

Accepted Manuscript

Kava Analogues as Agents for Treatment of Periodontal Diseases: Synthesis and Initial Biological Evaluation

Bin Cai, James S. Panek, Salomon Amar

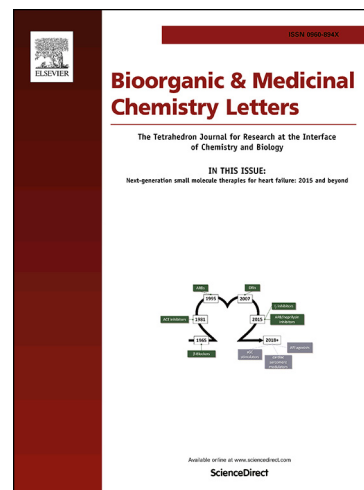
PII: S0960-894X(18)30428-1
DOI: <https://doi.org/10.1016/j.bmcl.2018.05.026>
Reference: BMCL 25843

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 6 February 2018
Revised Date: 11 May 2018
Accepted Date: 12 May 2018

Please cite this article as: Cai, B., Panek, J.S., Amar, S., Kava Analogues as Agents for Treatment of Periodontal Diseases: Synthesis and Initial Biological Evaluation, *Bioorganic & Medicinal Chemistry Letters* (2018), doi: <https://doi.org/10.1016/j.bmcl.2018.05.026>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



Graphical Abstract

To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

Kava Analogues as Agents for Treatment of Periodontal Diseases: Synthesis and Initial Biological Evaluation

Bin Cai, James S. Panek, and Salomon Amar

Leave this area blank for abstract info.





Kava Analogues as Agents for Treatment of Periodontal Diseases: Synthesis and Initial Biological Evaluation

Bin Cai^a, James S. Panek^{a,*}, Salomon Amar^{b,*}

^aDepartment of Chemistry, Boston University, Metcalf Center for Science and Engineering, 590 Commonwealth Avenue, Boston, Massachusetts 02215, United States

^bDepartment of Pharmacology, New York Medical College, Valhalla, NY, United States

ARTICLE INFO

Article history:

Received

Revised

Accepted

Available online

Keywords:

Kava

Periodontal disease

Cyclohexene benzoate

Buchwald coupling

ABSTRACT

Six kava analogues of the structural type 3-oxocyclohex-1-en-1-yl benzoates (and corresponding benzamides) were synthesized and evaluated for their affect on periodontal deconstruction in collagen anti-body primed oral gavage model of periodontitis. The compounds were prepared through an acylation or amidation of the enolizable cyclic 1,3-diketone. We have learned that three of the analogues are responsible for the reduction of inflammatory cell counts within soft tissue. These novel kava-like molecules where the lactone is replaced by an α,β -unsaturated ketone show promise in the prevention and treatment of inflammation and alveolar bone loss associated with periodontitis.

2009 Elsevier Ltd. All rights reserved.

Periodontal disease caused by bacterial accumulations or dental plaque on the teeth is highly prevalent in the United States. Gingivitis which is the most popular and mild form of periodontal disease affects 70%-80% of adults in the United States.¹⁻³ The more advanced form is periodontitis with affected US adult population over 47%.⁴ While gingivitis is characterized by inflammation of the gums, redness, swelling, and frequent bleeding, periodontitis is more severe and leads to tissue damage and alveolar bone loss.^{2,3} Studies in the past 10 years have also indicated correlations between periodontal disease and various systemic disorders and disease, including cardiovascular disease, diabetes mellitus, preterm low birth weight, and osteoporosis. The source of periodontal disease comes from approximately 500 different bacterial entities and various human viruses.⁵⁻⁸ Among them, three microaerophilic species (*Actinobacillus actinomycetemcomitans*, *Campylobacter rectus*, and *Eikenella corrodens*) and seven anaerobic species (*Porphyromonas gingivalis*, *Bacteroides forsythus*, *Treponema denticola*, *Prevotella intermedia*, *Fusobacterium nucleatum*, Eubacteria, and spirochetes) are frequently identified periodontal pathogens.

