Second Generation of Zafirlukast Derivatives with Improved Activity against the Oral Pathogen *Porphyromonas gingivalis*

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ABSTRACT: *Porphyromonas gingivalis* is a Gram-negative anaerobic pathogen that can trigger oral dysbiosis as an early event in the pathogenesis of periodontal disease. The FDA-approved drug zafirlukast (ZAF) was recently shown to display antibacterial activity against *P. gingivalis*. Here, 15 novel ZAF derivatives were synthesized and evaluated for their antibacterial activity against *P. gingivalis* and for their cytotoxic effects. Most derivatives displayed superior antibacterial activity against *P. gingivalis* compared to ZAF and its first generation derivatives along with little to no growth inhibition of other oral bacterial species. The most active compounds displayed bactericidal activity against *P. gingivalis* and less cytotoxicity than ZAF. The superior and selective antibacterial activity of ZAF derivatives against *P. gingivalis* along with an increased safety profile compared to ZAF suggest these new compounds, especially **14b** and **14e**, show promise as antibacterials for future studies aimed to test their potential for preventing/treating *P. gingivalis*-induced periodontal disease.

KEYWORDS: Oral microbiome species, dental plaque, oral dysbiosis, periodontal disease

Periodontal diseases are bacterial infections characterized by unresolved inflammation that leads to the destruction of the gum and bone that support the teeth.¹ In the United States, >47% of adults 30 years and older suffer from periodontal diseases.² An early event in the pathogenesis of periodontal diseases is oral dysbiosis, a phenomenon that describes changes in the abundance of specific bacterial species that are normally found in oral health. Among the triggers of oral dysbiosis, “key stone” pathogens such as *Porphyromonas gingivalis* have been identified. In addition to periodontal disease, *P. gingivalis* has been correlated to diseases such as cardiovascular diseases, diabetes, rheumatoid arthritis, and Alzheimer’s disease.^{3–6} Therefore, *P. gingivalis* is a plausible bacterial target for preventing/treating periodontal diseases with antibacterials. The lack of clear guidelines for selecting the antibiotic regimen for treating generalized aggressive forms of periodontal diseases has led to poor efficacy and development of drug-resistant bacteria.^{7–9} One of the current treatment options for chronic periodontitis includes scaling and root planning with antibiotic adjunctive therapy using doxycycline or a combination of amoxicillin and metronidazole.^{10–13} Although there are antibiotics currently used to treat chronic periodontitis, it is not feasible to use these antibiotics for a prolonged period of time.^{14–16} Therefore, there is a growing need for novel and specific antibacterials active against periodontopathogens such as *P. gingivalis* to prevent/treat chronic periodontitis.

An appealing option to uncover new antibacterials with a fast and affordable strategy is the repositioning of FDA-approved drugs for new applications to treat periodontitis.^{17,18} Drug repositioning is simply using existing drugs approved for the treatment of one disease to treat a different disease. Screening of a drug repositioning library has recently led to the discovery that the FDA-approved drug zafirlukast (ZAF) displayed specific antibacterial activity against *P. gingivalis* (Figure 1).^{8,19,20} This is significant given the need for developing new antimicrobials with specificity for oral pathogens associated with periodontal diseases without compromising the normal “good” oral bacteria associated with periodontal health. Additionally, ZAF has been shown to be a potential treatment option for *Mycobacterium tuberculosis* and West Nile virus infections.^{21,22} Inspired by these findings, we have been developing ZAF derivatives as novel drug candidates for targeting *P. gingivalis*. We previously identified three ZAF derivatives with promising anti-*P. gingivalis* activity.⁸ Herein, these first generation ZAF derivatives were used as starting points for further structure optimization on the ZAF

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Figure 1. Chemical structure of ZAF and overview of 15 second generation derivatives synthesized herein.

acylsulfonamide ring (e.g., addition of fluoro and nitro groups along with replacement of phenyl by a naphthyl moiety) with the goal of increasing inhibition of *P. gingivalis* growth and reducing cytotoxicity. The short 3–4 linear step synthesis of 15 ZAF derivatives is reported. The antibacterial activity against *P. gingivalis* and other oral bacterial species as well as the cytotoxic activity against human oral epithelial cells of these compounds is also presented.

