ACS Medicinal Chemistry Letters

pubs.acs.org/acsmedchemlett Letter

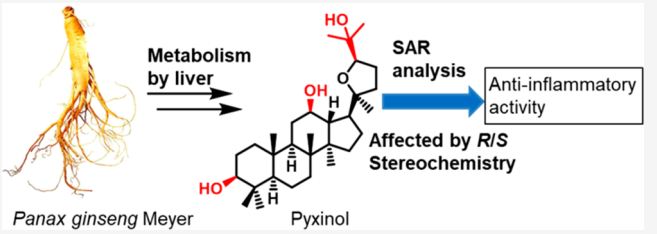
Synthesis and Structure–Activity Relationship of Pyxinol Derivatives as Novel Anti-Inflammatory Agents

Yixiao Sun,[§] Xiaojuan Fang,[§] Meng Gao, Conghui Wang, Hongyan Gao, Wenjing Bi, Hanhan Tang, Yetong Cui, Leiming Zhang, Huaying Fan, Hui Yu,* and Gangqiang Yang*

Cite This: https://dx.doi.org/10.1021/acsmedchemlett.9b00562 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Pyxinol, the main metabolite of 20*S*-protopanaxadiol in human liver, was chosen as a novel skeleton for the development of anti-inflammatory agents. Pyxinol derivatives modified at C-3, C-12, or C-25 and selected stereoisomers were designed, prepared, and investigated for *in vitro* anti-inflammatory activities. Structure–activity relationship (SAR), focused on skeleton, was analyzed based on their ability to inhibit lipopolysaccharide (LPS)-induced nitric oxide (NO) synthesis. The preliminary SAR results signified that the biological activity of the pyxinol derivatives is largely dependent on the *R*/*S* stereochemistry of pyxinol skeleton and the hydroxy at C-3 is a modifiable position. Among the tested compounds, the 3-oximinopyxinol (**4a**) exhibited the most potent NO-inhibitory activity and was even comparable to the steroid drug. Furthermore, compound **4a** also significantly decreased LPS-induced TNF-α and IL-6 synthesis and iNOS and COX-2 expressions via the NF-κB pathway. This study proves that pyxinol is an interesting skeleton for anti-inflammatory drug discovery.



KEYWORDS: *Pyxinol, ginsenoside, anti-inflammatory activity, structure–activity relationship, macrophages*

Inflammation is a primary defense response of the immune system and protects against infection and injury.[1] Macrophages are the key cells that produce inflammatory mediators, including nitric oxide (NO), prostaglandins (PGs), and proinflammatory cytokines, including tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6). However, prolonged inflammation can lead to immune over-reactions that cause tissue damage and lead to chronic diseases, such as Alzheimer's disease and diabetes.[2,3] Steroid drugs, such as hydrocortisone, are powerful at reducing inflammation, but they have many serious side effects, such as water and sodium retention and osteoporosis.[4] Other drugs are also limited by side effects.[5] Therefore, the development of new anti-inflammatory agents that are safer and more effective is ongoing. Natural products are a key source and play an important role in discovering next generation medicines.[6]

Ginseng, the roots of *Panax ginseng* Meyer, is well-known to extend life, enhance vitality and play roles against kinds of conditions, such as tumors, depression, and inflammation.[7,8] Ginsenosides, the widely orally administered components of ginseng, are recognized as its major active pharmacological components with various activities.[7] Their anti-inflammatory activities are well-known because of the structural similarity with cortisone, a steroid hormone with powerful anti-inflammatory activity. Some of them have been applied to clinical treatment. Previous studies and reviews have summarized that the protopanoxadiol type ginsenosides (Rb1, Rb2, Rd, and Rh2) exert anti-inflammatory activities by decreasing the generation of inflammatory mediators and proinflammatory cytokines and regulating the inflammatory signaling pathways.[8,9] 20*S*-Protopanoxadiol (PPD), the principal intestinal metabolite of ginsenosides, was recognized as the pharmacophore of ginsenosides and showed increased absorption in the gastrointestinal tract.[10,11] PPD also showed anti-inflammatory activity by inactivation of nuclear factor κB (NF-κB) and inducing heme oxygenase 1 expression.[12] These results suggested that the metabolites of ginsenosides could be promising candidates for inflammation treatment.

Pyxinol, an ocotillol-type triterpene shown in Figure 1, was first isolated from lichen in 1972 and recently was identified as the main metabolite of PPD in human liver.[13−15] Pyxinol was found to have better oral bioavailability and a lower metabolism burden in organisms compared with the parent compounds PPD and protopanoxadiol-type ginsenosides, suggesting its medicinal development value.[14,16] Therefore, pyxinol and its derivatives have been extensively studied for drug development against a variety of conditions, such as cardiovascular diseases,[17−19] tumors, and bacterial infec-

Received: November 30, 2019
Accepted: February 12, 2020
Published: February 12, 2020

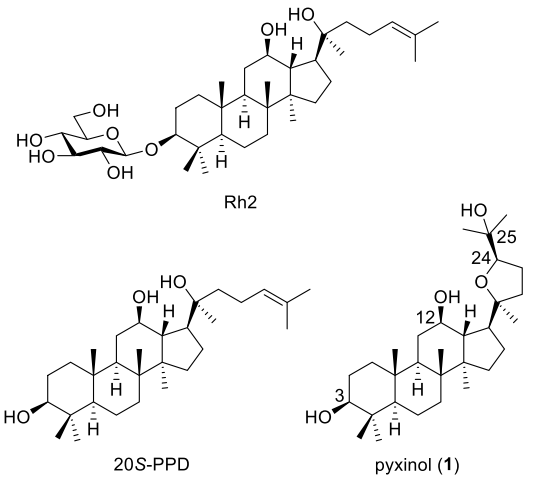


Figure 1. Chemical structures of ginsenosides.

tion.[20−24] Nevertheless, there are few reports of pyxinol or pyxinol analogs with anti-inflammatory activity.

