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Synthesis and PGE_2 inhibitory activity of novel diarylheptanoids

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ABSTRACT

Prostaglandin E_2 (PGE_2) is a lipid mediator of inflammation and its inhibition has become a popular drug target due to its harmful physiological roles. Diarylheptanoids are one class of compounds that have shown successful inhibition of PGE_2. This paper reports the synthesis and PGE_2 inhibitory activity of a series of analogues of a naturally occurring diarylheptanoid. The most efficacious compounds were examined for dose-dependent PGE_2 inhibition. Among several promising compounds, the lead candidate exhibited an IC_{50} value of 0.56 ng/μL or 1.7 μM with no detectable toxicity at the highest dose of 10 ng/μL.

Inflammation is an adaptive, physiological response to harmful stimuli that can include tissue damage and infection. Generally, the inflammatory response is beneficial and protective to cells and tissues. However, dysregulation of the inflammatory response can result in adverse effects and contributes to the pathogenesis of many diseases. The inflammatory response can be mediated through the conversion of arachidonic acid to the cyclooxygenases (COX-1 and COX-2), which produce thromboxanes (TX) and prostaglandins (PG) (Fig. 1).[1] The prostaglandins, PGI_2 and PGE_2, induce vasodilation and increase vascular permeability which can cause long term swelling and tissue damage.[2] Furthermore, PGE_2 has been shown to affect pain, tumorigenesis, neuronal functions, female reproduction, gastric mucosal protection, and kidney function.[2,3] Non-specific NSAIDs target both COX-1 and COX-2 with the intent of down-regulating the inflammatory response entirely, but also result in damage to the gastric mucosa.[4] More recently, COX-2 inhibitors or coxibs have been developed to preferentially inhibit COX-2 activity and avoid damaging the gastric mucosa. However, selective COX-2 inhibition results in reduced PGI_2 production by the vascular endothelium and predisposes individuals to increased vascular endothelial injury.[4] For this reason, individuals with pre-existing cardiovascular disease have an increased risk for cardiac ischemia, heart failure, hypertension, and cardiac arrhythmia when taking coxibs.[5,6] In an effort to avoid these harmful side effects, mPGES-1 (microsomal prostaglandin E_2 synthase-1) inhibitors have gained interest as a novel therapeutic target for the treatment of inflammation associated with a variety of diseases.[7] One class of natural products, that have shown anti-inflammatory activity by inhibition of mPGES-1, are diarylheptanoids.[8]

Diarylheptanoid natural products are produced as secondary plant metabolites and are recognized by two aromatic rings linked by a seven-carbon chain. They are primarily isolated from plants in the genus *Zingiber, Alnus, Alpinia, Curcuma,* and *Myrica.* Diarylheptanoids have become of increased interest due to their vast array of biological activities including anti-inflammatory, antioxidant, antitumor, antiosteoporotic, antibacterial, melanogenic, hepatoprotective, and neuroprotective properties.[9,10]

A diarylheptanoid molecule (1-(4″-methoxyphenyl)-7-(4′-hydroxyphenyl)-(*E*)-hept-2-ene, **1**, Fig. 2) was isolated from *Pleuranthodium racemigerum*, an Australian ginger plant.[11] Upon isolation, **1** was tested and showed moderate anti-inflammatory activity through inhibition of PGE_2 production.[11] The goal of this research was to synthesize and investigate the biological properties of next-generation diarylheptanoid molecules that possess greater anti-inflammatory than **1** with enhanced on-target activity. Herein, we describe the synthesis and anti-inflammatory activity of **1** and seven novel analogues (Fig. 2). The molecules were synthesized via a four or five step route with a cross metathesis reaction using Grubbs Catalyst™ 1st generation as the key step. Each final compound was tested for its inhibition of PGE_2 and the results were compared against **1**.