■ DESIGN AND SYNTHESIS OF SECOND GENERATION ZAF DERIVATIVES

In our first generation of ZAF derivatives,⁸ the primary focus was to optimize the positions of the substituents on the ZAF benzoyl ring. It was found that a compound with a 2-methoxy-5-indoyl organization displayed increased *P. gingivalis* growth inhibition compared to those with the 3-methoxy-4-indoyl from the parent ZAF (Figure 1), and this new substitution pattern was therefore kept constant on the benzoyl ring of the second generation of compounds presented herein. With the limited preliminary structure–activity relationship (SAR) that was performed in our previous study, it appeared that a methyl group on the indole nitrogen might be beneficial for *P. gingivalis* growth inhibition and that having a hydrogen versus a nitro group at the R_1 position on the indole ring might not have any effect on activity. To corroborate these very preliminary observations and to, more importantly, investigate the potential of having different acylsulfonamides, which was not investigated previously, we synthesized compounds **12a**, **12c**, **12f**, **13a–13f**, and **14a–14f**.

The preparation of compounds **12a**, **12c**, **12f**, **13a–13f**, and **14a–14f** was performed by a linear 3–4 step synthesis to modify the following parts of the ZAF scaffold: its indole (at the R_1 and R_2 positions), benzoyl (arrangement of the three different substituents “indole, methoxy, and acylsulfonamide”), and acylsulfonamide (R_3 position) rings (Scheme 1A). Briefly, the synthesis proceeded as follows. Commercially available 5-nitroindole underwent methylation to form compound **3** in 66% yield. The condensation reaction between the commercially available (indicated by a \$ sign in Scheme 1) indole (**1**) or 1-methylindole (**2**), or the synthesized 1-methyl-5-nitro-

indole (**3**) with methyl-5-formyl-2-methoxybenzoate (**4**) first generated compounds **5**, **6**, and **7** in 10–93% yields. This condensation proceeds via a unique mechanism, which involves resonance stabilized carbocation formation in the presence of trifluoroacetic acid (TFA), followed by C–C bond formation, reduction, protonation of hydroxyl group, and reduction with hydride from triethylsilane (Et_3SiH) to yield the methylene compounds **5**, **6**, and **7** (Scheme 1A and Figure S1). Hydrolysis of the ester group of **5**, **6**, and **7** to a carboxylic acid then afforded compounds **8**, **9**, and **10** in 85–96% yields. Finally, amide coupling of the carboxylic acids with various commercially available acylsulfonamides (**11a–11f**) yielded 15 novel ZAF derivatives **12a**, **12c**, **12f**, **13a–13f**, and **14a–14f** in 16%–quantitative yields. Two of the three first generation lead ZAF derivatives, **13g** and **14h** (previously published as **22b** and **23a**),⁸ were also resynthesized in 38% and 24% yields (Scheme 1B) for comparison study with the new second generation ZAF derivatives **12a**, **12c**, **12f**, **13a–13f**, and **14a–14f**. It is important to note that as **13g** and **14h** had been found to display superior growth inhibition of *P. gingivalis* compared to the parent ZAF from which they were derived, in this study, the second generation of compounds were mostly compared to **13g** and **14h**.

With these second generation ZAF derivatives aimed at increasing *P. gingivalis* growth inhibition and decreasing cytotoxicity, the goal was to answer the following four *a priori* questions in terms of SAR. The series/compound numbers used to answer these questions are provided in parentheses following the questions. The four questions are (i) is a methyl R_2 group on the indole nitrogen truly enhancing bacterial growth inhibition? (compounds **12a** vs **13a**, **12c** vs **13c**, and **12f** vs **13f**); (ii) what is the effect of adding an R_3 electron-withdrawing group or bulkiness to the acylsulfonamide ring when $R_1 = \text{H}$ and $R_2 = \text{Me}$? (compounds **13a** vs **13b** vs **13c** vs **13d** vs **13e** vs **13f**); (iii) does an electron-withdrawing group (e.g., $R_1 = \text{NO}_2$) on the indole ring affect the trend established for the acylsulfonamide substituent patterns (trend for compounds **14a** vs **14b** vs **14c** vs **14d** vs **14e** vs **14f** compared to that of series **13**?); and (iv) is an electron-withdrawing group (e.g., $R_1 = \text{NO}_2$) on the indole ring beneficial for bacterial growth inhibition? (pairwise comparison of series **13** vs **14**).