On the basis of above view for development of novel anti-inflammatory agents, pyxinol was first used as a lead compound in this study and different modifications were carried out at the C-3, C-12, or C-25 of pyxinol to yield 18 compounds including several stereoisomers. Their anti-inflammatory activities were assessed by measuring NO-inhibitory activity in lipopolysaccharide (LPS)-induced macrophages. The anti-inflammatory effects of the most potent derivative **4a** with an oxime moiety at C-3 were further confirmed by its inhibition of proinflammatory cytokines that were comparable to the clinical steroid drug hydrocortisone sodium succinate.

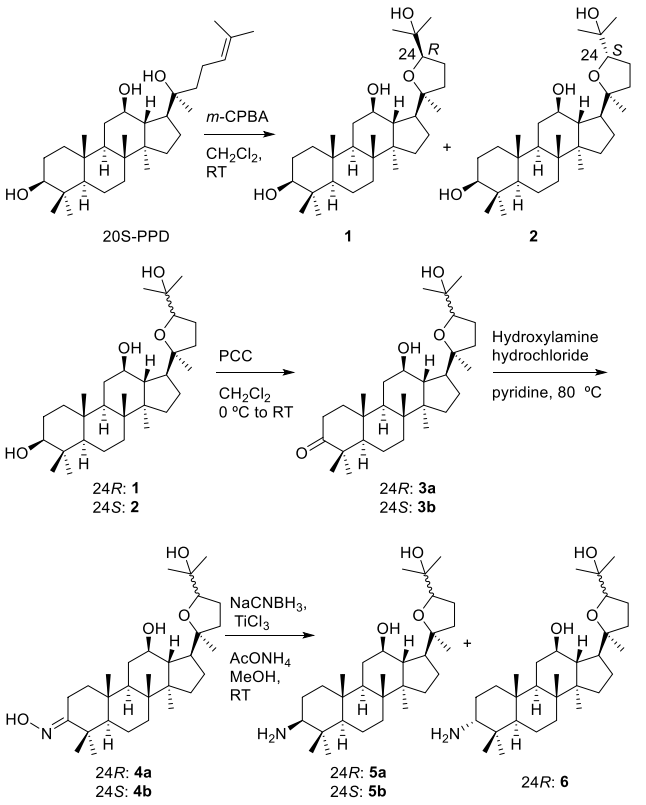
To explore the effects of hydroxy groups and stereochemistry of pyxinol on its anti-inflammatory activities, a series of pyxinol derivatives and selected stereoisomers were designed in which the hydroxy group at C-3, C-12, or C-25 was modified. Pyxinol (**1**) and its epimer 24*S*-pyxinol (**2**) were synthesized by epoxidization of the commercially available PPD and following nucleophilic addition *in situ* as previously described.[25] The modification of the hydroxy group at the C-3 of pyxinol gave the derivatives **3**−**6** as shown in Scheme 1 following our previously reported procedures.[20] The C-3 ester derivatives (**7a** and **7b**) were synthesized by selective esterification as shown in Scheme 2.

The modification of the hydroxy group at the C-12 of pyxinol is shown in Scheme 3. The oxidation of pyxinol with an excess of pyridinium chlorochromate (PCC) gave 3,12-diketopyxinol (**8**), subsequent selective reduction by $NaBH_4$ in isopropanol gave 12-ketopyxinol (**9**), and further oximation gave 12-oximinopyxinol (**10**). An unexpected stable imine (**11**) was attained by the reduction of oxime with $NaCNBH_3$ in the presence of $AcONH_4$ and titanium(III) chloride in methanol. Then reduction of the imine with $NaBH_4$ in methanol afforded the 12-aminopyxinol (**12**).

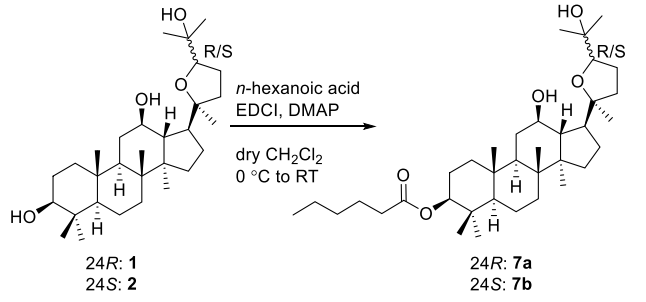
Another stereoisomer of pyxinol (**13**) was attained by reduction of **9** with $NaBH_4$ in methanol as shown in Scheme 4. The pyxinol derivative **16** modified at C-25 was synthesized as depicted in Scheme 5.

