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

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\textbf{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 \(\beta\)-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\textsubscript{50} values of 18.3 and 17.9 \(\mu\text{M}\), respectively.

Mast cells and basophils play crucial roles in type I allergy induced by antigens such as foods, dust, mites, pollen, cosmetics and medicines.\textsuperscript{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 (Fc\textsubscript{e}RI) on the surface membranes of mast cells or basophils.\textsuperscript{4} The interactions of multivalent antigens with IgE on the surfaces of mast cells lead to cross-linking of the Fc\textsubscript{e}RI-IgE complex, and triggers degranulation, the immediate release of granules containing histamine and serotonin as potent inflammatory mediators.\textsuperscript{5} These mediators induce a variety of biological processes, including inflammation of surrounding tissues, vasodilation, mucous secretion, and bronchoconstriction. \(\beta\)-Hexosaminidase enzyme, which is stored in the secretory granules of mast cells, is released concomitantly with histamine when mast cells are immunologically activated.\textsuperscript{6} The activity of \(\beta\)-hexosaminidase release into the medium has therefore been used as a marker of mast cell degranulation.\textsuperscript{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 \(\beta\)-hexosaminidase release activity.\textsuperscript{8}

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

Naturally occurring norlignan are a class of natural phenolic compounds with diphenylpentane carbon skeletons (C\textsubscript{6}–C\textsubscript{5}–C\textsubscript{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 \textit{Chamaecyparis obtuse} in 1965,\textsuperscript{15} and was found to display appreciable estrogen receptor binding activity\textsuperscript{16} and some antiplasmodial activity.\textsuperscript{17}

We recently found that nyasol (2) and its derivatives, isolated from \textit{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.\textsuperscript{18} In view of these interesting biological activities of norlignans, we report here the inhibitory activity of hinokiresinol 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 \(\beta\)-vinyl ketones 4a–d by the addition of Grignard reagent in the presence of Cu. The reduction of the ketones 4a–d with NaBH\textsubscript{4} yielded the alcohols 5a–d, which were reacted with 1 M HCl to produce...
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 (1H and 13C 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.
at a high concentration (>200 \( \mu M \)). The alcohols 5a and 5b, prepared from the corresponding compounds 4a and 4b, showed no activity, even though at a high concentration (>200 \( \mu M \)). Hinokiresinol (1a) and dimethylhinokiresinol (1b) were relatively less effective than norlignan 4a for \( \beta \)-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 \( \beta \)-hexosaminidase release. As shown in Figure 2, norlignan 4c displayed the most potent activity (IC\(_{50} = 18.3 \mu M \)) among the tested norlignans. The activity of 4c was about twofold stronger than that of second-generation H1-antihistamine ketotifen (6, IC\(_{50} = 35.2 \mu M \)), 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 \( \beta \)-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\(_{50} = 17.9 \mu M \)), whereas 4f with \( p \)-methoxy group on A-ring was significantly less biologically active (IC\(_{50} = 90.5 \mu M \)). Hence, these results indicate that \( \beta \)-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 \( \beta \)-hexosaminidase.

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

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