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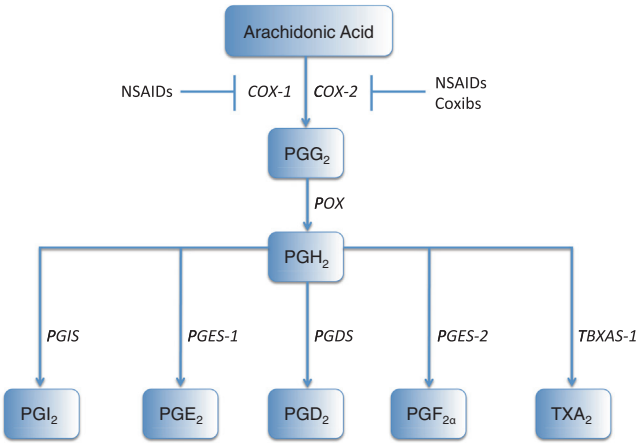


Fig. 1. Prostanoid Biosynthetic Pathway.

Synthesis of diarylheptanoids

To date, we have synthesized the natural product **1** and seven novel analogues to begin our investigation in developing a preliminary structure-activity relationship (Fig. 2). The synthetic route was adapted from Chang and involves a four- or five-step synthesis with a cross metathesis reaction creating the seven-carbon linker (Scheme 1).[12]

Our synthesis of **1** began with the reduction of commercially available 3-(4-hydroxyphenyl)propionic acid using a borane-tetrahydrofuran complex and trimethyl borate to afford the primary alcohol **9a** in a 99% yield. Initially, this reduction only produced a 41% yield, but based on the methodology described by Zhou and co-workers, we found increasing the equivalents of trimethyl borate dramatically improved the yield.[13] Compound **9a** was further reacted with carbon tetrabromide and triphenylphosphine in anhydrous dichloromethane to give the primary alkyl bromide **10a** in a 69% yield. The bromide **10a** was refluxed in anhydrous toluene for 48 h in the presence of excess allylmagnesium chloride to produce the Grignard product **11a** in a 63% yield. As an acidic proton is present in compound **10a**, excess Grignard reagent was used instead of adding a protecting group, as the yields for the reaction were similar regardless of a protecting group as reported by Chang in the original synthesis of **1**.[12] In addition, being the second step of the synthesis, we looked into improving the yield. The total reaction time for the Grignard reaction was reduced to 18 h from the original 48 h, developed by Chang, to decrease the amount of possible disproportionation of the Grignard reagent.[12] This shorter reaction time increased the yield of **11a** to 89%. Compound **1** was then produced through the cross metathesis reaction of the alkene **11a** with 4-allylanisole and Grubbs Catalyst™ 1st generation in anhydrous dichloromethane at 35 °C for 18 h. To aid in the removal of the catalyst, 50 equivalents of DMSO (relative to the catalyst) were added to the crude mixture, which was stirred for an additional 18 h.[14] While the mechanism remains unknown, it has been proposed that DMSO acts as a mild oxidant and converts the metal species into polar oxides that can be purified during column chromatography.[15] The final product was purified using MPLC to provide **1** in an 18% yield. Based on NMR integration, it was concluded that **1** was formed as a mixture of *E*- (85%) and *Z*-isomers (15%). Chang was able to separate the mixture of *E:Z* cross metathesis products by the use of $AgNO_3$-doped silica gel.[12] After several efforts to separate the *E:Z* product mixture using this method, we were unable to isolate either of the compounds and tested **1** as a set of diastereomers with *E* being the major product.

The first novel analogue of **1** synthesized was compound **2**, which lacked the alkene on the seven-carbon linker. This analogue was created in order to probe the importance of the alkene to its biological activity. Molecule **1** underwent a hydrogenation reaction with palladium on carbon to create **2** in a 60% yield.[16]

Our next novel analogues synthesized were compounds **3** and **4** (Scheme 1), which contain the phenol on the **A** ring in the *ortho*- rather than the *para*-position as in compounds **1** and **2**. Compound **3** was synthesized through the reduction of commercially available 3-(2-hydroxyphenyl)propionic acid using a borane-tetrahydrofuran complex and trimethyl borate to afford the primary alcohol **9b** in a 99% yield. While the reduction to provide alcohol **9b** was analogous to the alcohol **9a**, the bromination reaction to prepare **10b** gave much lower yields (33% at best). Based on the work of Tongkate et al., the equivalents of CBr_4 and PPh_3 were lowered and the reaction time was shortened to produce bromide **10b** in a 57% yield, which although low is almost double the initial yield.[17] Compound **10b** was further reacted with excess allylmagnesium chloride for 18 h to provide **11b** in an 83% yield. Molecule **3** was then produced through the cross metathesis reaction of the alkene **11b** with 4-allylanisole and Grubbs Catalyst™ 1st generation under the same reaction conditions that were used to produce compound **1**. The final product was purified using MPLC to give **3** in a 25% yield. Based on NMR integration, it was concluded that **3** was formed as a mixture of *E*- (82%) and *Z*-isomers (18%), which was used for biological testing without further purification. Compound **3** was then reacted with palladium on carbon under an atmosphere of hydrogen to afford **4** in a 60% yield.[16]

