

# A bisphenolic honokiol analog outcompetes oral antimicrobial agent cetylpyridinium chloride via a membrane-associated mechanism

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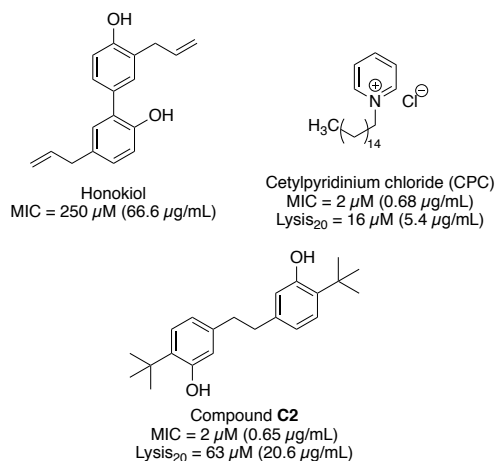
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Targeting *Streptococcus mutans* is the primary focus in reducing dental caries, one of the most common maladies in the world. Previously, our groups discovered a potent bactericidal biaryl compound that was inspired by the natural product honokiol. Herein, a structure activity relationship (SAR) study to ascertain structural motifs key to inhibition is outlined. Furthermore, mechanism studies show that bacterial membrane disruption is central to the bacterial growth inhibition. During this process it was discovered that analog **C2** demonstrated a 4-fold better therapeutic index compared to the commercially available antimicrobial cetylpyridinium chloride (CPC) making it a viable alternative for oral care.

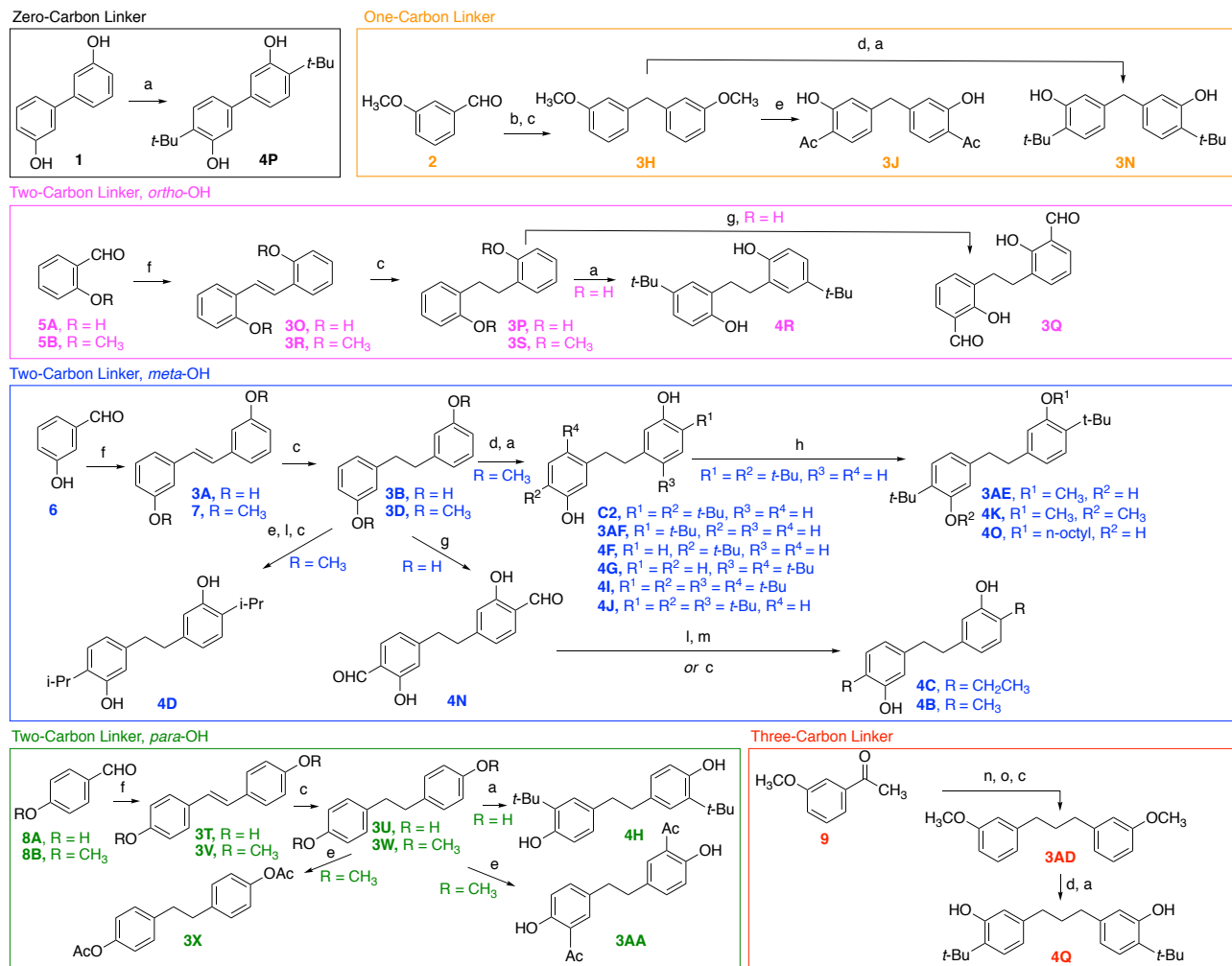
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*S. gordonii*, *S. sanguini*