■ ANTIBACTERIAL ACTIVITY

To answer the four *a priori* questions posed and to determine the potential of the second generation ZAF derivatives **12a**, **12c**, **12f**, **13a–13f**, and **14a–14f** as antibacterials compared to the first generation and the parent ZAF, they were first tested against *P. gingivalis* 381. A colorimetric water-soluble tetrazolium-1 (WST-1) assay was used to determine the antimicrobial effect of the 15 synthesized ZAF derivatives at 1, 10, and 100 μM . Their activity was compared to commercially available positive controls tetracycline (T, at 2.81 μM , which displayed 51% *P. gingivalis* growth inhibition) and ZAF (Z, at 25 and 50 μM , which displayed 70% and 68% *P. gingivalis* growth inhibition, respectively) (Figure 2, different blue bars), as well as to the first generation ZAF derivatives **13g** and **14h** (at 1, 10, and 100 μM). It is to be noted that, as expected, the DMSO control did not display any bacterial growth inhibition. Additionally, it is important to note that the second generation ZAF derivatives were used as a preliminary screening of antibacterial activity in order to identify lead compounds for further structure optimization along with future studies outside

Scheme 1. Synthetic Scheme for the Preparation of (A) Second Generation ZAF Derivatives 12a, 12c, 12f, 13a–13f, and 14a–14f and (B) First Generation ZAF Derivatives 13g and 14h^{8a}A. 2nd generation ZAF derivatives B. 1st generation ZAF derivatives used for comparison purpose

^aReaction conditions: (a) Et_3SiH , TFA, CH_2Cl_2 , 0 °C to rt, 10–93%; (b) MeOH:THF:H₂O/5:1:1, KOH, 65 °C, 85–96%; (c) acylsulfonamide, EDC·HCl, DMAP, CH_2Cl_2 , rt, 16%–quantitative yields.

the scope of this preliminary investigation. The concentrations of the ZAF control, 25 and 50 μM , were based on a previous publication, which showed that these concentrations were the minimum concentration that inhibited the growth of *P. gingivalis*.¹⁹ In order to test the ability of the ZAF derivatives to inhibit *P. gingivalis* growth, a wide range of concentrations, 1–100 μM , was used to determine their level of potency.

At a quick glance, when compared to the first generation compounds 13g and 14h, it was found that all second generation compounds in series 14 were generally better at inhibiting the growth of *P. gingivalis* at 1 μM . Overall, at 100 μM , 10 out of the 15 compounds (13b–13d, 13f, and 14a–14f) displayed 58–75% growth inhibition comparable or superior to the parent drug ZAF with 68% growth inhibition at

50 μM . In addition to these compounds, one more compound (13a) also displayed superior activity with 55% inhibition of *P. gingivalis* growth when compared to the effect of tetracycline with 51% growth inhibition. At 10 μM , six out of the 10 compounds (13c and 14a–14e) still exhibited 59–67% growth inhibition similar to that of the parent ZAF with 68–70% growth inhibition. Additionally, three more compounds (13b, 13d, and 14f) displayed 57%, 50%, and 57% growth inhibition, which were comparable to or better than the antibiotic tetracycline with 51% growth inhibition. At 1 μM , four of these compounds (14a, 14b, 14d, and 14e) displayed 53–62% growth inhibition, which was comparable or superior to the parent compound ZAF (68% at 50 μM), its derivatives

Figure 2. Percent growth inhibition of *P. gingivalis* 381 by ZAF derivatives **12a**, **12c**, **12f**, **13a–13f**, and **14a–14f**, as determined by a colorimetric WST-1 assay after 24 h. Negative control = DMSO. Positive controls = 2.81 μ M of tetracycline (T) or 25 and 50 μ M of ZAF (Z), and first generation ZAF derivatives **13g** and **14h**. For each ZAF derivative, the data represent the average from six independent replicates per condition of the growth inhibitory effect versus bacterial cultures grown in medium only. For the controls, the data represent the average from 36 independent replicates. *The first generation ZAF derivatives **13g** and **14h** were previously published as **22b** and **23a**.⁸

13g and **14h** (16% and 0%, respectively, at 1 μ M), and tetracycline (51% at 2.81 μ M).

To establish the importance of an *N*-methyl R_2 group on bacterial growth inhibition (question (i)), three pairs of ZAF derivatives (**12a** and **13a**, **12c** and **13c**, as well as **12f** and **13f** with 2-F-Ph, 4-F-Ph, and 2-naphthylsulfonamides, respectively) were generated (Scheme 1) and tested for growth inhibition of *P. gingivalis* (Figure 2). As previously observed, it was found that the compounds lacking the methyl group on the indole nitrogen (series 12) were much less efficient at

inhibiting bacterial growth at all three concentrations tested when compared to those with a methyl at the R_2 position (series 13). Having confirmed the importance of the *N*-methyl R_2 group, series 12 was not further expanded/pursued.