The next novel analogues synthesized were **5** and **6** (Scheme 1). These derivatives have the phenol on the **A** ring in the *para*-posi-

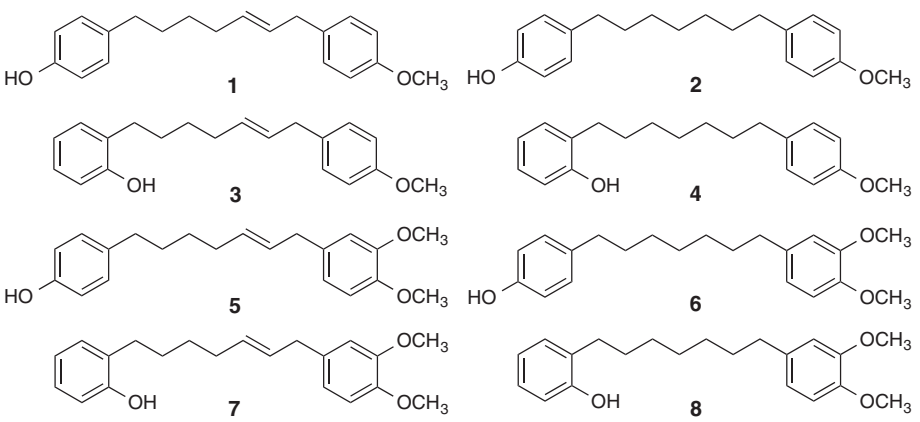
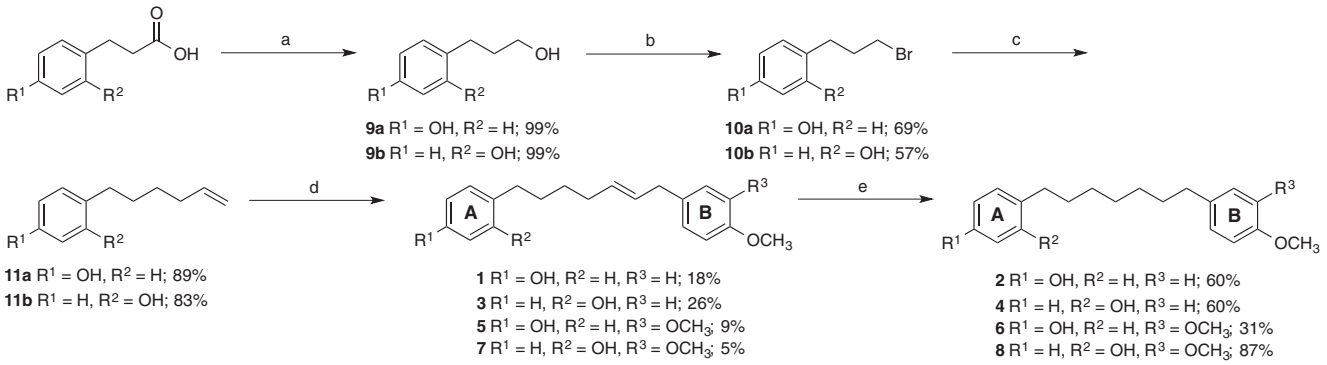


Fig. 2. Synthesized Diarylheptanoids.