The microbiota in the human oral cavity consists of many bacterial species acting as a defense against external pathogens.<sup>1</sup> The majority of these microorganisms exist in symbiosis with the host, but environmental changes can cause the equilibrium to shift and oral infections can form.<sup>2</sup> *Streptococcus mutans* naturally resides in the human oral cavity and is a common etiologic agent of dental caries,<sup>3,4</sup> which is one of the most costly diseases in the world and is implicated in other illnesses, such as diabetes and endocarditis.<sup>5,6</sup> As a result, research has focused on understanding this disease state by studying model organisms for oral pathogenesis.<sup>7</sup> Commensal streptococcus species, such as *S. gordonii* and *S. sanguinis*, initiate biofilm formation by binding to a thin film of glycoproteins, mucins, acidic proline-rich proteins, and bacterial cell debris on the enamel surface of a tooth, leading to the formation of dental plaque.<sup>8,9</sup> The acidogenic bacteria *S. mutans* and *Streptococcus sobrinus* then bind to the early colonizers via cell-to-cell interactions and form biofilms by producing extracellular polymeric substance matrices.<sup>10,11</sup> If not removed, these biofilms can produce sufficient lactic acid to erode dental enamel, which will eventually lead to dental caries after 6-24 months.<sup>12,13</sup> Many researchers have focused on the development of compounds that target *S. mutans* as potential therapeutics.<sup>14,15</sup> For example, the naturally occurring product

honokiol was isolated from *Magnolia officinalis* and reported to have potent activity, with a minimum inhibitory concentration (MIC) of 10  $\mu\text{g/mL}$  against *S. mutans*.<sup>16</sup> However, this activity was measured in an aerobic environment, which is not representative of the oral cavity.<sup>17</sup> We became interested in the honokiol scaffold after designing cross coupling methodology that enabled efficient synthesis of honokiol and analogs thereof.<sup>18</sup>



**Figure 1. Structures of relevant compounds active against *S. mutans*.**



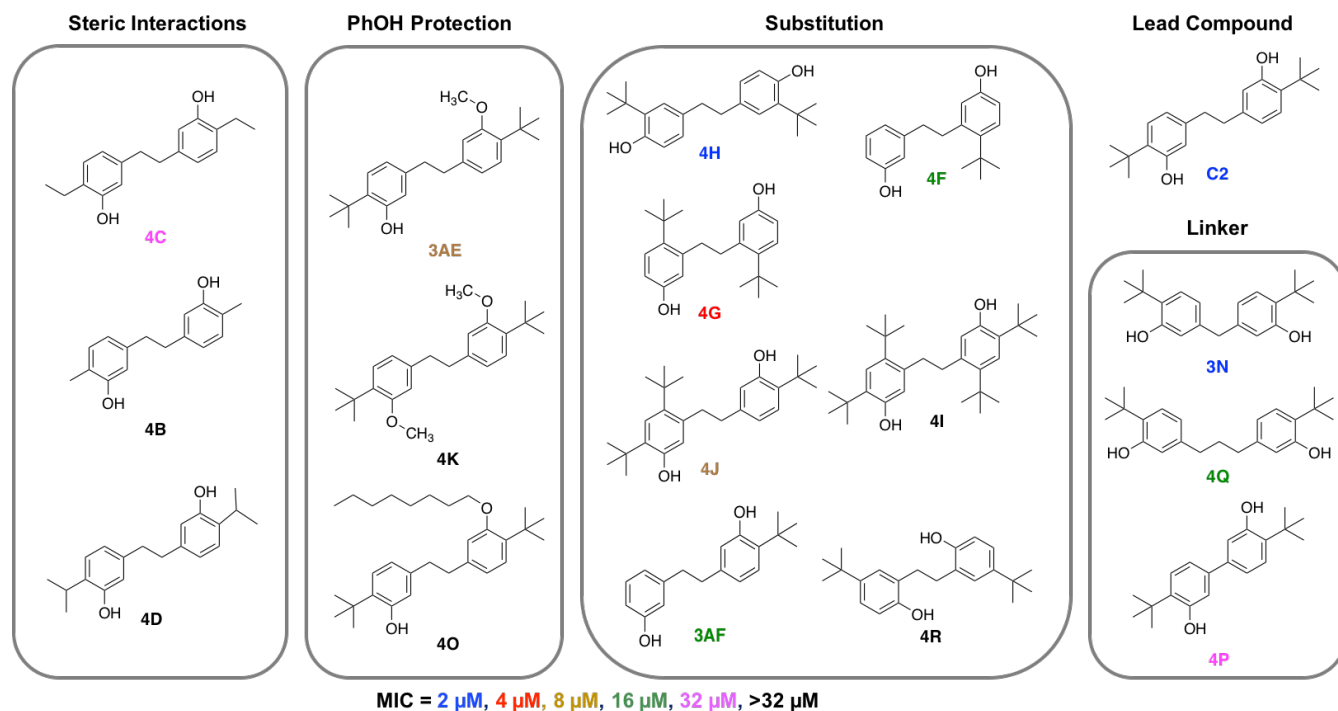
Scheme 1. Synthesis of honokiol analogs. (a) ROH, H<sub>2</sub>SO<sub>4</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 6-53%; (b) 0.5 M 3-MeOC<sub>6</sub>H<sub>4</sub>MgBr solution in THF, rt, 3 h, 97%; (c) Pd/C, EtOH, 26-98%; (d) BBr<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 90-98%; (e) CH<sub>3</sub>COCl, TiCl<sub>4</sub>, tol, 110 °C, 24-48%; (f) TiCl<sub>4</sub>, Zn, THF, 80 °C, 6 h, 21-56%; (g) CH<sub>2</sub>O, MgCl<sub>2</sub>, NEt<sub>3</sub>, THF, 80 °C, 16 h, 7-45%; (h) R-Br, K<sub>2</sub>CO<sub>3</sub>, acetone, 60 °C, 18 h, 17-49%; (i) CH<sub>3</sub>MgBr, THF, rt, 3 h, 56-70%; (m) BH<sub>3</sub>·SMe<sub>2</sub>, C<sub>6</sub>H<sub>5</sub>Cl, 130°C, 74%; (n) 2, 3-anisaldehyde, BF<sub>3</sub>·OEt<sub>2</sub>, 4 h, rt, 18%; and (o) NaBH<sub>4</sub>, CHCl<sub>3</sub>, MeOH, 2 h, rt, 8-18%.