The difficulty with treatment of periodontal disease has been the fact that there are no effective, orally available small molecule drugs. Conventional periodontal therapy comprises oral hygiene instruction and scaling and root planning. However, systemic or local risk factors such as deep pockets or furcations of multirooted teeth may complicate the treatment.⁹ To control the inflammation associated with periodontal disease, many anti-TNF- α drugs have been used as additive therapies.^{10,11} However, risks of such therapies outweigh potential benefits. Additionally, all current TNF- α blockers are protein therapeutics, which must be administered parenterally and cost significantly higher than small molecule drug formulations. Therefore, there is a pressing need for the



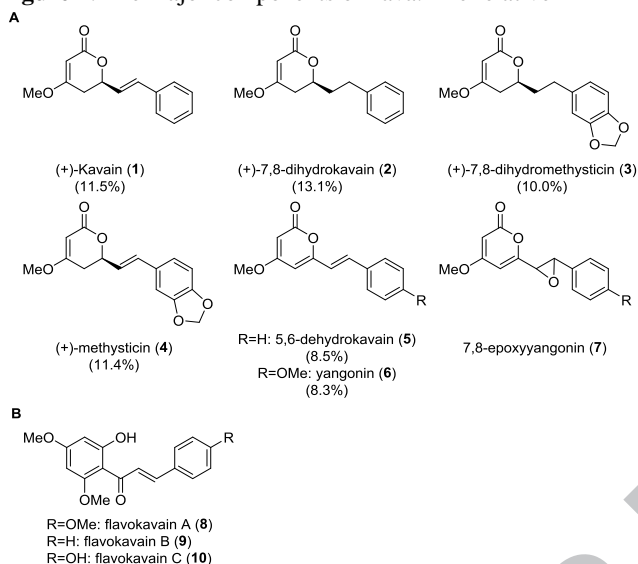
development of effective small molecule therapeutics for the definitive treatment of periodontal diseases. In line with our continued interest in identifying a novel class of oral TNF- α modulators, we recently discovered that Kava, a natural product extract from the *Piper methysticum* plant (in set), and its

* Corresponding author. Tel.: +1-617-353-2484; fax: +1-617-353-6466; e-mail: panek@bu.edu

* Corresponding author. Tel.: +1-914-594-3036; fax: +1-914-594-4362; e-mail: salomon_amar@nymc.edu

several analogues exhibited significant TNF- α reduction in vitro and in vivo.^{2,12} Moreover, our recent study of kava on a conventional oral gavage model of periodontitis has indicated kava as a useful compound in reducing alveolar bone loss and inflammation in *P. gingivalis*-induced periodontitis.³ The major chemical components of kava are seven lactones (Figure 1A) and three chalcones (Figure 1B).¹³ Although previous studies^{2,12} have demonstrated kava, a mixture of the ten major compounds, as a potent agent on TNF- α reduction, limitations including hepatic, neurologic, and dermatological toxicity, solubility and stability in vivo and cell permeability¹⁴ present opportunity for further medicinal chemistry optimization.

Figure 1. The major components of kava. The relative

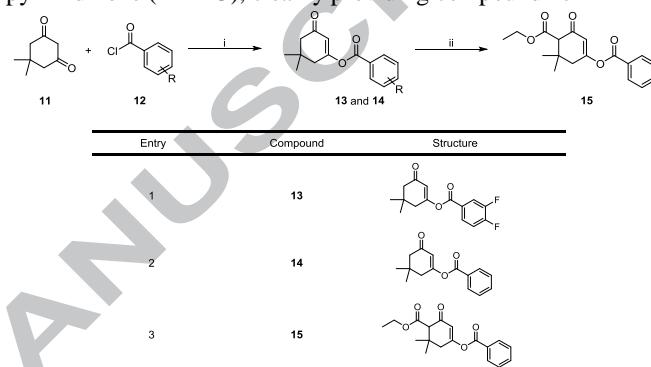


abundance of the major constituents are shown in parentheses.