Scheme 1. Reagents and conditions: (a) $B(OMe)_3$, BH_3-THF complex, THF, 0 °C; (b) CBr_4, PPh_3, DCM, 0° to rt; (c) allylmagnesium chloride, toluene, 110 °C; (d) 4-allylanisole *or* 4-allyl-1,2-dimethoxybenzene, Grubbs Catalyst™ 1st generation, DCM, 35 °C; (e) H_2, Pd/C, EtOH.

tion and an additional methoxy group on the **B** ring. Compound **5** was synthesized by reacting alkene **11a** with 4-allyl-1,2-dimethoxybenzene in the presence of Grubbs Catalyst™ 1st generation.[12] After purification, **5** was isolated in a 10% yield. Based on NMR integration, it was concluded that **5** was formed as a mixture of *E*- (81%) and *Z*-isomers (19%), which was used for biological testing without further purification. Compound **5** was then reacted with palladium on carbon under an atmosphere of hydrogen to afford **6** in a 31% yield.[16]

The last novel analogues synthesized were **7** and **8** (Scheme 1). These derivatives have the phenol on the **A** ring in the *ortho*-position and an additional methoxy group on the **B** ring. Compound **7** was synthesized by reacting alkene **11b** with 4-allyl-1,2-dimethoxybenzene in the presence of Grubbs Catalyst™ 1st generation.[12] After purification, **7** was isolated in a 5% yield and based on NMR integration, it was concluded that **7** was formed as a mixture of *E*- (89%) and *Z*-isomers (11%). This mixture of alkenes was used for biological testing without further purification. Compound **7** was then reacted with palladium on carbon under an atmosphere of hydrogen to afford **8** in an 87% yield.[16]

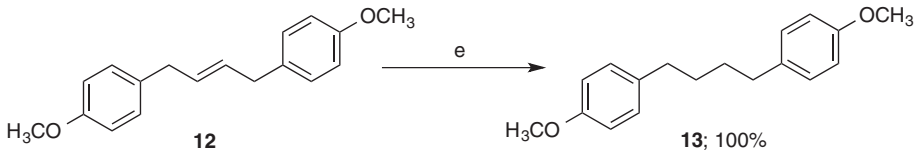
During the cross metathesis reactions to form **1** and **3**, we observed the formation of a major byproduct, 1,4-bis(4-methoxyphenyl)but-2-ene (**12**), which was formed by 4-allylanisole reacting with itself. This product was purified and tested alongside the diarylheptanoid molecules, in addition to the hydrogenated version (**13**). Compound **13** was formed, in a quantitative yield, by reacting **12** with palladium on carbon under an atmosphere of hydrogen (Scheme 2).

As shown above, the purified quantities of the cross metathesis reaction products are less than desirable. We found the low yields were attributed to two major factors. Firstly, the major impurity observed for each cross metathesis reaction was a byproduct formed by either the 4-allylanisole or 4-allyl-1,2-dimethoxybenzene undergoing a cross metathesis reaction with itself. As the desired product and byproduct share similarities in polarity and solubility, isolating pure diarylheptanoid proved to be challenging. Secondly, we found multiple purifications were necessary to obtain pure product. This was especially true for compounds **5** and **7**, as they have an additional oxygen containing functionality and coordinate more strongly with the catalyst. Despite favorable byproduct formation, multiple purifications and low yields, we obtained enough material for biological testing. These reactions may be applied to create more final compounds as the synthetic route is fairly short and does not require many expensive chemicals, excluding the catalyst.

PGE_2 inhibitory activity and biological properties of diarylheptanoids

Diarylheptanoid compounds **1**–**8** (Fig. 2), **12**, and **13** (Scheme 2) were tested for their ability to inhibit the production of prostaglandin E_2 (PGE_2) in 3 T3 murine fibroblast cells. As shown in Fig. 3, efficacy of the diarylheptanoid compounds at 1 ng/μl and 10 ng/μl ranged from no significant decrease (**1** and **4**) to significant reduction in PGE_2 (**3**, **5**, **6**, **8**, and **12**), as compared to vehicle control. The most efficacious inhibitor of PGE_2 production was **6**, which reduced PGE_2 levels to 33% of control at 10 ng/μl. Many of the compounds (**5**, **6**, **8**, **12**, and **13**) also exhibited a dose-dependent inhibition of PGE_2 production, while others did not, suggesting a difference in potency between the compounds. Furthermore, we determined a more in-depth dose-response curve for the four most efficacious compounds (**5**, **6**, **8**, and **12**) and identified their IC_{50} values to be 0.56, 9.6, 5.5, and 3.2 ng/μl or 1.7, 29, 17 and 12 μM, respectively (Fig. 4). The steepness of the slope of **5** suggests that it has the highest potency and a maximum efficacy comparable to **12**.