The NO-inhibitory activity of a compound is always related to its anti-inflammatory activity.[26−28] In order to avoid the cytotoxic interference of the synthesized compounds on NO-inhibitory effect, the cytotoxicity was evaluated. As shown in Figure 2a, compounds **1**−**11** and **16** at the test concentrations

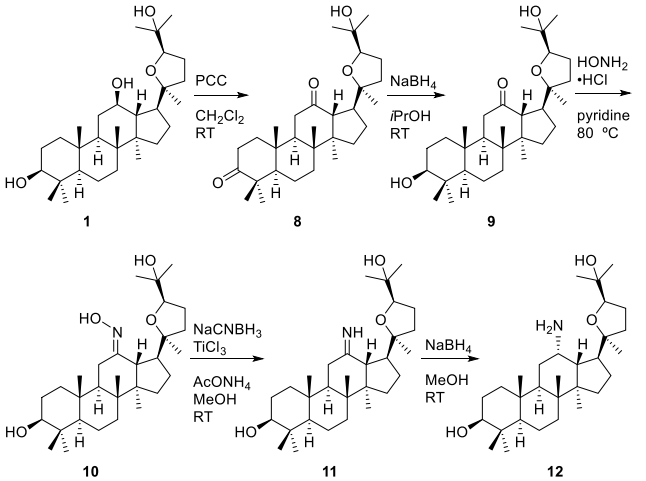
Scheme 1. Synthesis of Pyxinol Derivatives 1−6



Scheme 2. Synthesis of Pyxinol Derivatives 7a,b



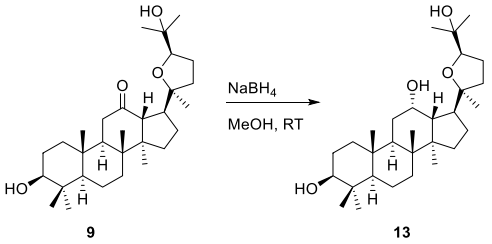
Scheme 3. Synthesis of Pyxinol Derivatives 8−12



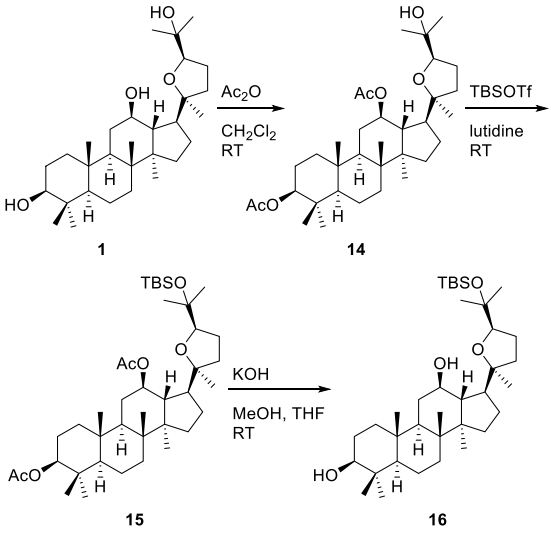
(20 μM) had no obvious effects on cytotoxicity in RAW264.7 cells, but compound **12** showed a notably significant

https://dx.doi.org/10.1021/acsmedchemlett.9b00562
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

Scheme 4. Synthesis of Isomer 13



Scheme 5. Synthesis of Pyxinol Derivative 16



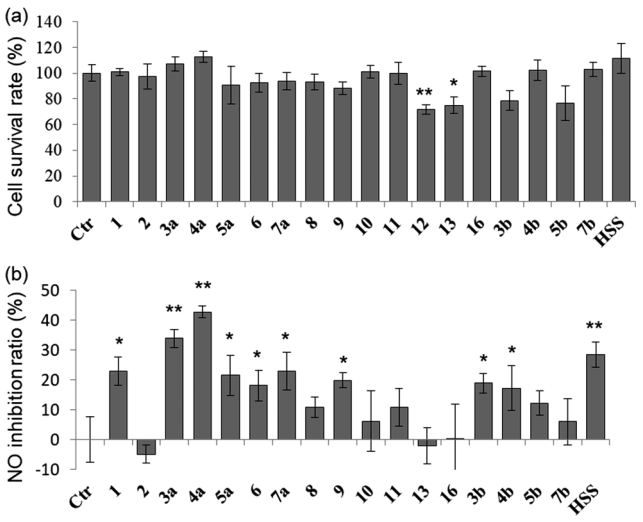


Figure 2. Inhibitory effects of pyxinol derivatives on LPS-induced NO generation in RAW264.7 cells: (a) effect of pyxinol derivatives and LPS on the cell survival by MTT assays; (b) inhibitory effect of pyxinol derivatives on LPS-induced NO generation. The cells were co-treated with pyxinol derivatives (20 μM) and LPS (1 μg/mL) for 24 h. Data were denoted as the mean ± SD (n = 3): (*) $p < 0.05$, (**) $p < 0.01$, relative to control group; Ctr, control; HSS, hydrocortisone sodium succinate.

cytotoxicity ($p < 0.01$). Therefore, compounds **1**−**11**, **13**, and **16** were further tested for their anti-inflammatory activities. The cells were co-treated with the pyxinol derivatives and LPS for 24 h. After that, the LPS-induced NO production was determined by the Griess assay. Hydrocortisone sodium succinate (20 μM) was the positive control (Figure S1). As shown in Figure 2b, relative to the control group, pyxinol (**1**) significantly inhibited the LPS-mediated NO release ($p < 0.05$) to a similar extent as that of the positive control. However, compound **2** had no obvious NO-inhibitory activity. Pyxinol and **2**, a pair of stereoisomers at C-24, were known as the main metabolites of PPD in human liver microsomes.[14,15] These results suggested that pyxinol may be the major active form of PPD *in vivo* on anti-inflammation and the stereochemistry at C-24 of pyxinol influences the NO-inhibitory capacity with an *R*-configuration favored.

