12 were aqueous stable compounds with poor antimicrobial activity at both pH 4 and pH 7.6 Unlike previous series,7 the oxazolidinone 11 showed pH independent biological activity. In addition, based on earlier experience, we had expected that the activity of lipophilic oxazolidinone analogs, such as 11, to be comparable to the sulfonic acid 12. But in fact, when the solubilizing group X was modified to a less polar hydroxyl or chloro group, a substantial increase in anti-bacterial activity was observed for both pHs.

Oxazolidinones as Activated Heterocycles

The oxazolidinone series showed the greatest activity of the three heterocyclic cores tested, with sulfonamide activity for both pHs in the chloro analog 11. In addition, unlike the chloramine series, the safety profile of these compounds, as estimated by the ratio of the cytotoxicity (CT50) against L929 cells vs. MBC, did not substantially decrease with more lipophilic analogs. Additional modifications incorporating neutral electron withdrawing functionality at the 4 and 5 positions of the oxazolidinone ring were tested. The electronic effects of these substituents had little effect on the aqueous activity or stability. Instead a wide range of lipophilic analogs showed potent anti-bacterial activity against Gram-negative and Gram-positive examples.

References


Abstract

The oxazolidinone series showed the greatest activity of the three heterocyclic cores tested, with sulfonamide activity for both pHs in the chloro analog 11. In addition, unlike the chloramine series, the safety profile of these compounds, as estimated by the ratio of the cytotoxicity (CT50) against L929 cells vs. MBC, did not substantially decrease with more lipophilic analogs. Additional modifications incorporating neutral electron withdrawing functionality at the 4 and 5 positions of the oxazolidinone ring were tested. The electronic effects of these substituents had little effect on the aqueous activity or stability. Instead a wide range of lipophilic analogs showed potent anti-bacterial activity against Gram-negative and Gram-positive examples.

Biological assay results

Compounds 5-23.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MBC or MFC (μg/mL)</th>
<th>pH 4</th>
<th>pH 7</th>
<th>pH 4</th>
<th>pH 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>ATCC 25922</td>
<td>256</td>
<td>128</td>
<td>64</td>
<td>32</td>
</tr>
<tr>
<td>S. aureus</td>
<td>ATCC 25923</td>
<td>128</td>
<td>64</td>
<td>64</td>
<td>32</td>
</tr>
<tr>
<td>C. albicans</td>
<td>ATCC 10231</td>
<td>256</td>
<td>128</td>
<td>64</td>
<td>32</td>
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<tr>
<td>CT50</td>
<td>µg/mL</td>
<td>512</td>
<td>128</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compared to E. coli</td>
<td>MBC</td>
<td>128</td>
<td>32</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Synthesis of N-Cl-Heterocycles

Reagents and conditions:

(a) Et2AlCN, NaSO3, DMF, H2O, 60°C; (b) NaBr, DMF, 80°C; (c) Raney-Ni, H2, MeOH, 24h; (d) Triphosgene, TEA, DCM, 0°C, 16h; (e) NaBH4, EtOH, 21h; (f) NaH, THF, 3h; (g) i. Ethyl chloroformate; ii. NaOH, 0°C-25°C; (h) NaBH4, EtOH, 21h; (i) NaBH4, EtOH, 21h; (j) NaBH4, EtOH, 21h; (k) NaBH4, EtOH, 21h; (l) Ethanethiol, K2CO3, MeOH, 24hr; (m) pentanethiol, tert-BuOH, THF -78 to 25°C; (n) tert-BuOH, THF, 100°C; (o) ClSO2Ph or ClSO2Me, TFA, 100°C; (p) i. tert-BuOH, THF, 100°C; ii. 85% HCl, 0°C-25°C; (q) i. tert-BuOH, THF, 100°C; ii. 85% HCl, 0°C-25°C; (r) i. tert-BuOH, THF, 100°C; ii. 85% HCl, 0°C-25°C; (s) i. tert-BuOH, THF, 100°C; ii. 85% HCl, 0°C-25°C; (t) i. tert-BuOH, THF, 100°C; ii. 85% HCl, 0°C-25°C.

Conclusions

- N-Chlorooxazolidin-2-ones with lipophilic sidechains, such as 11, 13, 16, and 21 have excellent activity against E. coli and S. aureus at both pH 4 and 7, and the safety profile of these compounds at pH 7, as measured by the ratio of the CT50/MBC vs. 1 h MBC (E.coli), is comparable to more polar analogs, differentiating this class from previous di- and mono-chloramine series.
- Compounds 16, 20, and 21 show good anti-fungal activity at pH 7 against C. albicans, and compound 16 has very good safety in the CT50 cell assay.
- Conclusions 15, 16, and 21 have bactericidal and fungicidal activity and are potential advanced candidates suitable for IND-enabling studies.