The diarylheptanoid compounds were also tested for inhibitory activity of NF-κB, a pro-inflammatory transcription factor that helps to regulate PGE_2 activity. To test NF-κB inhibition, 3T3 cells were transfected with an NF-κB-luciferase reporter containing a luciferase reporter gene under control of an NF-κB specific gene promoter. Curcumin, an extensively studied and structurally similar diarylheptanoid,[18] has been shown to inhibit transcriptional activation of NF-κB and was used as a positive control for the assay.[19] As expected, TNF-α-induced NF-κB reporter activity was significantly decreased by the addition of curcumin (10 ng/μl) (Fig. 5). However, none of the tested diarylheptanoid compounds



Scheme 2. Reagents and conditions: (e) H_2, Pd/C, EtOH.

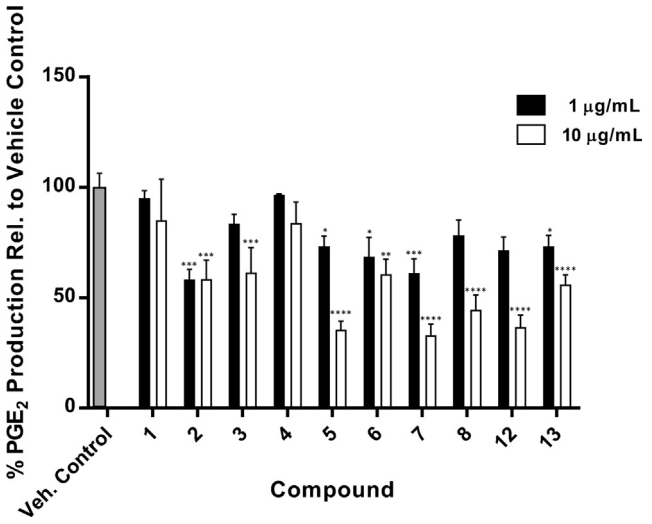


Fig. 3. 3T3 cells were treated with compounds 1–8, 12, and 13 at doses of 1 and 10 ng/μl, and media was collected for determination of PGE_2 secretion via ELISA. Many of the novel compounds show an inhibitory effect on PGE_2 production ($^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, $^{****}P < .0001$).

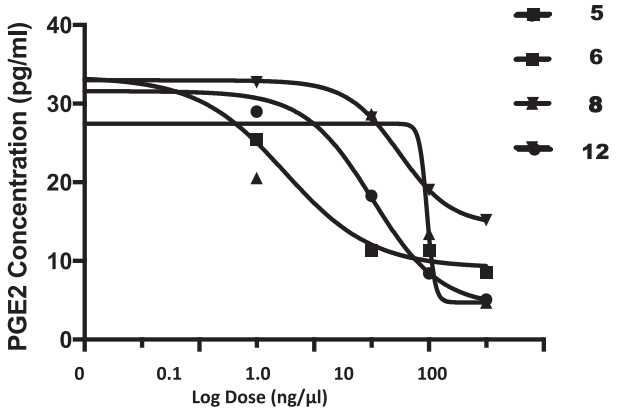


Fig. 4. 3 T3 cells were treated with compounds 5, 6, 8, and 12 at four different doses to determine IC_{50} values for inhibition of PGE_2 secretion. IC_{50} values were calculated with SigmaPlot using four-parameter logistic fit.

(all at 10 ng/μl) exhibited any inhibitory activity toward NF-κB, suggesting the possibility of enhanced on-target activity compared to curcumin.

Cellular toxicity for the compounds was measured by alamarBlue® cell viability assay. The active ingredient of alamarBlue®, resazurin, is reduced by viable cells to resorufin and can be used to quantify the number of viable cells through fluorescence intensity or absorbance.[20] In both cases, we observed a dose-dependent degree of cell death induced by H_2O_2 treatment, as expected. However, none of the tested diarylheptanoid compounds, administered at a dose of 10 ng/μl, resulted in any decrease in cell viability. This was consistent for both fluorescence and absorbance readouts of the assay (Fig. 6).