Furthermore, pyxinol derivatives **3a**, **4a**, **5a**, **6**, **7a**, and **9** also exhibited significant NO-inhibitory activity ($p < 0.05$) (Figure 2b). The stereoisomer of pyxinol (**13**) had no obvious NO-inhibitory activity. In addition to **9**, pyxinol derivatives **10**, **11**, and **16**, in which the hydroxy groups at C-12 or C-25 were modified, also had no significant NO-inhibitory activity. Interestingly, 3-oximinopyxinol (**4a**) exhibited the most potent NO-inhibitory activity that was even stronger than that of the positive control, hydrocortisone sodium succinate, at the same concentration. Fernández-Herrera et al. have reported similar result that incorporating oxime functionality has improved the biological activities of the steroids.[29] These results indicated that pyxinol is an interesting skeleton for anti-inflammatory drug development and its derivative **4a** is a promising candidate and worthy of more detailed studies.

As shown in Figure 2, in the absence of any obvious cytotoxicity, derivatives **3a**, **4a**, **5a**, **6**, and **7a** modified at the C-3 of pyxinol showed significant NO-inhibitory activity similar to pyxinol, while derivatives **10**, **11**, and **16** modified at C-12 or C-25 had no obvious NO-inhibitory activity. This suggested that the hydroxy moieties at C-12 and C-25 of pyxinol are important for its anti-inflammatory activity and that the C-3 position is amenable to modification. Stereoisomers **1**, **2**, and **13** displayed dramatically dissimilar NO-inhibitory activities, which suggested that the stereochemistry of the hydroxy moiety at C-12 and/or the isopropanol moiety at C-24 may considerably influence the binding of the bioactive compounds to their target in macrophages. This was further confirmed by the comparison that the NO-inhibitory activity of derivatives **3b**, **4b**, **5b**, and **7b** with 24*S*-configuration was lower than that of corresponding derivatives **3a**, **4a**, **5a**, and **7a** with 24*R*-configuration. Stereoisomers **5a** and **6** with epimerization at C-3 displayed similar NO-inhibitory activities, which further suggested that the C-3 of pyxinol is a modifiable position for the discovery of anti-inflammatory drugs.

On the basis of the NO-inhibitory activity of pyxinol derivatives, a probable structure−activity relationship (SAR) could be predicted as shown in Figure 3. The hydroxy group at C-3 is a modifiable position, and the anti-inflammatory activity of a pyxinol derivative incorporating oxime functionality at C-3 was improved. Hydroxy moieties at C-12 and C-25 are crucial for the activity. Furthermore, the stereochemistry at C-12 and C-24 considerably influences the NO-inhibitory activity with an *R*-configuration preferred.

In LPS-activated macrophages, overproduction of proinflammatory cytokines such as TNF-α and interleukins and the resulting tissue damage can be inhibited by anti-inflammatory agents.[30] To confirm the anti-inflammatory capacity of **4a**, its inhibition effects on 1 μg/mL of LPS-induced proinflammatory cytokines (TNF-α, IL-6) were evaluated in RAW264.7 cells. Hydrocortisone sodium succinate (20 μM) was the positive control. Compared with the basal level in macrophages, the

https://dx.doi.org/10.1021/acsmedchemlett.9b00562
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

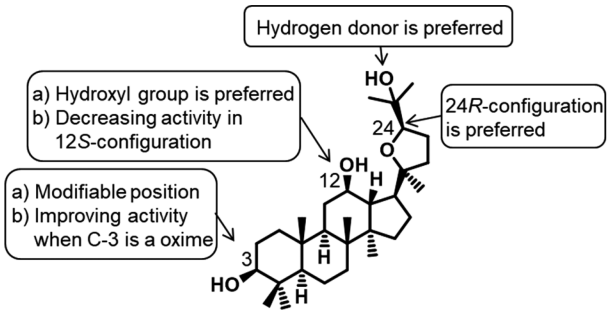


Figure 3. Preliminary structure−activity relationships of pyxinol derivatives.

LPS-stimulation considerably increased the TNF-α and IL-6 productions as expected (Figure 4). In the absence of any

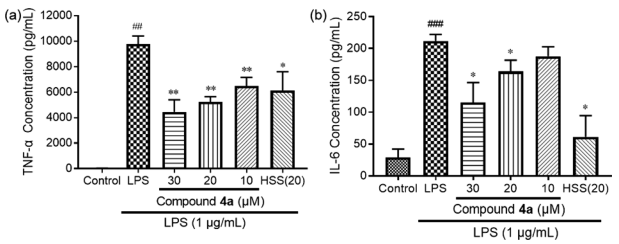


Figure 4. Inhibitory effect of **4a** on LPS-mediated (a) TNF-α and (b) IL-6 synthesis in RAW264.7 cells, which were pretreated with **4a** (10, 20, 30 μM) or HSS (20 μM) for 2 h before the LPS stimulation (1 μg/mL) for 4 h. The data are expressed as the mean ± SD (n = 3): (##) $p < 0.01$, (###) $p < 0.001$, relative to the control group; (*) $p < 0.05$, (**) $p < 0.01$, relative to LPS-stimulated group; HSS, hydrocortisone sodium succinate.

obvious cytotoxicity, the LPS-stimulated increase of TNF-α and IL-6 was suppressed concentration-dependently through pretreatment with **4a**. In particular, the inhibition effect of **4a** on LPS-stimulated TNF-α increase was better than that of hydrocortisone sodium succinate, a clinical steroid drug. These results indicated that **4a** can alleviate an over-immune-reaction by inhibiting the LPS-stimulated increase of proinflammatory cytokines.