We observed that the MIC of honokiol was 250  $\mu$ M (66.6  $\mu$ g/mL) when grown in biologically relevant conditions (5% CO<sub>2</sub>-supplemented).<sup>19</sup> From our analog library, 5,5'-(ethane-1,2-diyl)bis(2-(tert-butyl)phenol) (**C2**) was highly potent against *S. mutans* planktonic cells with an MIC of 2  $\mu$ M (0.65  $\mu$ g/mL; Figure 1). This new scaffold greatly improved upon the natural product, but it was unclear what structural features were causing this potent activity and what biological mechanism led to the potent effect. For this reason, 66 new analogs of **C2** were designed to assess the structure-activity relationships (SAR) of the lead compound. Moreover, this study aimed to gauge the potency and toxicity profiles of these biaryl analogs relative to commercial antimicrobials. Cetylpyridinium chloride (CPC) is a notable compound used in toothpastes, mouthwashes, throat sprays, and breath sprays, because CPC has been found to reduce the amount of plaque due to its high affinity for Gram-positive bacterial cell membranes, such as those of *S. mutans*.<sup>20-23</sup> However, CPC has also been shown to have toxic effects at higher concentrations.<sup>24</sup> Disclosed herein is the SAR report of analog **C2**, the identification of a mechanism of action associated

with the bacterial membrane, a similar inhibition profile of **C2** against *S. mutans* relative to CPC, and better performance of **C2** relative to CPC in a preliminary toxicity test.

Synthesis of analogs to probe the role of linker length, the phenol groups, ring substitution, and sterics were completed according to the methods shown in **Scheme 1**. Analog **4P** with a zero-carbon linker was generated from *meta*-biphenol **1** via Friedel-Crafts *ortho*-alkylation. The one-carbon linker skeleton was generated by Grignard addition and hydrogenation to **2**. Subsequent demethylation of **3H** followed by Friedel-Crafts *ortho*-acylation or *ortho*-alkylation provided **3J** and **3N**, respectively.

The two-carbon linker was explored with different positioning of the phenolic groups (*ortho*, *meta*, and *para*



**Figure 2.** Chemical structures of the synthesized analogs with inhibitory activity against *S. mutans*. Highlighted in this figure are key characteristics of the compounds that affect biological activity. The size of the groups on the ring, the length of the linker between rings, the positioning of *tert*-butyl groups on the ring, and the protection of the phenols all affect efficacy. All compounds with MIC < 250  $\mu$ M were  $\geq$ 90 pure as judged by UPLC.

relative the linker site). In all cases, the core was efficiently generated by McMurry coupling of the corresponding aldehyde (**5**, **6**, **8**) to generate alkenes **3O**, **3R**, **3A**, **7**, **3T**, and **3V**, which were hydrogenated using Pd/C to afford the corresponding alkanes **3P**, **3S**, **3B**, **3D**, **3U**, **3W**. Further combinations of phenol deprotection, Friedel-Crafts *ortho*-acylation, Friedel-Crafts *ortho*-formylation,<sup>25</sup> Friedel-Crafts *ortho*- and *para*-alkylation, *O*-alkylation, and *O*-acylation provided the remainder of the two-carbon linker derivatives.

