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New classes of Gram-positive selective antibacterials: Inhibitors of MRSA and surrogates of the causative agents of anthrax and tuberculosis

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Abstract

An antimicrobial phenolic stilbene, \((E)-3\)-hydroxy-5-methoxystilbene, 1 was recently isolated from the leaves of Comptonia peregrina (L.) Coulter and shown to possess inhibitory activity against several Gram-positive bacteria, including isolates of methicillin-resistant Staphylococcus aureus (MRSA), Mycobacterium bovis BCG, and avirulent Bacillus anthracis (Sterne strain), among others. These results prompted the design and synthesis of two new classes of compounds, phenoxy styrenes and phenothio styrenes, as analogs of the natural antimicrobial stilbene. These and additional stilb enoid analogs were synthesized using new, efficient, copper-mediated coupling strategies. Minimum inhibitory concentration (MIC) antimicrobial assays were performed on all compounds prepared. These preliminary structure–activity relationship studies indicated that both new classes of synthetic analogs, as well as the stilbenes, show promising activity against Gram-positive bacteria when at least one phenolic moiety is present, but not when absent. The potencies of the phenolic phenoxy styrenes and phenothio styrenes were found to be comparable to those of the phenolic stilbenes tested.

As part of our ongoing ethnopharmacological efforts to discover new molecules that might be used to combat clinically significant bacterial infections, we identified the phenolic stilbene, \((E)-3\)-hydroxy-5-methoxystilbene, 1 in the leaves of the shrub, Comptonia peregrina (L.) Coulter (‘sweet fern’).1 This plant has been used traditionally for its antimicrobial and other biological properties by a number of native North American cultures of the Great Lakes and Maritime regions.2 Although the cytotoxic compound, methyl-p-coumarate had previously been found in this plant,3 along with numerous other compounds isolated from the essential oil,4 stilbene 1 had not previously been reported to be present in C. peregrina. Interestingly, compound 1 was identified in the plants, Didymochnaena truncata (Sw.) J. Sm (Dryopteridaceae)5 and Alpinia katsumadai Hayata.6 However, the antimicrobial properties of this natural stilbene 1 were not reported in either of the prior studies.

After the purification of the natural product 1, a battery of minimum inhibitory concentration (MIC) assays was performed to assess the range of antimicrobial activity.7 Analysis of those studies indicated that 1 exhibited selective inhibition of the growth of numerous clinically relevant Gram-positive bacteria at reasonably low concentrations.8 For example, 1 was found to inhibit the growth of methicillin-resistant Staphylococcus aureus (MRSA) with an MIC of 32 μg/mL and of vancomycin-resistant enterococci (VRE) with an MIC of 16 μg/mL (Table 1).1 Currently, MRSA and VRE are two bacterial species of growing concern to the medical community because of the development of widespread resistance to existing frontline drugs.9–10 In addition, 1 also showed very good inhibitory activity against the Sterne strain of Bacillus anthracis (MIC 8 μg/mL), the attenuated variant of the causative species of anthrax, as well as promising activity against other related Bacillus species (Table 1). Furthermore, natural product 1 was found to be effective in inhibiting the growth of Mycobacterium bovis with an MIC of 26 μg/mL, which indicated it might serve as a promising lead scaffold in the development of drugs used to combat drug resistant Mycobacterium tuberculosis infections.

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Organic and medicinal chemists have found trans-stilbene scaffolds to be an intriguing structural class of compounds (e.g., see methoxylated and phenolic stilbenes, Fig. 1). These stilbene scaffolds may be considered to be ‘privileged structures’ because of their wide variety of biological activity. Indeed, trans-stilbene compounds are widely distributed throughout nature, and potential therapeutic properties. Specifically, resveratrol, as well as the promising antimicrobial and potential therapeutic properties. It was envisioned that functionalized analogs of E-phenoxystyrenes and E-phenothioxyrenes might fill this role; consequently, new synthetic approaches were developed to create these new agents. In addition, the synthetic methods developed for the preparation of the phenoxystyrenes and phenothioxyrenes was extended to the construction of several substituted E-stilbenoid analogs to explore the structure–antimicrobial activity relationships of these classes of compounds related to 1 in greater detail.