To investigate the effect of adding an R_3 electron-withdrawing group or bulkiness to the acylsulfonamide ring when R_1 = H and R_2 = Me (question (ii)), three additional compounds were prepared in series 13: **13b** with R_3 = 3-F-Ph, **13d** with R_3 = 3-NO₂-Ph, and **13e** with R_3 = 4-NO₂-Ph. The series 13 derivatives (**13a–13f**) were rationally designed to investigate the effect of replacing the R_3 = 2-Me on the acylsulfonamide of first generation **13g** with a weak electron-withdrawing group, the bioisostere fluorine, at the *ortho* (**13a**), *meta* (**13b**), and *para* (**13c**) positions. It was postulated that due to the weak electron-withdrawing effect fluorine has on the phenyl ring, a preferred conformational change in **13a–13c** could lead to increased antibacterial activity compared to the counterpart first generation **13g**, which was indeed the case with compound **13c** (with R_3 = 4-F-Ph) at all concentrations tested. Compound **13b** (with R_3 = 3-F-Ph) was also found to display 14%, 57%, and 73% growth inhibition at 1, 10, and 100 μ M, respectively, which were similar or superior to that of **13g** (16%, 56%, and 74%) at all concentrations tested. As addition of a weak electron-withdrawing group at the *meta* and *para* positions of the acylsulfonamide proved to be beneficial for *P. gingivalis* growth inhibition, the effect of a strong electron-withdrawing group, nitro, at these positions was also investigated. It was found that the R_3 = 3-NO₂-Ph of **13d** yielded 18–75% growth inhibition, which was better than the R_3 = 4-NO₂-Ph of **13e** with 0–27% growth inhibition. Finally, the phenyl ring of the acylsulfonamide was replaced by its larger naphthyl bioisostere to yield compound **13f**, which displayed intermediate activity compared to the other series 13 compounds. Overall, at all concentrations tested, it was found that compound **13c** (R_3 = 4-F-Ph) displayed better *P. gingivalis* growth inhibition than (>) **13d** (R_3 = 3-NO₂-Ph), which was better than (>) **13b** (R_3 = 3-F-Ph) > **13f** (R_3 = 2-naphthyl) > **13a** (R_3 = 2-F-Ph) > **13e** (R_3 = 4-NO₂-Ph).

SUMMARY OF SAR ACTIVITY:

Figure 3. Summary of SAR activity for the second generation of ZAF derivatives for series 12, 13, and 14.

Having established the trend for the acylsulfonamide substituent patterns in series 13 in terms of bacterial growth inhibition activity (question ii), the effect of an additional electron-withdrawing group (e.g., $R_1 = \text{NO}_2$) on the indole ring on that trend was investigated by generating the counterpart series 14a–14f (question iii). When comparing the compounds of series 14, it was found that 14e ($R_3 = 4\text{-NO}_2\text{-Ph}$) displayed better *P. gingivalis* growth inhibition than (>) 14b ($R_3 = 3\text{-F-Ph}$), which was similar to ($\approx$) 14a ($R_3 = 2\text{-F-Ph}$), which was better than (>) 14d ($R_3 = 3\text{-NO}_2\text{-Ph}$) > 14c ($R_3 = 4\text{-F-Ph}$) > 14f ($R_3 = 2\text{-naphthyl}$). Interestingly, when compared to the trend observed with series 13, the trend with series 14 was completely opposite with the exception of compounds with $R_3 = 2\text{-naphthyl}$ (13f and 14f), which were randomly placed in these series in terms of their activity. A summary of the SAR activity discussed above is presented in Figure 3.

Finally, to establish if an electron-withdrawing group (e.g., $R_1 = \text{NO}_2$) on the indole ring is beneficial for bacterial growth inhibition (question iv), a pairwise comparison of series 13 vs 14 was done. It was found that at 1 and 10 μM , the presence of the nitro group on the indole ring was generally beneficial for *P. gingivalis* growth inhibition, with all compounds in series 14 (with the exception of 14c) displaying comparable or superior activity to those in series 13. At 100 μM , this was also mostly true, but compounds 13b, 13d, and 13f displayed a weak improvement in activity over that of their series 14 counterparts. These novel results indicate the importance of an electron-withdrawing group on the indole, while in the first generation, because of the very limited SAR that was done, this conclusion could not be made.