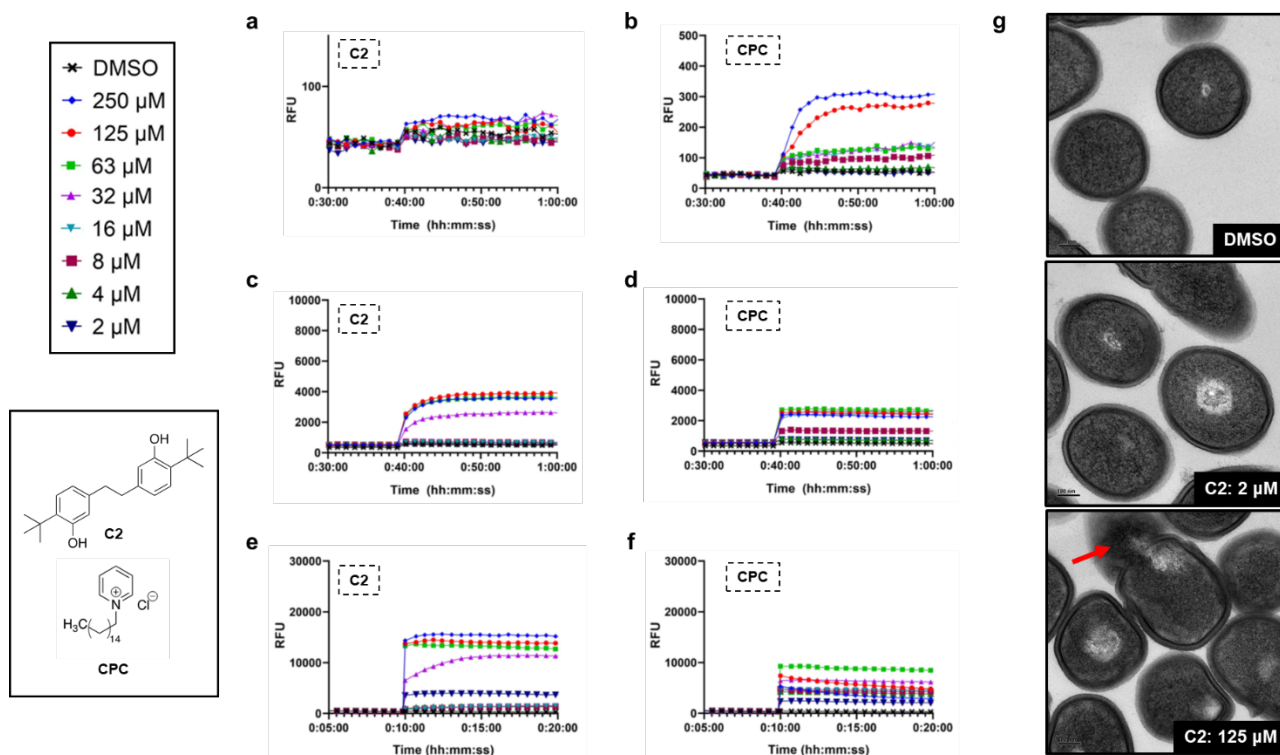
The three-carbon linker compound was obtained by aldol reaction of **9** with **2** followed by exhaustive reduction to obtain **3AD**. Phenol deprotection followed by Friedel-Crafts *ortho*-alkylation generated **4Q**.

Together, these methods permitted synthesis of 44 different compounds with varying linker sizes, electronics, steric interactions, and phenol substitution (see SI for structure list). Synthesized structures were supplemented with commercially available bibenzyl compounds (**3AG**–**3AR**); notably, none of the commercial analogs exhibited potency.

The synthesized analogs were first tested against *S. mutans* planktonic growth (Figure S1–S3). From this data, the SAR observations can be divided into key characteristic groups: steric interactions at the *ortho* position, phenol protection, positioning of aryl substituents, and linker length (Figure 2). Importantly, the corresponding monomer of **C2** (**4M**, see SI for structure) did not exhibit bioactivity, illustrating the importance of the biaryl motif for the observed inhibition. Analogs **3A** – **3M** and **3O** – **3AD**, none of which possessed alkyl groups on the aromatic rings, were inactive

proving the importance of the *tert*-butyl groups in compound **C2**. According to these results, there is a “goldilocks” response in terms of the steric functionality. Derivatives that contained smaller substituents *ortho* to the phenol (**4B**, **4C**, **4D**) also led to a decrease in efficacy, establishing that one *tert*-butyl substituent on each aromatic ring is optimal. (Figure 2).