Inflammatory mediators, such as PGs and NO, play pivotal roles in inflammatory diseases and are closely related to the expression of cyclooxygenase 2 (COX-2) and inducible NO synthase (iNOS).[31] The levels of COX-2 and iNOS were investigated in LPS-induced RAW264.7 cells by Western blotting. Hydrocortisone sodium succinate (20 μM) was the positive control. As shown in Figure 5, the LPS-stimulation obviously increased protein levels of COX-2 and iNOS as expected. Similar to the positive control, co-treatment with **4a** significantly decreased LPS-stimulated COX-2 and iNOS expressions, respectively. These inhibitions were in a dose-dependent manner. COX-2 and iNOS are positively modulated by the NF-κB pathway in inflammation.[32] The LPS-activated NF-κB pathway involves phosphorylation and degradation of iκB. Compound **4a** could suppress the LPS-induced iκB degradation, as well as its phosphorylation, similar to the positive control (hydrocortisone sodium succinate) (Figure 6). However, unlike that of hydrocortisone sodium succinate, the anti-inflammatory activities of **4a** could not be blocked by the RU-486 (the glucocorticoid receptor antagonist), even though their core structures were similar

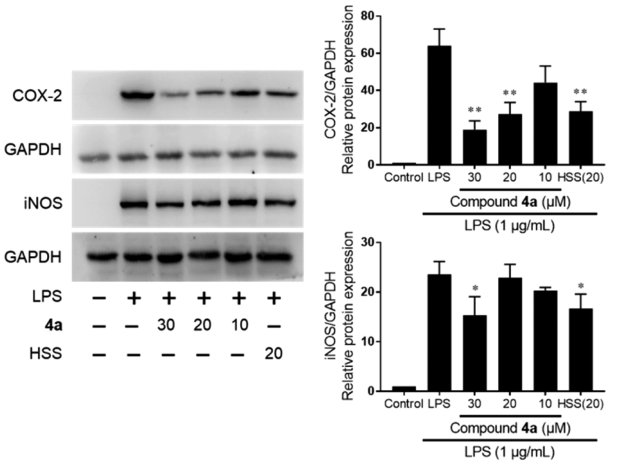


Figure 5. Effect of **4a** on LPS-stimulated COX-2 and iNOS protein expressions in RAW264.7 cells co-treated with LPS (1 μg/mL) and **4a** (10, 20, 30 μM) or HSS (20 μM) for 24 h. The data are expressed as the mean ± SD (n = 3) of three independent experiments: (*) $p < 0.05$, (**) $p < 0.01$, relative to LPS-stimulated group; HSS, hydrocortisone sodium succinate.

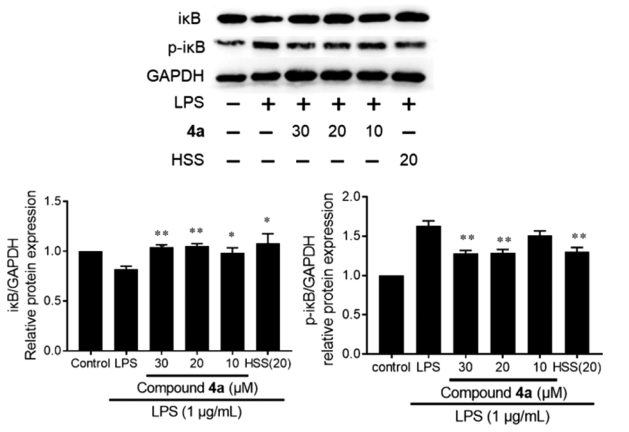


Figure 6. Effect of **4a** on LPS-stimulated NF-κB signaling pathway in RAW264.7 cells co-treated with LPS (1 μg/mL) and **4a** (10, 20, 30 μM) or HSS (20 μM) for 2 h. The data are expressed as the mean ± SD (n = 3): (*) $p < 0.05$, (**) $p < 0.01$, relative to LPS-stimulated group; HSS, hydrocortisone sodium succinate.

(data not shown). It suggested that the anti-inflammatory activities of **4a** are not associated with glucocorticoid receptor. Ahn et al. reported that the ginsenosides have valid binding affinity with NF-κB to exert their anti-inflammatory activity by molecular docking study.[33] It is interesting to further study the target of pyxinol analogs on anti-inflammatory activities. Anyway, our results revealed that **4a** could modulate the expression of proinflammatory proteins by inhibiting the LPS-activated NF-κB pathway in macrophages.

Inflammatory mediators, such as PGs and NO, are the pivotal signal transduction molecules of inflammation and are regulated by endogenous enzymes. PGs are mainly synthesized via mediation of COX-2 in LPS-stimulated macrophages.[34] Therefore, blocking protein expression of COX-2 is a valid approach to alleviate the inflammation by inhibiting PGs production. Additionally, NO is synthesized endogenously through a series of biochemical reactions by NO synthases. In inflammatory responses, NO is primarily regulated by iNOS.[35] The increased protein expression of iNOS can also cause

https://dx.doi.org/10.1021/acsmedchemlett.9b00562
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

chronic inflammation.[36] Therefore, blocking the protein expression of iNOS is one of the effective ways to treat inflammatory diseases. Compound **4a** could block both COX-2 and iNOS expression (Figure 5) to inhibit inflammatory mediators (Figure 2) in LPS-activated macrophages. These results all demonstrated that **4a** is a promising anti-inflammatory agent.