In sum, we have described the synthesis of several novel diarylheptanoid analogues of **1**. Biological results demonstrate that compounds **3**, **5**, **6**, **8**, and **12** inhibit PGE_2 production with more efficacy than **1**. In addition, the lack of NF-κB inhibition of our diarylheptanoids suggests possibly improved on target specificity versus curcumin, which needs to be further investigated. Based on the PGE_2 inhibitory activity, the compounds that contain a dimethoxy functional group on the **B** ring (**5**–**8**) show a greater potency. When comparing the dimethoxy compounds (**5**–**8**), the

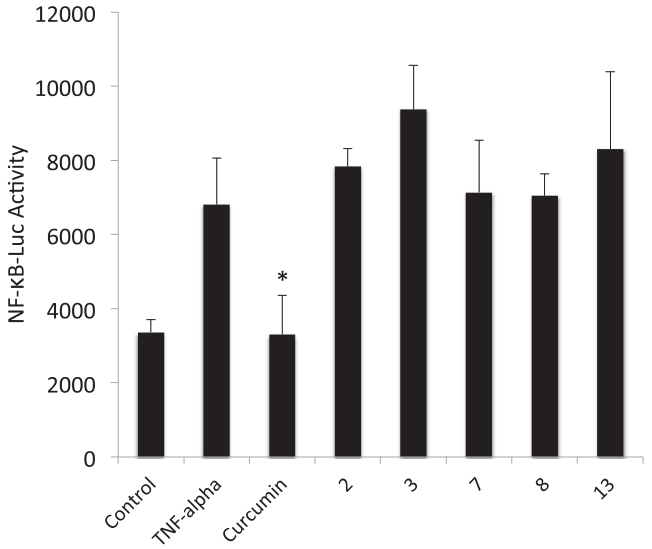


Fig. 5. Curcumin, but not the tested diarylheptanoid compounds reduced TNF-α-induced NF-kB transcriptional activity in 3T3 cells at 10 ng/μl. $^{*}P \leq .05$ vs. TNF-α treated cells.

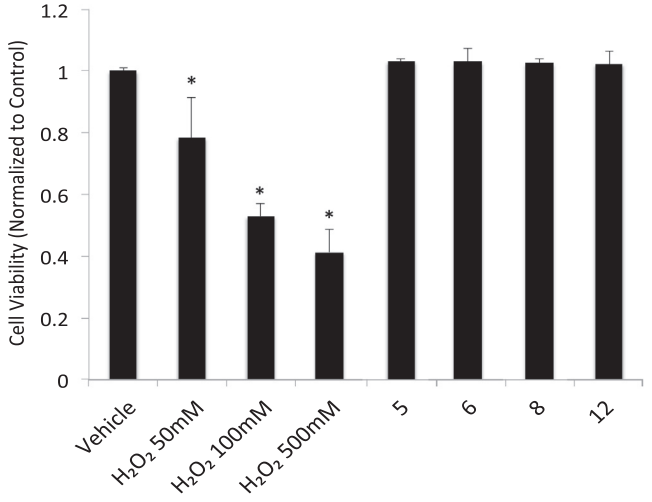


Fig. 6. AlamarBlue® was used to assess 3T3 cell viability following six hours treatment with compounds 5, 6, 8, and 12 at 10 ng/μl. While a six-hour treatment with H_2O_2 resulted in a dose-dependent decrease in cell viability, none of the diarylheptanoid compounds induced any toxicity. $^{*}P \leq .05$ vs. Vehicle control.

phenol on the **A** ring shows preference for the *para* position. The alkene on the seven-carbon linker does not appear to be crucial for the observed inhibition of PGE_2 as **6** was the most efficacious. In contrast, the alkene on the linker does appear to be necessary for the activity of **12** as compared to **13**. The information gathered from this preliminary structure-activity relationship will be used to guide the synthesis of future diarylheptanoid analogues in order to create a more potent lead compound. Further characterization of the diarylheptanoid compounds is also necessary to help discern their biological target(s). In addition, molecules with scaffolds similar to compound **12** (1,4-bis(4-methoxyphenyl)but-2-ene) should be further investigated.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmcl.2017.12.046.

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