In previous work, the bis-hydroxyl functionality of **C2** was found to be a significant feature of the active structure. Here, we discover that potency can be maintained if one hydroxyl group is alkylated with a small group such as a methyl (**3AE**). If both hydroxyls are alkylated, as is the case with **4K**,



**Figure 3.** Comparison of the mechanism of action of **C2** and CPC. a-b) Cellular membrane depolarization measured DiBAC<sub>4</sub>(3) recorded for 1 hour. c-d) Cellular lysis measured with PI recorded for 1 hour. e-f) Confirmation of cellular membrane permeability measured with SYTOX<sup>™</sup> Green for 20 minutes. g) TEM images of DMSO vehicle control and **C2** (2 and 125 μM) treated *S. mutans* cells.

there is a more drastic drop in activity, leading to the conclusion that the hydroxyl groups are creating important binding contacts, most likely through hydrogen bonds. Larger alkylating groups, as found in **4O**, also cause large decreases in potency, possibly due to a lack of hydrogen donors as well as changes in overall solubility.

Altering the substituents on the aryl rings can alter the projection of the key functional groups, and these changes can result in drastic changes in inhibition. Analogs **4H** and **4G** show that slight modification in the substitution pattern allows potency to be retained. However, larger changes, such as in **4R**, cause slight decreased inhibition due to hydroxyl proximity to the linker. We postulate that **4R** is not able to bind as well as **C2** to the target due to misalignment of the hydrogen bonding. Additionally, the optimal number of *tert*-butyl groups was found to be two (**C2**, **4G**), as analogs with additional (**4J**, **4I**) and fewer (**3AF**, **4F**) *tert*-butyl substituents have lower bioactivity. Finally, the testing of analogs with differing linker lengths (**4Q**, **4P**) showed that the ideal length is one (**3N**) or two carbons (**C2**).

We hypothesized that these antimicrobials were working via a membrane-specific mechanism due to similar SAR trends seen in our group's (W.M.W.) previous work.<sup>26,27</sup> Further, attempts at selecting for a resistant mutant of *S. mutans*, after treatment of **C2**, were unsuccessful. Todd Hewitt Broth (THB) agar plates with a range of concentrations of **C2** (MIC, 2X MIC, 4X MIC and 8X MIC) were plated with *S. mutans* cells. Three colonies were isolated and tested, but resistance to the activity of **C2** was not observed. Failure to select for resistant colonies is a hallmark

of membrane-targeting mechanisms.<sup>28</sup> For that reason, the effect of our compounds on bacterial membrane integrity was investigated. Depolarization (DiBAC<sub>4</sub>(3)) and cell lysis (Propidium Iodide, PI) of the bacterial cell membrane were analyzed between 2-250 μM (Figure 3a-d, Figure S4-S12). Analog **C2** (Figure 3a) did not have a significant depolarization response when compared to the DMSO vehicle control (Figure S12) and the commercial oral antimicrobial, CPC (Figure 3b). CPC, known to act via a lytic mechanism, showed higher levels of membrane depolarization, causing a response as low as 8 μM (Figure 3b). Nevertheless, both **C2** and CPC demonstrated a lytic response, denoted by an increase in PI fluorescence (Figure 3c and 3d). To visualize the membrane effects of **C2**, TEM images were obtained of compound treated cells, and lysis was observed at high concentrations (Figure 3g, red arrow).

| Compound | <i>S. mutans</i> | <i>S. gordonii</i> | <i>S. sanguinis</i> | <i>S. sobrinus</i> | <i>S. Mutans</i><br>MBC | Lysis <sub>20</sub> | Therapeutic<br>Index (TI) |
|----------|------------------|--------------------|---------------------|--------------------|-------------------------|---------------------|---------------------------|
| 3N       | 2 $\mu$ M        | 2 $\mu$ M          | 2 $\mu$ M           | 4 $\mu$ M          | 2 $\mu$ M               | 63 $\mu$ M          | 32                        |
| 4B       | >250 $\mu$ M     | 250 $\mu$ M        | 250 $\mu$ M         | >250 $\mu$ M       | -                       | -                   | -                         |
| 4G       | 4 $\mu$ M        | 1 $\mu$ M          | 1 $\mu$ M           | 2 $\mu$ M          | 4 $\mu$ M               | 32 $\mu$ M          | 8                         |
| 4H       | 2 $\mu$ M        | 4 $\mu$ M          | 8 $\mu$ M           | 8 $\mu$ M          | 2 $\mu$ M               | 63 $\mu$ M          | 32                        |
| 4I       | >250 $\mu$ M     | >250 $\mu$ M       | 63 $\mu$ M          | 250 $\mu$ M        | -                       | -                   | -                         |
| 4K       | >250 $\mu$ M     | >250 $\mu$ M       | >250 $\mu$ M        | >250 $\mu$ M       | -                       | -                   | -                         |
| 4P       | 32 $\mu$ M       | 32 $\mu$ M         | 32 $\mu$ M          | 32 $\mu$ M         | -                       | -                   | -                         |
| 4R       | 8 $\mu$ M        | 8 $\mu$ M          | 8 $\mu$ M           | 8 $\mu$ M          | 8 $\mu$ M               | -                   | -                         |
| CPC      | 2 $\mu$ M        | 1 $\mu$ M          | 0.5 $\mu$ M         | 0.5 $\mu$ M        | 8 $\mu$ M               | 16 $\mu$ M          | 8                         |
| C2       | 2 $\mu$ M        | 1 $\mu$ M          | 1 $\mu$ M           | 1 $\mu$ M          | 4 $\mu$ M               | 63 $\mu$ M          | 32                        |
| Honokiol | 250 $\mu$ M      | -                  | -                   | -                  | -                       | >500 $\mu$ M        | N/A                       |