Pyxinol, the primary *in vivo* metabolite of PPD which acted as the pharmacophore of ginsenosides, was first chosen as the lead compound for the development of novel anti-inflammatory agents. Its derivatives were designed and synthesized by structural modification at the C-3, C-12, or C-25 of pyxinol based on the structure characteristics. The inhibitory activities of the pyxinol derivatives toward LPS-induced NO production were evaluated. The preliminary SAR results of these pyxinol derivatives indicated that pyxinol is responsible for the inhibitory activity of the LPS-induced NO release, the hydroxy at C-3 is a modifiable position, and the activity is improved when the C-3 is an oxime. Additionally, hydroxy groups at C-12 and C-25 are essential for the activity, and the stereochemistries at C-12 and C-24 significantly affect the NO-inhibitory activity with an *R*-configuration preferred. Among the tested compounds, 3-oximinopyxinol (**4a**) displayed the strongest inhibitory activity on LPS-induced NO synthesis and was even comparable to the clinical steroid drug. Furthermore, the results indicated that compound **4a** can reduce LPS-induced production of TNF-α and IL-6 and the protein expressions of COX-2 and iNOS via NF-κB pathway. Further studies on the mechanism responsible for the anti-inflammatory action and structural optimization are ongoing.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.9b00562.

Figure S1, experimental protocols, NMR spectra, and mass spectra (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Hui Yu − *College of Food Engineering, Ludong University, Yantai 264025, China*; Email: zoehuihui@hotmail.com

Gangqiang Yang − *School of Pharmacy, Key Laboratory of Molecular Pharmacology and Drug Evaluation (Yantai University), Ministry of Education, Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shandong, Yantai University, Yantai 264005, China*; orcid.org/0000-0001-6030-3570; Phone: +86-535-6706095; Email: oceanygq@ytu.edu.cn

Authors

Yixiao Sun − *School of Pharmacy, Key Laboratory of Molecular Pharmacology and Drug Evaluation (Yantai University), Ministry of Education, Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shandong, Yantai University, Yantai 264005, China*

Xiaojuan Fang − *School of Pharmacy, Key Laboratory of Molecular Pharmacology and Drug Evaluation (Yantai University), Ministry of Education, Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shandong, Yantai University, Yantai 264005, China*

Meng Gao − *School of Pharmacy, Key Laboratory of Molecular Pharmacology and Drug Evaluation (Yantai University), Ministry of Education, Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shandong, Yantai University, Yantai 264005, China*

Conghui Wang − *School of Pharmacy, Key Laboratory of Molecular Pharmacology and Drug Evaluation (Yantai University), Ministry of Education, Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shandong, Yantai University, Yantai 264005, China*

Hongyan Gao − *School of Pharmacy, Key Laboratory of Molecular Pharmacology and Drug Evaluation (Yantai University), Ministry of Education, Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shandong, Yantai University, Yantai 264005, China*

Wenjing Bi − *School of Pharmacy, Key Laboratory of Molecular Pharmacology and Drug Evaluation (Yantai University), Ministry of Education, Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shandong, Yantai University, Yantai 264005, China*

Hanhan Tang − *School of Pharmacy, Key Laboratory of Molecular Pharmacology and Drug Evaluation (Yantai University), Ministry of Education, Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shandong, Yantai University, Yantai 264005, China*

Yetong Cui − *School of Pharmacy, Key Laboratory of Molecular Pharmacology and Drug Evaluation (Yantai University), Ministry of Education, Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shandong, Yantai University, Yantai 264005, China*

Leiming Zhang − *School of Pharmacy, Key Laboratory of Molecular Pharmacology and Drug Evaluation (Yantai University), Ministry of Education, Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shandong, Yantai University, Yantai 264005, China*

Huaying Fan − *School of Pharmacy, Key Laboratory of Molecular Pharmacology and Drug Evaluation (Yantai University), Ministry of Education, Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shandong, Yantai University, Yantai 264005, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsmedchemlett.9b00562

Author Contributions

[§]Y.S. and X.F. contributed equally. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding

This study was supported by Key Research Project of Yantai City (Grant 2018ZHGY085), Key Research Project of Shandong Province (Grant 2019GSF108241), and National Natural Science Foundation of China (Grant 21502164).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors are grateful to National Demonstration Center for Experimental Pharmacy Education (Yantai University) for the support.

■ ABBREVIATIONS

COX-2, cyclooxygenase 2; IL-6, interleukin-6; iNOS, inducible NO synthase; LPS, lipopolysaccharide; NF-κB, nuclear factor κB; NO, nitric oxide; PCC, pyridinium chlorochromate; PG, prostaglandin; PPD, 20*S*-protopanoxadiol; SAR, structure−activity relationship; TNF-α, tumor necrosis factor α; TLC, thin layer chromatography