These results indicated that the six most active second generation ZAF derivatives against *P. gingivalis* were 13b, 13c, 13d, 13f, 14b, and 14e. These compounds were then further tested against other selected oral Gram-positive and Gram-negative bacterial species to determine their antibacterial specificity profile (Figure 4). The oral bacterial species chosen were representative of those more abundant in health (*Actinomyces naeslundii*, *Streptococcus sanguinis*, and *Veillonella parvula*) and in periodontal diseases (*Aggregatibacter actinomycetemcomitans*). Overall, the compounds displayed little to no antibacterial activity against these other oral bacterial species, with the exception of 20% growth inhibition with *S. sanguinis* and *A. naeslundii*. These data suggest that the six most active second generation ZAF derivatives seem to display specificity against *P. gingivalis*. Future studies using an expanded number of oral bacterial species or whole subgingival plaque samples from healthy and periodontitis patients will be useful to further validate the specificity of these new ZAF compounds, but not necessary at this time.

Further investigation into the activity of the six most active ZAF derivatives (13b, 13c, 13d, 13f, 14b, and 14e) was performed to determine if they displayed bactericidal activity by colony forming unit (CFU) assays (Figure 5). *P. gingivalis* 381 was incubated with the antibiotic tetracycline (T, 2.81 μM) and ZAF (Z, 25 and 50 μM) as positive controls and bacteria only with DMSO as a negative control. *P. gingivalis* was exposed to ZAF derivatives 13b, 13c, 13d, 13f, 14b, and 14e (1, 10, and 100 μM) for 24 h in media followed by bacterial growth in blood agar plates for 5 days under anaerobic conditions and CFUs determination. All six compounds exhibited bactericidal activity at 100 μM with 100% growth inhibition. Compounds 14b and 14e were also

Figure 4. Percent growth inhibition of oral bacterial species by ZAF derivatives 13b, 13c, 13d, 13f, 14b, and 14e, as determined by a colorimetric WST-1 assay after 24 h. Negative control = DMSO. Positive controls = 25 and 50 μM of ZAF (Z) or penicillin/streptomycin –1X (P/S) (100 U/mL of penicillin and 100 $\mu\text{g/mL}$ of streptomycin). These data show the average from six independent replicates per condition of the growth inhibitory effect versus bacterial cultures grown in medium only.

bactericidal at 10 μM and 1 μM ; however, at 10 μM , compounds 13b, 13c, 13d, and 13f were not. Even at 1 μM , compound 13d revealed moderate antibacterial activity with 30% growth inhibition. At all three concentrations tested, the second generation compounds 14b and 14e proved to display a higher percent growth inhibition by CFU assays than their first generation counterpart 14h. At 100 μM , compounds 13b, 13c, 13d, and 13f displayed higher percent growth inhibition by CFU assays than their first generation counterpart 13g, and most of them showed better antimicrobial activity than 13g at 10 μM .

Figure 5. Bactericidal effect of ZAF derivatives **13b**, **13c**, **13d**, **13f**, **14b**, and **14e** determined by colony forming unit (CFU) assays. Negative control = DMSO. Positive controls = tetracycline (T, 2.81 μ M) or ZAF (Z, 25 and 50 μ M). The data shows the calculated percentage of inhibitory effect from six independent replicates per condition by comparison of the treated bacteria groups (CFUs/mL) versus bacteria grown in medium only (CFUs/mL) using the formula $100 \times [\text{CFUs/mL of (control)} - \text{CFUs/mL (experimental)}] / \text{CFUs/mL (control)}$. For the controls, the data represent the average from 12 independent replicates per condition.

■ CYTOTOXIC EFFECT OF ZAF DERIVATIVES IN ORAL EPITHELIAL CELLS

The first line of defense against periodontopathogens is oral epithelial cells. To determine the value of drugs as potential antibacterials, their selectivity toward pathogenic bacterial species with little to no cytotoxicity toward host cells has to be determined. The cytotoxic effect of the six most active second generation ZAF derivatives **13b**, **13c**, **13d**, **13f**, **14b**, and **14e** was therefore evaluated against immortalized oral keratinocyte (OKF6) cells. To examine the effect of various treatments on OKF6 cell morphology and density, the cells were visualized by microscopy (Figure 6). There was a higher density of OKF6 cells in the control and DMSO wells, in contrast to the lower

density in the ZAF and staurosporine (STS, a pro-apoptotic chemical) wells. Wells containing compounds **13b** and **13d** had the highest density of cells, followed closely by compound **13c**. A decrease in cell density was seen in the presence of compounds **13f**, **14b**, and **14e**, indicating a slight increase in cytotoxicity against OKF6 cells, nowhere near that of the ZAF and STS positive controls. Untreated OKF6 cells along with cells treated with DMSO displayed epithelial-like morphology, i.e., elongated and flat.²³ OKF6 cells treated with compounds **13b**, **13c**, **13d**, and **13f**, resembled the large and flattened cells seen in the untreated OKF6 cells, which are also all in close proximity to one another. This is in direct contrast to the OKF6 cells treated with controls STS and ZAF as well as with compounds **14b** and **14e** that seem to be smaller in size, more round in shape, and at an increased distance from one another.

Cytotoxicity determined by flow cytometry revealed 76–93% cell viability with ZAF derivatives **13b**, **13c**, **13d**, **13f**, **14b**, and **14e** at the concentration of 1 μ M, and 49–92% cell viability at 10 μ M (Figure 7). Of note, all ZAF derivatives displayed less cytotoxicity than the parent drug ZAF. Overall, at 1 μ M, it was found that compound **13b** ($R_3 = 3\text{-F-Ph}$) displayed less cytotoxicity than (<) **13c** ($R_3 = 4\text{-F-Ph}$), which was less toxic than (<) **13d** ($R_3 = 3\text{-NO}_2\text{-Ph}$) < **13f** ($R_3 = 2\text{-naphthyl}$) < **14e** ($R_3 = 4\text{-NO}_2\text{-Ph}$) < **14b** ($R_3 = 3\text{-F-Ph}$). At 10 μ M, a similar trend was observed. As desired, these second generation of ZAF derivatives achieved the overall goal of increasing *P. gingivalis* growth inhibition and decreasing cytotoxicity compared to the first generation compounds and ZAF parent drug. Briefly, at all concentrations tested, compounds in series **13** all displayed less cytotoxicity when compared to their first generation counterpart **13g**. Compounds in series **14** also displayed similar or decreased cytotoxicity when compared to their first generation counterpart **14h** (Note: the viability for **13g** and **14h** used for comparison is not shown in this paper.)

In summary, 15 novel ZAF derivatives (**12a**, **12c**, **12f**, **13a**–**13f**, and **14a**–**14f**) were synthesized. Six of these (**13b**, **13c**, **13d**, **13f**, **14b**, and **14e**) displayed increased antibacterial activity against *P. gingivalis* and decreased cytotoxicity against OKF6 cells when compared to the parent ZAF and its first

Figure 6. Images representative of the whole well used to evaluate the effect of ZAF derivatives **13b**, **13c**, **13d**, **13f**, **14b**, and **14e** on cell morphology of oral epithelial cells (OKF6). Cells treated with medium only or DMSO were used as negative controls, while cells treated with 8 μ M of staurosporine (STS) or 25 μ M of ZAF were used as positive controls. Cells were treated with 10 μ M of ZAF derivatives **13b**, **13c**, **13d**, **13f**, **14b**, and **14e** for 24 h.

Figure 7. Cell viability assay used to evaluate the effect of ZAF derivatives **13b**, **13c**, **13d**, **13f**, **14b**, and **14e** on apoptosis and necrosis of oral epithelial cells (OKF6) after 24 h. Negative control = DMSO. Positive controls = 8 μ M of staurosporine (STS) or 25 μ M of ZAF (Z). Two independent experiments analyzing at least 10,000 events per condition in duplicate were used to generate the FACS data.

generation derivatives **13g** and **14h**. It was found that the most promising compounds all contained a methyl group on the nitrogen of the indole ring. It was also discovered that, at low concentrations (1 and 10 μ M), the presence of an electron-withdrawing group (e.g., $R_1 = \text{NO}_2$) on the indole ring, as in series **14**, was beneficial for bacterial growth inhibition and seemed to determine which acylsulfonamides imparted the best antibacterial activity (with complete opposite trends observed with series **13** ($R_3 = 4\text{-F-Ph} > 3\text{-NO}_2\text{-Ph} > 3\text{-F-Ph} > 2\text{-F-Ph} > 4\text{-NO}_2\text{-Ph}$) and **14** ($R_3 = 4\text{-NO}_2\text{-Ph} > 2\text{-F-Ph} \approx 3\text{-F-Ph} > 3\text{-NO}_2\text{-Ph} > 4\text{-F-Ph}$) when comparing which sulfonamides result in better growth inhibition). These promising results pave the way for future investigations, outside the scope of this study, seeking to identify the mechanism of action as well as further optimization of these compounds for *in vivo* studies using animal models of *P. gingivalis*-induced periodontal disease. These lengthy studies are currently underway in our laboratory and will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsmmedchemlett.9b00614>.

