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Synthesis of norlignans and in vitro inhibitory activity of antigen-induced degranulation

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ABSTRACT

The synthesis and biological evaluation of a series of novel norlignans are described. Norlignans were evaluated for their inhibitory activity on the release of β-hexosaminidase, a marker of degranulation, from RBL-2H3 cells induced by the IgE–antigen complex. The results showed that norlignans **4c** and **4e** potently inhibited degranulation, with IC_{50} values of 18.3 and 17.9 μM, respectively.

Mast cells and basophils play crucial roles in type I allergy induced by antigens such as foods, dust, mites, pollen, cosmetics and medicines.[1–3] When the human body is stimulated by antigens, B cells and plasma cells produce and release antigen-specific immunoglobulin E (IgE) antibodies that bind to high affinity IgE receptors (FcεRI) on the surface membranes of mast cells or basophils.[4] The interactions of multivalent antigens with IgE on the surfaces of mast cells lead to cross-linking of the FcεRI–IgE complex, and triggers degranulation, the immediate release of granules containing histamine and serotonin as potent inflammatory mediators.[5] These mediators induce a variety of biological processes, including inflammation of surrounding tissues, vasodilation, mucous secretion, and bronchoconstriction. β-Hexosaminidase enzyme, which is stored in the secretory granules of mast cells, is released concomitantly with histamine when mast cells are immunologically activated.[6] The activity of β-hexosaminidase release into the medium has therefore been used as a marker of mast cell degranulation.[7] Rat basophilic leukemia 2H3 (RBL-2H3) cells, tumor analog of mast cells, have been used as mast cell models in vitro for screening the effects of unknown compounds on histamine release and β-hexosaminidase release activity.[8]

Norlignans are abundant in the heartwood of many coniferous trees and in some monocotyledonous plants,[9] and possess a wide spectrum of biological activities such as anti-cancer/anti-inflammatory,[10] anti-complement,[11] anti-fungal activity,[12] testosterone 5α-reductase inhibition,[13] and cyclic AMP phosphodiesterase inhibition.[14]

Naturally occurring norlignan are a class of natural phenolic compounds with diphenylpentane carbon skeletons (C_6–C_5–C_6). Hinokiresinol (**1a**), the E-isomer of nyasol (**2**), is a typical example of such a norlignan (Fig. 1). Hinokiresinol (**1a**) was first isolated from the heartwood of *Chamaecyparis obtuse* in 1965,[15] and was found to display appreciable estrogen receptor binding activity[16] and some antiplasmodial activity.[17]

We recently found that nyasol (**2**) and its derivatives, isolated from *Anemarrhena asphodeloides*, act as powerful inhibitors of antigen-induced degranulation, and have the potential to be useful therapies for allergic disorders such as asthma and atopic dermatitis.[18] In view of these interesting biological activities of norlignans, we report here the inhibitory activity of hinokiresionol derivatives including synthetic intermediates on antigen-induced degranulation.

General routes for the preparation of hinokiresinol derivatives are outlined in Scheme 1. Chalcones **3a–d**, prepared from the corresponding acetophenones and aldehydes through Claisen–Schmidt condensation, were smoothly converted to β-vinyl ketones **4a–d** by the addition of Grignard reagent in the presence of CuI. The reduction of the ketones **4a–d** with $NaBH_4$ yielded the alcohols **5a–d**, which were reacted with 1 M HCl to produce

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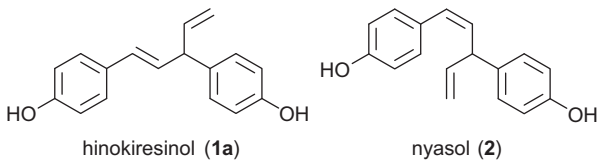
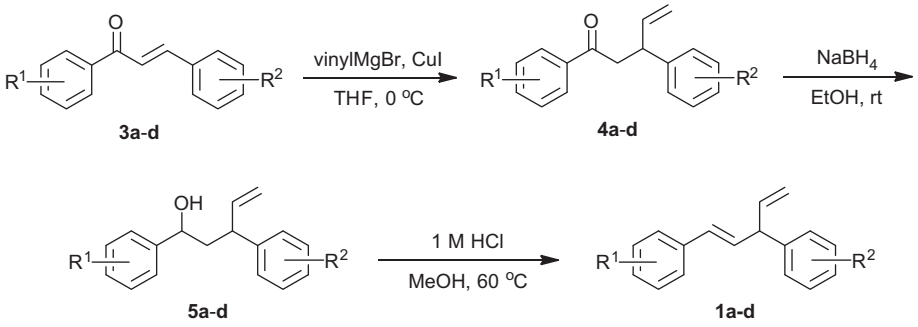


Figure 1. Structures of hinokiresinol (**1a**) and nyasol (**2**).

hinokiresinol derivatives **1a**–**d** with high *trans*-stereoselectivity (*trans*:*cis* = 20 > 1). The structures of synthesized compounds were determined by the characterization of spectroscopic data (^{1}H and ^{13}C NMR) and mass spectroscopy analysis.

The inhibitory activity of antigen-induced degranulation by synthesized norlignans was tested in an in vitro β-hexosaminidase release inhibition assay using RBL-2H3 cells stimulated by DNP-BSA, according to described protocols,[19] and the results of their inhibitory activities are summarized in Figure 2. 1,3-Bis(4-hydroxyphenyl)pent-4-en-1-one (**4a**) inhibited 50% of β-hexosaminidase release activity at a concentration of 34.6 μM. On the other hand, compound **4b** with a conversion of the bis-hydroxyl groups in **4a** to bis-methoxy groups had lower activity



Scheme 1. Strategy for the synthesis of hinokiresinol derivatives.

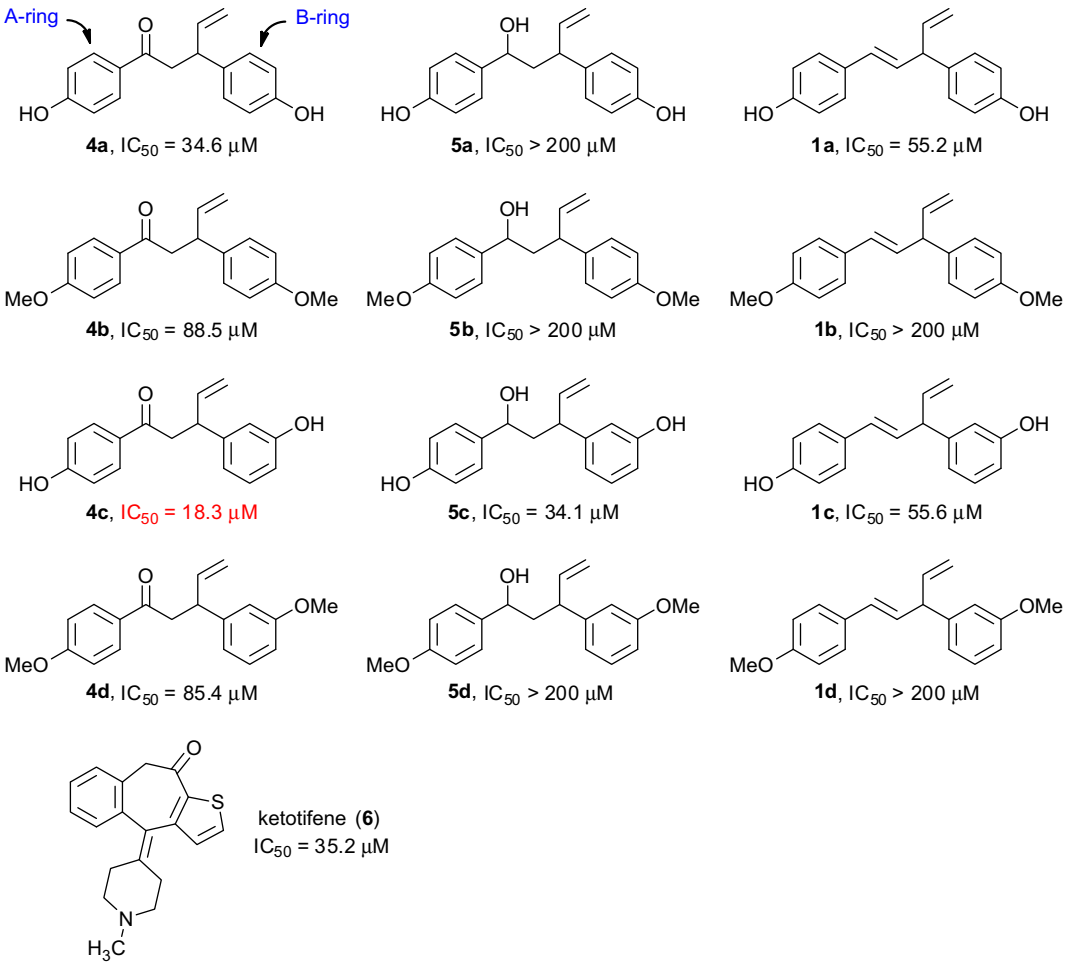


Figure 2. Structures and inhibitory activities of norlignans (**1a**–**d**, **4a**–**d**, and **5a**–**d**) and ketotifene (**6**) on β-hexosaminidase release from RBL-2H3 cells stimulated by DNP–BSA.

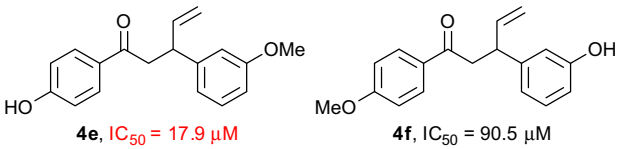


Figure 3. Structures and inhibitory activities of compounds **4e** and **4f** on β-hexosaminidase release from RBL-2H3 cells stimulated by DNP–BSA.

(IC_{50} = 88.5 μM). The alcohols **5a** and **5b**, prepared from the corresponding compounds **4a** and **4b**, showed no activity, even though at a high concentration (>200 μM). Hinokiresinol (**1a**) and dimethylhinokiresinol (**1b**) were relatively less effective than norlignan **4a** for β-hexosaminidase release inhibition. To investigate the effects of B-ring substituents of **4a**, *m*-hydroxylated derivatives (**1c**, **4c** and **5c**) and *m*-methoxylated derivatives (**1d**, **4d** and **5d**) were examined for inhibitory activity of β-hexosaminidase release. As shown in Figure 2, norlignan **4c** displayed the most potent activity (IC_{50} = 18.3 μM) among the tested norlignans. The activity of **4c** was about twofold stronger than that of second-generation H_1-antihistamine ketotifen (**6**, IC_{50} = 35.2 μM), used to treat allergic conjunctivitis.[20]

To further explore the effects of the *m*-hydroxyl moiety on the B-ring and the *p*-hydroxyl moiety on the A-ring, we examined the β-hexosaminidase release inhibitory activity of compounds **4e** and **4f**, as shown in Figure 3. Interestingly, compound **4e** with *m*-methoxy group on B-ring showed comparable activity (IC_{50} = 17.9 μM), whereas **4f** with *p*-methoxy group on A-ring was significantly less biologically active (IC_{50} = 90.5 μM). Hence, these results indicate that β-vinyl ketone structures with a *p*-hydroxyl moiety on A-ring and *m*-substituted groups (OH or OMe) on the B-ring are crucial for inhibitory activity of β-hexosaminidase release.

In conclusion, we synthesized various norlignans and evaluated their inhibitory activities against β-hexosaminidase release from RBL-2H3 cells stimulated by DNP-BSA. In general, the β-vinyl ketone series were more potent than the β-vinyl alcohol and hinokiresinol series. In particular, β-vinyl ketones **4c** and **4e** showed about twofold stronger inhibitory activity than the well-known anti-allergic drug ketotifen. These results show that β-vinyl ketones represent a new class of strong β-hexosaminidase release inhibitors. Further synthesis and biological evaluation of functionalized β-vinyl ketones are currently under way to elucidate their potential therapeutic uses.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.04.033.

References and notes

1. Stevens, R. L.; Austen, K. F. *Immunol. Today* **1989**, *10*, 381.
2. Wuthrich, B. *Int. Arch. Allergy Appl. Immunol.* **1989**, *90*, 3.
3. Bruhns, P.; Fremont, S.; Daeron, M. *Curr. Opin. Immunol.* **2005**, *17*, 662.
4. Gould, H. J.; Sutton, B. J.; Beavil, A. J.; Beavil, R. L.; McCloskey, N.; Coker, H. A.; Fear, D.; Smurthwaite, L. *Annu. Rev. Immunol.* **2003**, *21*, 579.
5. (a) Metcalfe, D. D.; Baram, D.; Mekori, Y. A. *Physiol. Rev.* **1997**, *77*, 1033; (b) Corry, D. B.; Kheradmand, F. *Nature* **1999**, *402*, B18.
6. Lobe, T. E.; Schwartz, M. Z.; Richardson, C. J.; Rassin, D. K.; Gourley, W. K.; Srivastava, S. K.; Storozuk, R. B. *J. Pediatr. Surg.* **1983**, *18*, 449.
7. Matsuda, H.; Morikawa, T.; Ueda, K.; Managi, H.; Yoshikawa, M. *Bioorg. Med. Chem.* **2002**, *10*, 3123.
8. Choi, O. H.; Adelstein, R. S.; Beaven, M. A. *J. Biol. Chem.* **1994**, *269*, 536.
9. Suzuki, T.; Umezawa, T. *J. Wood Sci.* **2007**, *53*, 273.
10. (a) Wróbel, A.; Eklund, P.; Bobrowska-Hägerstrand, M.; Hägerstrand, H. *Anticancer Res.* **2010**, *30*, 4423; (b) Song, M.-C.; Yang, H.-J.; Han, M.-W.; Kim, D.-K.; Kwon, B.-M.; Lee, K.-T.; Baek, N.-I. *J. Appl. Biol. Chem.* **2008**, *51*, 66.
11. Min, B.-S.; Oh, S.-R.; Ahn, K.-S.; Kim, J.-H.; Lee, J.; Kim, D.-Y.; Kim, E.-H.; Lee, H.-K. *Planta Med.* **2004**, *70*, 1210.
12. Iida, Y.; Oh, K.-B.; Saito, M.; Matsuoka, H.; Kurata, H.; Natsume, M.; Abe, H. *J. Agric. Food Chem.* **1999**, *47*, 584.
13. Matsuda, H.; Sato, N.; Yamazaki, M.; Naruto, S.; Kubo, M. *Biol. Pharm. Bull.* **2001**, *24*, 586.
14. Nikaido, T.; Ohmoto, T.; Noguchi, H.; Kinoshita, T.; Saitoh, H.; Sankawa, U. *Planta Med.* **1981**, *43*, 18.
15. Hirose, Y.; Oishi, N.; Nagaki, H.; Nakatsuka, T. *Tetrahedron Lett.* **1965**, *6*, 3665.
16. Minami, E.; Taki, M.; Takaishi, S.; Iijima, Y.; Tsutsumi, S.; Akiyama, T. *Chem. Pharm. Bull.* **2000**, *48*, 389.
17. (a) Lassen, P. R.; Skytte, D. M.; Hemmingsen, L.; Nielsen, S. F.; Freedman, T. B.; Nafie, L. A.; Christensen, S. B. *J. Nat. Prod.* **2005**, *68*, 1603; (b) Skytte, D. M.; Nielsen, S. F.; Chen, M.; Zhai, L.; Olsen, C. E.; Christensen, S. B. *J. Med. Chem.* **2006**, *49*, 436.
18. (a) Bak, J. P.; Kim, J. B.; Park, J. H.; Yang, Y. J.; Kim, I. S.; Choung, E. S.; Kang, S. C. *J. Korean Soc. Appl. Biol. Chem.* **2011**, *54*, 367; (b) The IC_{50} value of natural nyasol (**2**), isolated from Anemarrhena asphodeloides, on β-hexosaminidase release inhibition is 11.5 μM.
19. Choi, O. H.; Kim, J. H.; Kinet, J. P. *Nature* **1996**, *380*, 634.
20. Kidd, M.; McKenzie, S. H.; Steven, I.; Cooper, C.; Lanz, R. *Br. J. Ophthalmol.* **2003**, *87*, 1206.