■ REFERENCES

(1) Medzhitov, R. Origin and physiological roles of inflammation. *Nature* **2008**, *454* (7203), 428−35.
(2) Kokiko-Cochran, O. N.; Godbout, J. P. The Inflammatory Continuum of Traumatic Brain Injury and Alzheimer's Disease. *Front. Immunol.* **2018**, *9*, 672−672.
(3) Donath, M. Y.; Shoelson, S. E. Type 2 diabetes as an inflammatory disease. *Nat. Rev. Immunol.* **2011**, *11* (2), 98−107.
(4) Song, Y. Q.; Zhao, F.; Zhang, L. M.; Du, Y.; Wang, T.; Fu, F. H. Ginsenoside Rg1 exerts synergistic anti-inflammatory effects with low doses of glucocorticoids in vitro. *Fitoterapia* **2013**, *91*, 173−179.
(5) Pereira-Leite, C.; Nunes, C.; Jamal, S. K.; Cuccovia, I. M.; Reis, S. Nonsteroidal Anti-Inflammatory Therapy: A Journey Toward Safety. *Med. Res. Rev.* **2017**, *37* (4), 802−859.
(6) Newman, D. J.; Cragg, G. M. Natural Products as Sources of New Drugs from 1981 to 2014. *J. Nat. Prod.* **2016**, *79* (3), 629−61.
(7) Helliwell, R. M.; ShioukHuey, C. O.; Dhuna, K.; Molero, J. C.; Ye, J. M.; Xue, C. C.; Stokes, L. Selected ginsenosides of the protopanaxdiol series are novel positive allosteric modulators of P2X7 receptors. *Br. J. Pharmacol.* **2015**, *172* (13), 3326−3340.
(8) Kim, J. H.; Yi, Y. S.; Kim, M. Y.; Cho, J. Y. Role of ginsenosides, the main active components of Panax ginseng, in inflammatory responses and diseases. *J. Ginseng Res.* **2017**, *41* (4), 435−443.
(9) Yang, Y.; Lee, J.; Rhee, M. H.; Yu, T.; Baek, K. S.; Sung, N. Y.; Kim, Y.; Yoon, K.; Kim, J. H.; Kwak, Y. S.; Hong, S.; Kim, J.-H.; Cho, J. Y. Molecular mechanism of protopanaxadiol saponin fraction-mediated anti-inflammatory actions. *J. Ginseng Res.* **2015**, *39* (1), 61−68.
(10) Ren, H.-c.; Sun, J.-g.; Wang, G.-j.; A, J.-y.; Xie, H.-t.; Zha, W.-b.; Yan, B.; Sun, F.-z.; Hao, H.-p.; Gu, S.-h.; Sheng, L.-s.; Shao, F.; Shi, J.; Zhou, F. Sensitive determination of 20(S)-protopanaxadiol in rat plasma using HPLC−APCI-MS: Application of pharmacokinetic study in rats. *J. Pharm. Biomed. Anal.* **2008**, *48* (5), 1476−1480.
(11) Kim, D.-H. Gut microbiota-mediated pharmacokinetics of ginseng saponins. *J. Ginseng Res.* **2018**, *42* (3), 255−263.
(12) Lee, S. H.; Seo, G. S.; Ko, G.; Kim, J. B.; Sohn, D. H. Anti-inflammatory activity of 20(S)-protopanaxadiol: enhanced heme oxygenase 1 expression in RAW 264.7 cells. *Planta Med.* **2005**, *71* (12), 1167−70.
(13) Yosioka, I.; Yamauchi, H.; Kitagawa, I. Lichen Triterpenoids. V. On the Neutral Triterpenoids of Pyxine endochrysina NYL. *Chem. Pharm. Bull.* **1972**, *20* (3), 502−513.
(14) Li, L.; Chen, X.; Li, D.; Zhong, D. Identification of 20(S)-protopanaxadiol metabolites in human liver microsomes and human hepatocytes. *Drug Metab. Dispos.* **2011**, *39* (3), 472−83.
(15) Wang, W.; Wang, L.; Wu, X.; Xu, L.; Meng, Q.; Liu, W. Stereoselective formation and metabolism of 20(s)-protopanaxadiol ocotillol type epimers in vivo and in vitro. *Chirality* **2015**, *27* (2), 170−6.
(16) Wang, W.; Wu, X.; Wang, L.; Meng, Q.; Liu, W. Stereoselective Property of 20 (S)-Protopanaxadiol Ocotillol Type Epimers Affects Its Absorption and Also the Inhibition of P-Glycoprotein. *PLoS One* **2014**, *9* (6), No. e98887.
(17) Wang, T.; Meng, Q.; Zhang, J.; Bi, Y.; Jiang, N. Study on the structure-function relationship of 20(S)-panaxadiol and its epimeric derivatives in myocardial injury induced by isoproterenol. *Fitoterapia* **2010**, *81* (7), 783−7.
(18) Bi, Y.; Wang, T.; Meng, Q.; Zhang, J.; Wang, L.; Li, Q.; Zhao, F.; Sun, H. Synthesis and Myocardial Ischemia Protective Effect of Ocotillol-Type Derivatives. *Rec. Nat. Prod.* **2012**, *6* (3), 242−254.
(19) Yang, G. Q.; Yang, Y. T.; Yang, Q.; Li, Y.; Jiang, Y. T.; Fu, F. H.; Wang, H. B. Novel Fluorescent Pyxinol-Based Probes: Design, Synthesis and Biological Evaluation. *Chin. J. Org. Chem.* **2017**, *37* (8), 2109−2114.
(20) Ren, Q.; Yang, G.; Guo, M.; Guo, J.; Li, Y.; Lu, J.; Yang, Q.; Tang, H.; Li, Y.; Fang, X.; Sun, Y.; Qi, J. G.; Tian, J.; Wang, H. Design, synthesis, and discovery of ocotillol-type amide derivatives as orally available modulators of P-glycoprotein-mediated multidrug resistance. *Eur. J. Med. Chem.* **2019**, *161*, 118−130.
(21) Liu, J.; Xu, Y. R.; Yang, J. J.; Wang, W. Z.; Zhang, J. Q.; Zhang, R. M.; Meng, Q. G. Discovery, semisynthesis, biological activities, and metabolism of ocotillol-type saponins. *J. Ginseng Res.* **2017**, *41* (3), 373−378.
(22) Bi, Y.; Yang, J.; Ma, C.; Liu, Z. Y.; Zhang, T. T.; Zhang, X. C.; Lu, J.; Meng, Q. G. Design, synthesis and in vitro NO-releasing activities of ocotillol-type furoxans. *Pharmazie* **2015**, *70* (4), 213−218.
(23) Zhou, Z.; Ma, C.; Zhang, H.; Bi, Y.; Chen, X.; Tian, H.; Xie, X.; Meng, Q.; Lewis, P. J.; Xu, J. Synthesis and biological evaluation of novel ocotillol-type triterpenoid derivatives as antibacterial agents. *Eur. J. Med. Chem.* **2013**, *68*, 444−453.
(24) Bi, Y.; Yang, X.; Zhang, T.; Liu, Z.; Zhang, X.; Lu, J.; Cheng, K.; Xu, J.; Wang, H.; Lv, G.; Lewis, P. J.; Meng, Q.; Ma, C. Design, synthesis, nitric oxide release and antibacterial evaluation of novel nitrated ocotillol-type derivatives. *Eur. J. Med. Chem.* **2015**, *101*, 71−80.
(25) Yang, G. Q.; Li, Y.; Yang, Q.; Yue, X.; Yao, L.; Jiang, Y. T. Simple and Efficient Synthesis of Pseudoginsenoside HQ. *Chin. J. Org. Chem.* **2017**, *37* (6), 1530−1536.
(26) Li, B.; Cai, S.; Yang, Y. A.; Chen, S. C.; Chen, R.; Shi, J. B.; Liu, X. H.; Tang, W. J. Novel unsaturated glycyrrhetic acids derivatives: Design, synthesis and anti-inflammatory activity. *Eur. J. Med. Chem.* **2017**, *139*, 337−348.
(27) Zhao, F.; Gao, Z. T.; Jiao, W. H.; Chen, L. P.; Chen, L.; Yao, X. S. In vitro Anti-Inflammatory Effects of Beta-Carboline Alkaloids, Isolated from Picrasma quassioides, through Inhibition of the iNOS Pathway. *Planta Med.* **2012**, *78* (18), 1906−1911.
(28) Sun, C. P.; Qiu, C. Y.; Zhao, F.; Kang, N.; Chen, L. X.; Qiu, F. Physalins V-IX, 16,24-cyclo-13,14-seco withanolides from Physalis angulata and their antiproliferative and anti-inflammatory activities. *Sci. Rep.* **2017**, *7*, 4057.
(29) Zeferino-Díaz, R.; Olivera-Castillo, L.; Dávalos, A.; Grant, G.; Kantún-Moreno, N.; Rodriguez-Canul, R.; Bernès, S.; Sandoval-Ramírez, J.; Fernández-Herrera, M. A. 22-Oxocholestane oximes as potential anti-inflammatory drug candidates. *Eur. J. Med. Chem.* **2019**, *168*, 78−86.
(30) Xia, Q.; Bao, X.; Sun, C.; Wu, D.; Rong, X.; Liu, Z.; Gu, Y.; Zhou, J.; Liang, G. Design, synthesis and biological evaluation of novel 2-sulfonylindoles as potential anti-inflammatory therapeutic agents for treatment of acute lung injury. *Eur. J. Med. Chem.* **2018**, *160*, 120−132.
(31) Tsatsanis, C.; Androulidaki, A.; Venihaki, M.; Margioris, A. N. Signalling networks regulating cyclooxygenase-2. *Int. J. Biochem. Cell Biol.* **2006**, *38* (10), 1654−61.
(32) Lee, S. M.; Kim, N.-H.; Lee, S.; Kim, Y. N.; Heo, J. D.; Jeong, E. J.; Rho, J.-R. Deacetylphylloketal, a New Phylloketal Derivative from a Marine Sponge, Genus Phyllospongia, with Potent Anti-Inflammatory Activity in In Vitro Co-Culture Model of Intestine. *Mar. Drugs* **2019**, *17*, 634.