(i) Materials and instrumentation for chemistry, (ii) all experimental protocols for the synthesis and characterization of compounds, (iii) experimental protocols for all biological experiments, and (iv) all ^1H and ^{13}C NMR spectra along with HPLC traces for the final compounds tested (PDF)

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Author Contributions

The manuscript was written by K.C.H. and S.G.-T., and comments on the manuscript were provided by O.A.G. The figures were generated by K.C.H. and S.G.-T. All experiments were performed by K.C.H. All authors participated in the design of experiments and data interpretation and have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

CFU, colony forming unit; DMSO, dimethyl sulfoxide; FACS, flow cytometry analysis; FITC, fluorescein isothiocyanate; OKF6, immortalized oral keratinocyte cells; P/S, penicillin/streptomycin; SAR, structure–activity relationship; STS, staurosporine; T, tetracycline; Et_3SiH , triethylsilane; TFA, trifluoroacetic acid; WST-1, water-soluble tetrazolium-1; ZAF or Z, zafirlukast

■ REFERENCES

- (1) Hegde, R.; Awan, K. H. Effects of periodontal disease on systemic health. *DM, Dis.-Mon.* **2019**, *65*, 185–192.
- (2) Eke, P. I.; Dye, B. A.; Wei, L.; Thornton-Evans, G. O.; Genco, R. J. CDC Periodontal Disease Surveillance workgroup: James Beck, Gordon Douglass, Roy Page. Prevalence of periodontitis in adults in the United States: 2009 and 2010. *J. Dent. Res.* **2012**, *91*, 914–920.
- (3) Kim, J.; Amar, S. Periodontal disease and systemic conditions: A bidirectional relationship. *Odontology* **2006**, *94*, 10–21.
- (4) Fernandez-Solari, J.; Barrionuevo, P.; Mastroradi, C. A. Periodontal disease and its systemic associated diseases. *Mediators Inflammation* **2015**, *2015*, 153074.
- (5) Dominy, S. S.; Lynch, C.; Ermini, F.; Benedyk, M.; Marczyk, A.; Konradi, A.; Nguyen, M.; Haditsch, U.; Raha, D.; Griffin, C.; Holsinger, L. J.; Arastu-Kapur, S.; Kaba, S.; Lee, A.; Ryder, M. I.; Potempa, B.; Mydel, P.; Hellvard, A.; Adamowicz, K.; Hasturk, H.; Walker, G. D.; Reynolds, E. C.; Faull, R. L. M.; Curtis, M. A.; Dragunow, M.; Potempa, J. *Porphyromonas gingivalis* in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors. *Sci. Adv.* **2019**, *5*, No. eaau3333.

- (6) Mikuls, T. R.; Payne, J. B.; Yu, F.; Thiele, G. M.; Reynolds, R. J.; Cannon, G. W.; Markt, J.; McGowan, D.; Kerr, G. S.; Redman, R. S.; Reimold, A.; Griffiths, G.; Beatty, M.; Gonzalez, S. M.; Bergman, D. A.; Hamilton, B. C., 3rd; Erickson, A. R.; Sokolove, J.; Robinson, W. H.; Walker, C.; Chandad, F.; O'Dell, J. R. Periodontitis and *Porphyromonas gingivalis* in patients with rheumatoid arthritis. *Arthritis Rheumatol.* **2014**, *66*, 1090–1100.
- (7) Sum, J.; O'Rourke, V. J. Factors affecting periodontal disease referral and the adherence to guidelines among general dentists. *Aust. Dent. J.* **2018**, *63*, 394–401.
- (8) Thamban Chandrika, N.; Fosso, M. Y.; Alimova, Y.; May, A.; Gonzalez, O. A.; Garneau-Tsodikova, S. Novel zafirlukast derivatives exhibit selective antibacterial activity against *Porphyromonas gingivalis*. *MedChemComm* **2019**, *10*, 926–933.
- (9) Levi, P. A., Jr; Rudy, R. J.; Jeong, Y. N.; Coleman, D. K. *Non-surgical control of periodontal diseases: A comprehensive handbook*, 1st ed.; Springer, 2016; pp 163–173.
- (10) Moreno Villagrana, A. P.; Gomez Clavel, J. F. Antimicrobial or subantimicrobial antibiotic therapy as an adjunct to the nonsurgical periodontal treatment: A meta-analysis. *ISRN Dent.* **2012**, *2012*, 581207.
- (11) Mestnik, M. J.; Feres, M.; Figueiredo, L. C.; Soares, G.; Teles, R. P.; Fermiano, D.; Duarte, P. M.; Faveri, M. The effects of adjunctive metronidazole plus amoxicillin in the treatment of generalized aggressive periodontitis: A 1-year double-blinded, placebo-controlled, randomized clinical trial. *J. Clin. Periodontol.* **2012**, *39*, 955–961.
- (12) Mombelli, A.; Almaghlouth, A.; Cionca, N.; Courvoisier, D. S.; Giannopoulou, C. Differential benefits of amoxicillin-metronidazole in different phases of periodontal therapy in a randomized controlled crossover clinical trial. *J. Periodontol.* **2015**, *86*, 367–375.
- (13) Mombelli, A.; Cionca, N.; Almaghlouth, A. Does adjunctive antimicrobial therapy reduce the perceived need for periodontal surgery? *Periodontol.* **2000** **2011**, *55*, 205–216.
- (14) Shaikh, S.; Fatima, J.; Shakil, S.; Rizvi, S. M.; Kamal, M. A. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. *Saudi J. Biol. Sci.* **2015**, *22*, 90–101.
- (15) Diniz, C. G.; Farias, L. M.; Carvalho, M. A.; Rocha, E. R.; Smith, C. J. Differential gene expression in a *Bacteroides fragilis* metronidazole-resistant mutant. *J. Antimicrob. Chemother.* **2004**, *54*, 100–108.
- (16) Katlam, S.; Deshmukh, Y. A.; Jadhav, P. R. Comparative study of oxytetracycline and doxycycline on calcium chelation: *In-vitro* assay. *Int. J. Basic Clin. Pharmacol.* **2017**, *6*, 1160–1164.
- (17) Chong, C. R.; Sullivan, D. J., Jr. New uses for old drugs. *Nature* **2007**, *448*, 645–646.
- (18) Ashburn, T. T.; Thor, K. B. Drug repositioning: Identifying and developing new uses for existing drugs. *Nat. Rev. Drug Discovery* **2004**, *3*, 673–683.
- (19) Gerits, E.; Van der Massen, I.; Vandamme, K.; De Cremer, K.; De Brucker, K.; Thevissen, K.; Cammue, B. P. A.; Beullens, S.; Fauvar, M.; Verstraeten, N.; Michiels, J.; Roberts, M. *In vitro* activity of the antiasthmatic drug zafirlukast against the oral pathogens *Porphyromonas gingivalis* and *Streptococcus mutans*. *FEMS Microbiol. Lett.* **2017**, *364*.
- (20) Kelloway, J. S. Zafirlukast: The first leukotriene-receptor antagonist approved for the treatment of asthma. *Ann. Pharmacother.* **1997**, *31*, 1012–1021.
- (21) Pinault, L.; Han, J. S.; Kang, C. M.; Franco, J.; Ronning, D. R. Zafirlukast inhibits complexation of Lsr2 with DNA and growth of *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* **2013**, *57*, 2134–2140.
- (22) Martinez, A. A.; Espinosa, B. A.; Adamek, R. N.; Thomas, B. A.; Chau, J.; Gonzalez, E.; Keppetipola, N.; Salzedo, N. T. Breathing new life into West Nile virus therapeutics; discovery and study of zafirlukast as an NS2B-NS3 protease inhibitor. *Eur. J. Med. Chem.* **2018**, *157*, 1202–1213.
- (23) Dickson, M. A.; Hahn, W. C.; Ino, Y.; Ronfard, V.; Wu, J. Y.; Weinberg, R. A.; Louis, D. N.; Li, F. P.; Rheinwald, J. G. Human keratinocytes that express hTERT and also bypass a p16^{INK4a}-enforced mechanism that limits life span become immortal yet retain normal growth and differentiation characteristics. *Mol. Cell. Biol.* **2000**, *20*, 1436–1447.