A widely used and well-established method for early-stage discovery chemistry to optimize potent compounds to achieve drug-like properties is the structure-activity-relationship (SAR) study. In the present study, the correlations between the structural features of designed analogues and biological properties and/or activities of targets are presented. These correlations often provide useful information for specific interactions between the substructures of compounds and biological systems. More importantly, efforts are directed to identify sites of the molecules that can tolerate modifications without losing significant activities while displaying a range of potencies. Through the chemical modifications of these sites, compounds are modified in order to improve potency and pharmaceutical properties. Our previous efforts² on the initial SAR study generated a set of kavain analogues. Interestingly, among them, one ring-opened analogue which bears an α,β -unsaturated ester surprisingly showed $\geq 50\%$ suppression of TNF- α secretion. Additionally, medicinal chemistry¹⁵⁻¹⁷ efforts have pointed out the effect of the methyl group (and 4,4-dimethyl substitution in compounds **13-15**) in improving potency in drug discovery. In light of these findings, the present work details our synthesis of newly designed analogues to explore the above factors, preliminary structure-activity-relationship (SAR) study, evaluation of these analogues (not sure about biological term), and identification of a potent compound kava-241.¹⁸ In that regard, we sought to enhance the hydrolytic stability of the second generation of kava analogues by replacing the lactone with an α,β -unsaturated ketone (Scheme 1). The structural variations allowed the rapid production of six cyclohexanones substituted with a benzoate or benzamide at the C6-position of the cyclic enone (Schemes 1

and 2), and through these structural modifications we have gained enhanced bioactivity.

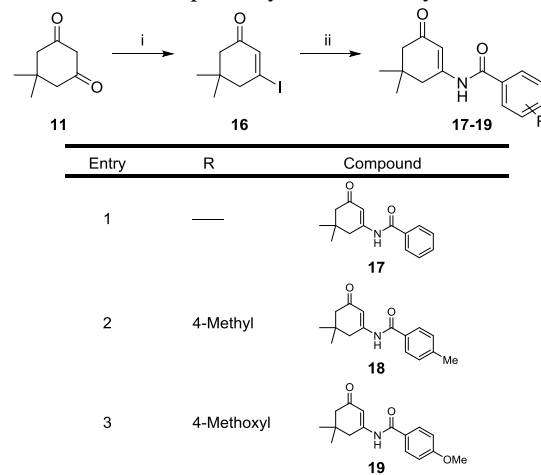
The synthetic approach towards compounds **13** and **14** is based on the O-acylation¹⁹ of the highly enolizable cyclic 1,3-diketones. Accordingly, treatment of 1,2-dichloroethane solution of commercially available 1,3-cyclohexanedione with various benzoyl chlorides in the presence of pyridine efficiently provided the O-acylated enol derivatives **13** and **14** (Scheme 1). They were further transformed to β -ketoesters through the C-acylation of in situ generated lithium enolate using Mander's reagent²⁰ (ethyl cyanofornate). Thus, compound **14** was treated with lithium bis(trimethylsilyl)amide solution (LHMDS) to generate lithium enolates. The subsequent nucleophilic attack (C-acylation) of the enolate with ethyl cyanofornate was facilitated by additive 1,3-Dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMPU), cleanly providing compound **15**



(Scheme 1) without trace of O-acylation products.

Scheme 1. Synthesis of compounds **13-15**. Reagents and conditions: (i) pyridine, DCE, rt; (ii) ethyl cyanofornate, DMPU, LHMDS, THF, -78 °C, 1h.