Initially, it was decided to prepare gram quantities of the active natural stilbene 1, and this was accomplished using a Horner–Wadsworth–Emmons process, as shown in Scheme 1. This novel approach to creating the substituted stilbenes was convenient and inexpensive, and the relatively high yields easily produced gram quantities of the desired targets. The condensation of the benzyl bromide-derived phosphorate and 3,5-dimethoxybenzaldehyde, under basic conditions, yielded a mixture of stilbenes 1a in a Z:E ratio of approximately 1:4 (Scheme 1). The pure E-3,5-dimethoxy-stilbene 1a was obtained from this mixture by crystallization from methanol and water. Subsequent mono-demethylation of (E)-1a using BBr3 at −78 °C gave the desired phenolic stilbene 1 in good yield.

To begin construction of derivatives of 1 for SAR studies, a practical synthesis was developed using a Negishi cross-coupling process that was directly applied to thiophenes and to give the 2- and 3-styrylthiophenes and 9 (Scheme 2). This approach, gratifyingly, gave only the desired (E)-alkene isomers, thereby eliminating the need for the tedious separation of the unwanted (Z)-isomer. Importantly, the phenolic 2- and 3-styrylthiophenes and 9, respectively, were produced efficiently using this one-pot process. Thus, coupling between the TBDPS-protected aryvinyl iodide 7 and the thiophenyl zinc reagent, followed by deprotection using TBAF–THF, afforded the target phenolic styrylthiophenes without the requirement of an extra demethylation step.

It was then felt extension of the vinyl linkage of the stilbenes by addition of either an oxygen or sulfur heteroatom would yield novel, unnatural phenoxystyrene and phenothioxyrene scaffolds that might exhibit enhanced antimicrobial activity. Therefore, the phenolic phenoxystyrene analogs and the phenolic phenoxystyrene analog were prepared employing copper(I)-catalyzed coupling reactions that were recently developed in this laboratory (Scheme 3). This process involved the cross-coupling between the aryl vinyl iodide and the corresponding phenols or thiophenols. In similar fashion to the sequence outlined in Scheme 2, this copper-mediated coupling reaction and the subsequent deprotection could be carried out conveniently in one reaction vessel. Overall, this approach provided an efficient and high-yielding access to these new classes of phenolic stilbene analogs.

Motivated by the positive results obtained from the reactions outlined in Scheme 3, the corresponding oxy-functionalized, dimethoxy phenoxystyrene and phenothioxyrene analogs were prepared employing copper(I)-catalyzed coupling reactions that were recently developed in this laboratory (Scheme 3).

![Figure 1. General structures of methoxylated and phenolic (E)-stilbenes.](image-url)
was obtained after coupling between Fenylic deprotection. Similarly, the dimethoxy phenoxystyrene 26 O-protected monophenol 27 was evaluated under acidic (1 M HCl), basic (1 M NaOH), and neutral aqueous media to assess chemical stability. After stirring under these conditions for 36 h, it was found that all of the novel phenoxy compounds presented in Scheme 3. The apparent versatility of this coupling method for this class of compounds prompted the modification of the asymmetric phenoxystyrene scaffold by placing the hydroxy/methoxy groups on the phenoxy ring rather than the styryl ring, as represented in compound 28 and subsequent deprotection. Similarly, the dimethoxy phenoxystyrene 29 was obtained after coupling between 28 and 3,5-dimethoxyphenol 27.

All compounds prepared in Schemes 2–5 were purified by flash column chromatography using silica gel and an appropriate solvent system. After purification of the phenoxystyrene and phenothiostyrene analogs, 28 after coupling with phenylvinyl iodide and the same phenols, or thiophenol presented in Scheme 3.

Scheme 2. Reagents and conditions: (a) i—3 or 4, n-BuLi, −78 °C, 30 min, ZnCl2, 0 °C, 1 h; ii—2, Pd2(dba)3, THF, rt, 2 h; (b) i—3 or 4, n-BuLi, −78 °C, 30 min, ZnCl2, 0 °C, 1 h; ii—7, Pd2(dba)3, THF, rt, 2 h; iii—TBAF THF, rt, 2 h.

Scheme 3. Reagents and conditions: (a) ArXH, Cu(I)Cl, NMM, Cs2CO3, toluene, reflux, 12–16 h; (b) TBAF THF, rt, 2 h.

also prepared using the same copper(I) mediated coupling approach (Scheme 4). These cross-coupling reactions were carried out between dimethoxy aryl vinyl iodide 2 and the same phenols, or thiophenol presented in Scheme 3.

The apparent versatility of this coupling method for this class of compounds prompted the modification of the asymmetric phenoxystyrene scaffold by placing the hydroxy/methoxy groups on the phenoxy ring rather than the styryl ring, as represented in compound 28 and subsequent deprotection. Similarly, the dimethoxy phenoxystyrene 29 was obtained after coupling between 28 and 3,5-dimethoxyphenol 27.

Scheme 4. Reagents and conditions: (a) ArXH, Cu(I)Cl, NMM, Cs2CO3, toluene, reflux, 12–16 h.

Scheme 5. Reagents and conditions: (a) TBDPSCI, imidazole, DMF, −20 °C, 10 min; (b) Cu(I)Cl, NMM, Cs2CO3, toluene, reflux, 16 h; (c) TBAF THF, rt, 2 h; (d) Cu(I)Cl, NMM, Cs2CO3, toluene, reflux, 12 h.

sayed for the ability to inhibit the growth of five selected strains of clinically significant bacteria. The results of these MIC (minimum inhibitory concentration) assays are presented in Table 2. The in vitro MIC determinations for each compound were performed according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines. For safety reasons, Gram-positive microorganisms, Bacillus cereus and Mycobacterium smegmatis were used as surrogates for the more virulent strains of B. anthracis and M. tuberculosis, respectively. All of the analogs were also tested for their ability to inhibit the growth of Escherichia coli as a representative Gram-negative microorganism.

Analysis of the results reported in Table 2 illustrates a number of interesting antimicrobial structure–activity features of the stilbenoid compounds and their extended, heteroatom-containing phenoxy styrene and phenothio styrene analogs. As expected, none of the compounds exhibited antimicrobial activity against the representative Gram-negative species, E. coli. This is in line with the results of preliminary assays performed using the natural stilbene 1 in which no Gram-negative bacteria were affected by this agent (see data in Table 1).

From examination of the data in Table 2, it is clear that the presence of at least one phenolic group is required for activity in this class of compounds. All of the dimethoxylated compounds, 1a, 5–6, 17–23, and 29, were devoid of inhibitory activity against the Gram-positive (and Gram-negative) species investigated. However, natural product 1 and all of its analogs that contain a meta-hydroxy group 8–16, 26 exhibited inhibitory activity against Gram-positive organisms, even when this moiety was located in the phenoxy ring as opposed to the styryl ring, as seen in compound 26. These results are not surprising because phenolic compounds are viewed as one of the major classes of natural antimicrobial agents.

Perhaps the most exciting finding in this study is that both the phenoxy styrene and phenothio styrene analogs 10–16, 26 of the phenolic stilbenes 1, 8–9 are roughly equivalent in their potency in the MIC assays performed. Although none of these vinyl ether or vinyl thioether analogs was significantly better at inhibition of the growth of Gram-positive microorganisms than the original natural
Table 2
Minimum inhibitory concentration (MIC µg/mL) values for natural stilbene 1 and the synthetic analogs

| Compound | S. aureus ATCC 29213 | MRSA MC-1 | B. cereus | M. smegmatis | E. coli |
|----------|-----------------------|------------|-----------|--------------|--------|
| 1        | 16                    | >512       | 16        | 64           | >512   |
| 1a       | >512                  | >512       | >512      | >512         | >512   |
| 5        | >512                  | >512       | >512      | >512         | >512   |
| 6        | >512                  | >512       | >512      | >512         | >512   |
| 8        | 16                    | 32         | 32        | 128          | 64     |
| 9        | 32                    | 32         | 64        | 128          | 64     |
| 10       | 32                    | 64         | 32        | 128          | 64     |
| 11       | 16                    | 32         | 64        | >128         | >512   |
| 12       | 16                    | 32         | 32        | 64           | >512   |
| 13       | 16                    | 32         | 64        | >128         | >512   |
| 14       | 16                    | 64         | 64        | 128          | >512   |
| 15       | 64                    | 64         | 64        | 128          | >512   |
| 16       | 16                    | 32         | 16        | 128          | >512   |
| 17       | >512                  | >512       | >512      | >512         | >512   |
| 18       | >512                  | >512       | >512      | >512         | >512   |
| 19       | >512                  | >512       | >512      | >512         | >512   |
| 20       | >512                  | >512       | >512      | >512         | >512   |
| 21       | >512                  | >512       | >512      | >512         | >512   |
| 22       | >512                  | >512       | >512      | >512         | >512   |
| 23       | >512                  | >512       | >512      | >512         | >512   |
| 24       | >512                  | >512       | >512      | >512         | >512   |
| 25       | >512                  | >512       | >512      | >512         | >512   |
| 26       | >512                  | >512       | >512      | >512         | >512   |
| 29       | >512                  | >512       | >512      | >512         | >512   |

* MIC values represent the mean of three experiments. ATCC, American Type Culture Collection; MRSA, methicillin-resistant Staphylococcus aureus.

In conclusion, the antibacterial activity of a phenolic (E)-stilbene 1 isolated from C. peregrina has been evaluated and demonstrated to be active against a range of Gram-positive bacteria, including drug resistant S. aureus and enterococci spp., but not Gram-negative bacteria. Importantly, 1 was active against M. bovis (BCG) and against B. anthracis, as well as three related Bacillus species. When the phenolic stilbene scaffold was modified with other aryl moieties or extended by one heteroatom to create the novel aryl moieties or extended by one heteroatom to create the novel phenoxystyrene and phenothiostyrene derivatives, these agents are highly encouraging in consideration of the viability of using phenoxystyrene and phenothiostyrene scaffold structural analogs of bioactive antimicrobial stilbenes to treat disease because they are stable at low and high pH values. Indeed, these novel scaffolds may serve as excellent starting points for further chemical optimization and the development of new drugs that are certainly needed to combat threatening MRSA, tuberculosis, and anthrax infections. Furthermore, because trans-stilbenes have been shown to possess such a diverse array of biological activity, further studies should promote additional SAR studies of stilbene compounds for additional therapeutic areas.

In conclusion, the antibacterial activity of a phenolic (E)-stilbene 1 isolated from C. peregrina has been evaluated and demonstrated to be active against a range of Gram-positive bacteria, including drug resistant S. aureus and enterococci spp., but not Gram-negative bacteria. Importantly, 1 was active against M. bovis (BCG) and against B. anthracis, as well as three related Bacillus species. When the phenolic stilbene scaffold was modified with other aryl moieties or extended by one heteroatom to create the novel phenoxystyrene and phenothiostyrene derivatives, these agents also demonstrated promising antimicrobial activity, given that at least one phenolic moiety was present, as in compounds 8–16 and 26. Consequently, further exploration of these novel scaffolds is warranted from both synthetic and microbiological perspectives. Additional SAR studies of these novel homologous stilbene compounds and more extensive microbiological characterization of the active agents are currently underway.

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