**Table 1.** Biological activity of honokiol analogs against oral microbiome bacteria. MIC for *S. mutans*, *S. gordonii*, *S. sanguinis* and *S. sobrinus* are reported in  $\mu$ M. MBCs were executed with *S. mutans* and reported based off CFU/mL counts of bacterial cell viability. Hemolysis values were calculated with defibrinated sheep blood and reported as Lysis<sub>20</sub> values which refer to 20% cell lysis compared to the Triton X control.

To further understand the differences between C2 and CPC, we utilized SYTOX™ Green Nucleic Acid Stain to measure membrane permeabilization. The bacterial membrane was permeabilized by C2 from 32  $\mu$ M to 250  $\mu$ M (Figure 3e). C2 has a more striking effect, responding with a higher maximum fluorescence, when compared to CPC (Figure 3e, f). With these data points in consideration, it appears that C2 affects the membrane stability and fluidity, which is evidenced in both the lytic (Figure 3c) and permeabilization activity (Figure 3e). The lack of depolarization events leads to the conclusion that C2 does not interfere with the membrane potential. Thus, the inhibitory activity must stem from a more physical mode of action, akin to the mechanism demonstrated by bithionol and nTZDpa.<sup>29</sup> Other active analogs were tested and demonstrate similar membrane effects (See SI).

We calculated minimum bactericidal concentration (MBC) values to determine if the analog promoted cell permeabilization led to bacteriostatic or bactericidal cell inhibition to better understand the overall mechanism. Colony Forming Units (CFU) were used to determine viability with the MBC referring to a 3-Log reduction in growth, corresponding to 99.9% bacterial death (Table 1). In accordance with our previous work, the active compounds demonstrated bactericidal mechanisms, since the MBCs were within 4X of the MIC.

Due to the bactericidal effect, the toxicity of C2 relative to CPC was explored. Hemolysis values were determined using defibrinated sheep blood (Table 1). Interestingly, CPC exhibits significant hemolytic activity with a Lysis<sub>20</sub> of 16  $\mu$ M. Based on these results, CPC has a therapeutic index (TI) of 8, which is expressed as the ratio Lysis<sub>20</sub>/MIC. Conversely, equally potent analog C2 has a Lysis<sub>20</sub> of 63  $\mu$ M, yielding a higher TI of 32 again hinting at a mechanism of action that is disparate from that of CPC. This finding establishes the potential of compound C2 for use in dental health agents based on its superior TI.

With the perspective of being used as a dental agent, we were curious if these analogs would also inhibit commensal bacteria in the oral microbiome. The inhibition values

reported above were all determined with *S. mutans*, one of the major agents in dental caries, but only one bacteria implicated in cavity formation. In an attempt to understand the broader impact of these compounds on the oral microbiome, the most potent analogs were tested against commensal bacteria *S. gordonii* and *S. sanguinis*, and the pathogenic species *S. sobrinus*. The analogs demonstrated comparable activity against these strains (Table 1).

Through this SAR study, the critical structural attributes of *S. mutans* inhibitor C2 have been delineated and a potential mechanism of action has been outlined. We have shown that C2 causes inhibition at high concentrations by disrupting membrane permeability of *S. mutans*. Through MBC calculations, it was determined that C2 is acting in a bactericidal fashion. While membrane effects are prevalent, other possible targets contributing to polypharmacology cannot be ruled out; future work will focus on confirming these findings.

In summary, the results presented in this work establish the potential of this new class of synthetically accessible bisphenolic compounds for antibacterial oral care. C2 possesses favorable properties for further development and an improved therapeutic index when compared to the ubiquitous CPC. Future work will investigate its translational potential as a next generation oral care product and will be reported in due course.

## ASSOCIATED CONTENT

### Supporting

### Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

Synthetic protocols, characterization, and biological assay protocols (PDF)

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### Author Contributions

C.O. and A.E.S. contributed equally. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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The authors declare the following competing financial interest(s): M.C.K. and W.M.W. have filed a patent on the technology disclosed.



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## REFERENCES

- (1) Forssten, S. D.; Björklund, M.; Ouwehand, A. C. Streptococcus Mutans, Caries and Simulation Models. *Nutrients*. **2010**, *2* (3), 290-298 DOI 10.3390/nu2030290.
- (2) Sakamoto, M.; Umeda, M.; Benno, Y. Molecular Analysis of Human Oral Microbiota. *Journal of Periodontal Research*. **2005**, *40* (3), 277-285 DOI 10.1111/j.1600-0765.2005.00793.x.
- (3) Lamont, R. J.; Eglund, P. G. Dental Caries. In *Molecular Medical Microbiology: Second Edition*; **2014**, 945-955 DOI 10.1016/B978-0-12-397169-2.00052-4.
- (4) Simón-Soro, A.; Mira, A. Solving the Etiology of Dental Caries. *Trends in Microbiology*. **2015**, *23* (2), 76-82 DOI 10.1016/j.tim.2014.10.010.
- (5) Marsh, P. D. Are Dental Diseases Examples of Ecological Catastrophes? *Microbiology*. **2003**, 279-294 DOI 10.1099/mic.0.26082-0.
- (6) Dewhirst, F. E.; Chen, T.; Izard, J.; Paster, B. J.; Tanner, A. C. R.; Yu, W.-H.; Lakshmanan, A.; Wade, W. G. The Human Oral Microbiome. *J. Bacteriol*. **2010**, *192* (19), 5002-5017 DOI 10.1128/JB.00542-10.
- (7) Kilian, M.; Chapple, I. L. C.; Hannig, M.; Marsh, P. D.; Meuric, V.; Pedersen, A. M. L.; Tonetti, M. S.; Wade, W. G.; Zaura, E. The Oral Microbiome - An Update for Oral Healthcare Professionals. *Br. Dent. J.* **2016**, *221* (10), 657-666 DOI 10.1038/sj.bdj.2016.865.
- (8) Davey, M. E.; O'toole, G. A. Microbial Biofilms: From Ecology to Molecular Genetics. *Microbiol. Mol. Biol. Rev.* **2000**, *64* (4), 847-867 DOI 10.1128/mmbr.64.4.847-867.2000.
- (9) Ahn, S. J.; Ahn, S. J.; Wen, Z. T.; Brady, J.; Burne, R. A. Characteristics of Biofilm Formation by Streptococcus Mutans in the Presence of Saliva. *Infect. Immun.* **2008**, *76* (9), 4259-4268 DOI 10.1128/IAI.00422-08.
- (10) de Soet, J. J.; Holbrook, W. P.; van Amerongen, W. E.; Schipper, E.; Homburg, C. H.; de Graaff, J. Prevalence of Streptococcus Sobrinus in Relation to Dental Caries in Children from Iceland and The Netherlands. *ASDCJ. Dent. Child.* **1990**, *57* (5), 337-342 DOI 10.3109/08910609309141332.
- (11) Tanzer, J. M.; Livingston, J.; Thompson, A. M. The Microbiology of Primary Dental Caries in Humans. *J. Dent. Educ.* **2001**, *65* (10), 1028-1037.
- (12) Balakrishnan, M.; Simmonds, R. S.; Tagg, J. R. Dental Caries Is a Preventable Infectious Disease. *Australian Dental Journal*. **2000**, *45* (4), 235-45 DOI 10.1111/j.1834-7819.2000.tb00257.x.
- (13) Koo, H.; Xiao, J.; Klein, M. I.; Jeon, J. G. Exopolysaccharides Produced by Streptococcus Mutans Glucosyltransferases Modulate the Establishment of Microcolonies within Multispecies Biofilms. *J. Bacteriol*. **2010**, *192* (12), 3024-3032 DOI 10.1128/JB.01649-09.
- (14) Solinski, A. E.; Koval, A. B.; Brzozowski, R. S.; Morrison, K. R.; Fraboni, A. J.; Carson, C. E.; Eshraghi, A. R.; Zhou, G.; Quivey, R. G.; Voelz, V. A.; et al. Diverted Total Synthesis of Carolacton-Inspired Analogs Yields Three Distinct Phenotypes in Streptococcus Mutans Biofilms. *J. Am. Chem. Soc.* **2017**, *139* (21), 7188-7191 DOI 10.1021/jacs.7b03879.
- (15) Yang, H.; Abouelhassan, Y.; Burch, G. M.; Kallifidas, D.; Huang, G.; Yousaf, H.; Jin, S.; Luesch, H.; Huigens, R. W. A Highly Potent Class of Halogenated Phenazine Antibacterial and Biofilm-Eradicating Agents Accessed Through a Modular Wohl-Aue Synthesis. *Sci. Rep.* **2017**, *7* (1), 2003 DOI 10.1038/s41598-017-01045-3.
- (16) Wang, X.; Wang, Y.; Geng, Y.; Li, F.; Zheng, C. Isolation and Purification of Honokiol and Magnolol from Cortex Magnoliae Officinalis by High-Speed Counter-Current Chromatography. *J. Chromatogr. A* **2004**, *1036* (2), 171-175 DOI 10.1016/j.chroma.2004.02.073.
- (17) Sakaue, Y.; Domon, H.; Oda, M.; Takenaka, S.; Kubo, M.; Fukuyama, Y.; Okiji, T.; Terao, Y. Anti-Biofilm and Bactericidal Effects of Magnolia Bark-Derived Magnolol and Honokiol on Streptococcus Mutans. *Microbiol. Immunol.* **2016**, *60* (1), 10-16 DOI 10.1111/1348-0421.12343.
- (18) Lee, Y. E.; Cao, T.; Torruellas, C.; Kozlowski, M. C. Selective Oxidative Homo- and Cross-Coupling of Phenols with Aerobic Catalysts. *J. Am. Chem. Soc.* **2014**, *136* (19), 6782-6785 DOI 10.1021/ja500183z.
- (19) Solinski, A. E.; Ochoa, C.; Lee, Y. E.; Paniak, T.; Kozlowski, M. C.; Wuest, W. M. Honokiol-Inspired Analogs as Inhibitors of Oral Bacteria. *ACS Infect. Dis.* **2018**, *4* (2), 118-122 DOI 10.1021/acsinfectdis.7b00178.
- (20) Albert-Kiszely, A.; Pjetursson, B. E.; Salvi, G. E.; Witt, J.; Hamilton, A.; Persson, G. R.; Lang, N. P. Comparison of the Effects of Cetylpyridinium Chloride with an Essential Oil Mouth Rinse on Dental Plaque and Gingivitis - a Six-Month Randomized Controlled Clinical Trial. *J. Clin. Periodontol.* **2007**, *34* (8), 658-667 DOI 10.1111/j.1600-051X.2007.01103.x.
- (21) Ayad, F.; Prado, R.; Mateo, L. R.; Stewart, B.; Szweczyk, G.; Arvanitidou, E.; Panagakos, F. S. A Comparative Investigation to Evaluate the Clinical Efficacy of an Alcohol-Free CPC-Containing Mouthwash as Compared to a Control Mouthwash in Controlling Dental Plaque and Gingivitis: A Six-Month Clinical Study on Adults in San Jose, Costa Rica. *J. Clin. Dent.* **2011**, *22* (6), 204-212.
- (22) Stookey, G. K.; Beiswanger, B.; Mau, M.; Isaacs, R. L.; Witt, J. J.; Gibb, R. A 6-Month Clinical Study Assessing the Safety and Efficacy of Two Cetylpyridinium Chloride Mouthrinses. *Am. J. Dent.* **2005**, *24A*-28A.
- (23) Mankodi, S.; Bauroth, K.; Witt, J. J.; Bsoul, S.; He, T.; Gibb, R.; Dunavent, J.; Hamilton, A. A 6-Month Clinical Trial to Study the Effects of a Cetylpyridinium Chloride Mouthrinse on Gingivitis and Plaque. *Am. J. Dent.* **2005**, *9A*-14A.
- (24) Lin, G. H. Y.; Voss, K. A.; Davidson, T. J. Acute Inhalation Toxicity of Cetylpyridinium Chloride. *Food Chem. Toxicol.* **1991**, *29* (12), 851-854 DOI 10.1016/0278-6915(91)90113-L.
- (25) Hofsløkken, N. U.; Skattebøl, L. Convenient Method for the Ortho-Formylation of Phenols. *Acta Chem. Scand.* **1999**, *53* (4), 258-262 DOI 10.3891/acta.chem.scand.53-0258.
- (26) Kim, W.; Zhu, W.; Hendricks, G. L.; Van Tyne, D.; Steele, A. D.; Keohane, C. E.; Fricke, N.; Conery, A. L.; Shen, S.; Pan, W.; et al. A New Class of Synthetic Retinoid Antibiotics Effective against Bacterial Persisters. *Nature* **2018**, *556*, 103-107 DOI 10.1038/nature26157.
- (27) Kim, W.; Steele, A. D.; Zhu, W.; Csatory, E. E.; Fricke, N.; Dekarske, M. M.; Jayamani, E.; Pan, W.; Kwon, B.; Sinita, I. F.; et al. Discovery and Optimization of NTZDpa as an Antibiotic Effective Against Bacterial Persisters. *ACS Infect. Dis.* **2018**, *4* (11), 1540-1545 DOI 10.1021/acsinfectdis.8b00161.
- (28) Hurdle, J. G.; O'Neill, A. J.; Chopra, I.; Lee, R. E. Targeting Bacterial Membrane Function: An Underexploited Mechanism for Treating Persistent Infections. *Nature Reviews Microbiology*. **2011**, *9* (1), 62-75 DOI 10.1038/nrmicro2474.
- (29) Kim, W.; Zou, G.; Hari, T. P. A.; Wilt, I. K.; Zhu, W.; Galle, N.; Faizi, H. A.; Hendricks, G. L.; Tori, K.; Pan, W.; et al. Selective Membrane-Targeting Repurposed Antibiotic with Activity against Persistent Methicillin-Resistant *Staphylococcus aureus*. *PNAS*, **2019**, *116* (33), 16529-16534 DOI 10.1073/pnas.1904700116.

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