Encouraged by the screening results that electron-donating group on the aryl ring plays a beneficial role in TNF- α reduction² and the assumption that amide would be more stable in vivo, we synthesized three enamides (**17-19**) utilizing Buchwald's copper-mediated coupling²¹⁻²⁵ between vinyl iodide **16** and commercially available benzamides. Treatment of commercially available 5,5-dimethylcyclohexane-1,3-dione with triphenylphosphine, iodine, and triethylamine in acetonitrile efficiently afforded vinyl iodide **16**, which subsequently participated in a copper-mediated cross-coupling reaction (Scheme 2). Thus, **16** and the benzamides were successfully coupled in the presence of substoichiometric amounts of CuI, *N,N*-dimethylglycine, and CS_2CO_3 to produce three enamides **17-19** respectfully. Unfortunately the series of

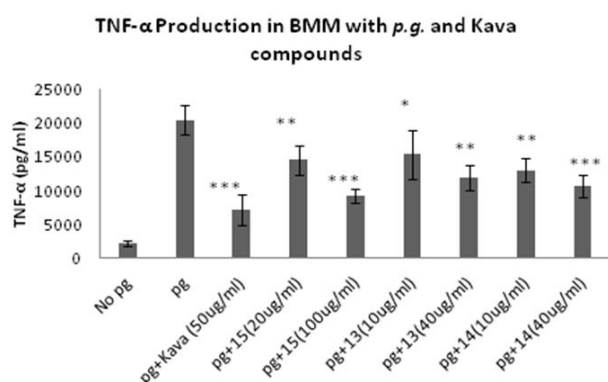


vinyl amides proved to be inactive in the initial TNF- α production assay, which of course requires further evaluation.

Scheme 2. Synthesis of vinyl amides **17-19**. Reagents and conditions: (i) PPh_3 , I_2 , NEt_3 , CH_3CN , rt, 3d. (ii) benzamide, CuI , *N,N*-dimethylglycine, CS_2CO_3 , dioxane, 60 °C, 12h.

Given that kava was found to be anti-inflammatory, we selected our screening strategy involving a cell-based assay wherein control experiments cells were exposed to *P. gingivalis* and responded by producing a potent pro-inflammatory cytokine TNF- α . The analogs in schemes 1 and 2 were screened using our cell-based assay with two optimal concentrations of the given compound at concentration depicted in Figure 2 using parent Kava as a positive control. Briefly, bone-marrow macrophages were harvested as previously described²⁶ and cultured during 7 days in RPMI medium supplemented with L929 and 10 000 U/liter penicillin, 100 mg/liter streptomycin and 10% fetal bovine serum in a humidified atmosphere (5% CO_2) at 37°. According to experimental design, cells were infected by *P. gingivalis* (MOI=20) and/or treated with 50 to 200 $\mu\text{g}\cdot\text{ml}^{-1}$ of synthesized Kava compound. As shown in figure 2 compound **15** significantly reduced *P. gingivalis* stimulated TNF- α to the same extent as the parent compound Kava at concentration of 100 $\mu\text{g}/\text{mL}$.

Figure 2. The mouse macrophages were collected from wild



type mice and recovered in the RPMI-1640 medium with 10% FBS for 5 days, and exposed with *P. gingivalis* (MOI: 20 *P. gingivalis* cells for 1 macrophage cell) with or without Kava compounds (at concentrations indicated above) for 4h. The supernatants from each test group were collected and TNF- α levels were subsequently assayed by ELISA (Invitrogen), following the manufacturer's instructions. Data were analyzed and then graphed where *** $p < 0.0005$; ** $p < 0.01$; * $p < 0.05$.

In summary, we have synthesized a new class of kava analogues (**13-15**) bearing an α,β -unsaturated cyclohexanone substituted with a benzoate at the C6-position. These analogues were then evaluated for their effect on periodontal destruction in a collagen antibody primed oral gavage model of periodontitis. Among them, compound **15** was found to significantly reduce *P. gingivalis* induced TNF- α production.¹⁸ Unfortunately the corresponding vinyl amide analogs were devoid of activity in the initial screening. Nevertheless, our preliminary results suggest they (**13-15**) are promising leads as potential therapeutic agents of periodontal diseases in the future. However, future studies should focus on determining the most effective dosing regimen and expanding the SAR study to further optimize the potencies and pharmaceutical properties of this series of compounds.

Acknowledgments

We gratefully acknowledge financial support from the NIH grants RO1HL076801 and RO1DE014079 to SA. We thank Dr. Paul Ralifo and Dr. Norman Lee at the Boston University Chemical Instrumentation Center for helpful discussions and assistance with NMR and HRMS experiments.

Supplementary data

Supplementary data associated with this article can be found in the online version

References

- Eke PI, Thornton-Evans, GO, Wei L, Borgnakke WS, Dye BA. *J Dent Res.* 2010;89:1208-1213.
- Pollastri MP, Whitty A, Merrill JC, Tang X, Ashton TD, Amar S. *Chem Biol Drug Des.* 2009;74:121-128.
- Yuan H, Gupte R, Zelkha S, Amar S. *J Clin Periodontol.* 2011;38:1029-1036.
- Eke PI, Dye, BA, Wei L, Thornton-Evans, GO, Genco RJ. *J Dent Res.* 2012;91:914-920.
- Su C, Lichtenstein GR. *Adv Drug Deliv Rev.* 2005;57:237-245.
- Cannon GW, Holden WL, Juhaeri J, Dai W, Scarazzini L, Stang P. *J Rheumatol.* 2004;31:1906-1911.
- Hanauer SB. *Curr Opin Gastroenterol.* 1999;15:308-314.
- Singh YN. *J Ethnopharmacol.* 1992;37:13-45.
- Bouchard P, Carra MC, Boillot A, Mora F, Range H. *J Clin Periodontol.* 2017;44:125-131.
- Jamieson DD, Duffield PH. *Clin Exp Pharmacol Physiol.* 1990;17:495-507.
- Tonks A. *BMJ* 2003; 326:700-702.
- Tang X, Amar S. *Toxicol Res. (Camb)* 2016;5:188-196.
- Folmer F, Blasius R, Morceau F, Tabudravu J, Dicato M, Jaspars M, Diederich M. *Biochem Pharmacol.* 2006;71:1206-1218.
- Gounder R. *Pac Health Dialog.* 2006;13:131-135.
- Schönherr H, Cernak T. *Angew Chem Int Ed Engl.* 2013;52:12256-12267.
- Cai B, Panek JS, Amar S. *J Med Chem.* 2016; 59:6878-6890.
- Talele TT. *J Med Chem.* 2017 ASAP.
- Alshammari A, Patel J, Al-Hashemi J, Cai B, Panek JS, Huck O, Amar S. *J Clin Periodontol.* 2017;44:1123-1132.
- Akhrem AA, Budai SI, Khlebnicova TS, Petrushevich II, Lakhvich FA. *Synthesis.* 1978;1978:925-927.
- Crabtree SR, Chu WL, A, Mander LN. *Synlett.* 1990;1990:169-170.
- Pan X, Cai Q, Ma D. *Org Lett.* 2004;6:1809-1812.
- Jiang L, Job GE, Klapars A, Buchwald SL. *Org Lett.* 2003; 5:3667-3669.
- Klapars A, Huang X, Buchwald SL. *J Am Chem Soc.* 2002;124:7421-7428.
- Kwong FY, Buchwald SL. *Org Lett.* 2003;5:793-796.
- Klapars A, Antilla JC, Huang X, Buchwald S L. *J Am Chem Soc.* 2001;123:7727-7729.
- Zhang X, Goncalves R, Mosser DM. *Curr Protoc Immunol.* 2008;1;14.1.1-14.1.14.

Highlights

- New kava analogues as agents for treatment of periodontal diseases.
- **15** was found to significantly reduce *P. gingivalis* induced TNF- α production.
- Corresponding vinyl amide analogues were inactive.

ACCEPTED MANUSCRIPT