https://dx.doi.org/10.1021/acsmedchemlett.9b00562
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

(33) Ahn, S.; Siddiqi, M. H.; Noh, H.-Y.; Kim, Y.-J.; Kim, Y.-J.; Jin, C.-G.; Yang, D.-C. Anti-inflammatory activity of ginsenosides in LPS-stimulated RAW 264.7 cells. *Sci. Bull.* **2015**, *60* (8), 773−784.
(34) Tsatsanis, C.; Androulidaki, A.; Dermitzaki, E.; Gravanis, A.; Margioris, A. N. Corticotropin releasing factor receptor 1 (CRF1) and CRF2 agonists exert an anti-inflammatory effect during the early phase of inflammation suppressing LPS-induced TNF-alpha release from macrophages via induction of COX-2 and PGE2. *J. Cell. Physiol.* **2007**, *210* (3), 774−83.
(35) Kong, F. H.; Lee, B. H.; Wei, K. 5-Hydroxymethylfurfural Mitigates Lipopolysaccharide-Stimulated Inflammation via Suppression of MAPK, NF-B and mTOR Activation in RAW 264.7 Cells. *Molecules* **2019**, *24* (2), 275.
(36) Suschek, C. V.; Schnorr, O.; Kolb-Bachofen, V. The role of iNOS in chronic inflammatory processes in vivo: is it damage-promoting, protective, or active at all? *Curr. Mol. Med.* **2004**, *4* (7), 763−75.

https://dx.doi.org/10.1021/acsmedchemlett.9b00562
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX