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Synthesis and anti-inflammatory activity of indole glucosinolates

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ABSTRACT

The nitronate and nitrovinyl methods to synthesize indole glucosinolates (GLs) have been investigated. The results were applied to generally the most prevalent natural indole glucosinolates to synthesize 4-methoxyglucobrassicin (MGB) and neo-glucobrassicin (NGB) in moderate overall yield for the first time. The anti-inflammatory activity of the synthetic indole GLs was determined by inhibition of TNF-α secretion in LPS-stimulated THP-1 cells. The data showed that glucobrassicin (GB) exhibited higher activity than other synthetic indolyl GLs.

1. Introduction

Indole glucosinolates (GLs) (Fig. 1) are β-thioglucoside *N*-hydroxysulfates with an indole ring as a side chain (R). Indole GLs are natural compounds which have been found in parts of plants of Brassicaceae (Cruciferae), Capparidaceae, Tovariaceae and Resedaceae.[1,2] There have been around thirteen indole GLs isolated and identified.[2] However, of the indole GLs, glucobrassicin (GB, (indol-3-yl)methyl glucosinolate), 4-methoxyglucobrassicin (MGB, (4-methoxy(indol-3-yl))methyl glucosinolate) and neo-glucobrassicin (NGB, (1-methoxy(indol-3-yl))methyl glucosinolate) have been of interest because of their stability (in room temperature and atmosphere conditions), presence in common vegetables (Savoy cabbage, Brussels sprouts, Calabrese and cauliflower) as well as useful biological activity.[1] These indole GLs and/or the hydrolysis products of these compounds have potentially useful biological effects which, include effects on the activity of drug metabolizing enzyme systems, effects on chemical carcinogenesis in experimental animals, and other effects (goitrogenicity and nitrosation of indole compounds).[1,3]

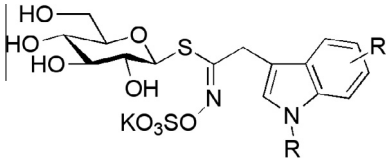

Figure 1. A general structure of indole glucosinolates.

The first synthesis of GB was reported using the so-called nitronate pathway.[4] This method was then developed to synthesize labeled GB and indole desulfoglucosinolates.[5,6] Indole GLs were also synthesized using the nitrovinyl pathway. Cassel et al. reported the synthesis of GB and its derivatives.[7] Following this method, GB was obtained in 11% overall yield from the starting material, indole-3-carbaldehyde.

The indole GLs have potentially useful biological and medicinal properties, however, previous studies have only synthesized GB and desulfo-indole GLs.[8] The syntheses of NGB, MGB and the

Abbreviations: DBU, 1,5-diazabicyclo [5.4.0]undecene; DCM, dichloromethane; DME, 1,2-dimethoxyethane; DMF, *N,N*-dimethylformamide; DMF.DMA, dimethylformamide dimethyl acetal; ELISA, enzyme-linked immunosorbent assay; ESI, electrospray ionization; FTMS, Fourier transform mass spectrometry; GB, glucobrassicin; GLs, glucosinolates; HESI, heated electrospray ionization; HRMS, high resolution mass spectrometry; IR, infra-red; LPS, lipopolysaccharides; MGB, 4-methoxyglucobrassicin; MS, mass spectrometry; NCS, *N*-chlorosuccinimide; NGB, neo-glucobrassicin; NMR, nuclear magnetic resonance; PMA, phorbol-12-myristate-13-acetate; THF, tetrahydrofuran; TLC, thin layer chromatography; TOMAC, tri(*n*-octyl)methyl ammonium chloride; TNF-α, tumor necrosis factor alpha; UV, ultra-violet.

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anti-inflammatory activity of indole GLs have not been reported. Here, we report comparison of the nitronate and nitrovinyl pathways in the synthesis of indole GLs and success in the synthesis of GB, MGB and NGB, and their bioassay as anti-inflammatory compounds. These three exemplary natural products were chosen for their prevalence in Brassica species and general stability.

2. Results

2.1. Synthesis of indole glucosinolates

2.1.1. Synthesis of 3-(2-nitroethyl)indole and derivatives

From previous studies,[4,7] the aldoxime pathway could not be applied for the synthesis of an indole hydroxymoyl chloride. An attempt to synthesize indole chlorooxime following the aldoxime pathway was unsuccessful. This may be because in the presence of NCS, the indole rings are decomposed. Study of the reaction mixture by NMR and MS showed that there was no indole ring or final product present. These results were well aligned with the literature,[4,6] and also confirmed that hydroxymoyl chlorides of indole rings should be synthesized by the nitronate or nitrovinyl pathways rather than the aldoxime pathway.

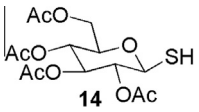
To synthesize 3-(nitroethenyl)indole, the starting material 1-methoxyindole-3-carbaldehyde **1** was synthesized. The compound can be formed by several methods. Selvakumar and Rajulu reported the synthesis of indole **1** from 1-fluoro-2-nitrobenzene.[9] However, the limitation of this method is a low yield of **1** (only 26% yield over five steps). Pedras and Okinyo reported the synthesis of **1** via the oxidation of indole by Na_2WO_4/H_2O_2 in considerable yield (40% yield over three steps).[10] The disadvantage of this method is use of the toxic chemical dimethyl sulfate.

After considering the limitations of these pathways, Somei's method was chosen to synthesize the aldehyde (Scheme 1).[11–13] The 1-hydroxyindole **4** was synthesized from 2-nitrotoluene **2** by a two-step method.[13] The compound **2** was reacted with dimethylformamide dimethyl acetal (DMF.DMA) in the presence of 1,5-diazabicyclo [5.4.0] undecene (DBU) in dry DMF to yield the enamine **3**. After evaporation of the solvent in vacuo, the residual enamine **3** was cyclised by treatment with zinc powder in ether solution in the presence of NH_4Cl to form 1-hydroxyindole **4**. The indole **4** was then reacted with iodomethane in the presence of NaOH and tri(*n*-octyl)methyl ammonium chloride (TOMAC) to yield the indole **5** (70% overall yield). Conventional Vilsmeier–Haack reaction of **5** with dry DMF and phosphorus oxychloride readily afforded 1-methoxyindole-3-carbaldehyde **1** in 96% yield.[14] As a result, the overall yield of **1** was improved to 67% yield compared to 26% yield by Selvakumar's method and 40% yield by Pedras' method.[9,10]

The synthesis of 3-(nitroethyl)indoles employed a literature method (Scheme 2).[4,6,12] The conventional Henry reaction of the aldehydes **1**, **6** and **7** (compounds **6** and **7** were commercially available) with nitromethane in the presence of ammonium acetate at reflux for 2 h yielded the nitroalkenes **8**–**10** in 79%, 78% and 99% yields, respectively. The nitrovinyl group was then reduced by $NaBH_4$ in THF and MeOH to form the nitroalkanes **11**–**13** in 75–79% yield.[15] The 3-(nitroethyl)indoles were then used for chlorination and coupling processes.

2.1.2. Synthesis of indolylthiohydroxymates

To evaluate the efficiency of the nitronate and nitrovinyl pathways in the synthesis of indole GLs, the coupling of the nitrovinyl or nitroethyl indoles with 2,3,4,6-tetra-*O*-acetyl-l-thio-β-D-glucopyranose **14** (**14** was synthesized by literature methods)[16,17] was carried out by both methods. In the nitrovinyl pathway the coupling was conducted following Cassel's method (Scheme 3).[7] In the first step, the nitroalkenes **8**–**10** were chlorinated using $TiCl_4$ in the presence of triethylsilane to form indole hydroxymoyl chlorides, which are not stable and not well isolated using column chromatography. Therefore, the hydroxymoyl chlorides were directly coupled with thiol **14** in the presence of catalytic triethylamine in DCM/Et_2O (2:1). After work-up and purification by flash column chromatography on silica gel, the indole thiohydroxymates **15**, **16** and **17** were obtained in 9%, 34% and 10% yield, respectively. The compounds **15** and **17** were produced in low yield.



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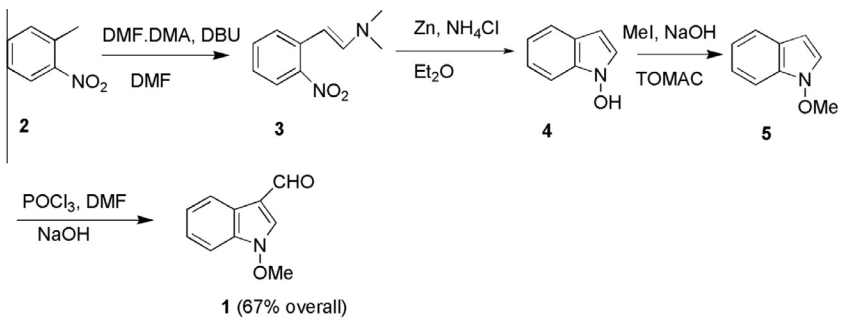
In contrast, for compound **9**, where the nitrogen atom of the indole ring was protected by a methoxy group, the conversion to **16** was higher.

On the other hand, the application of the nitronate method for synthesis of thiohydroxymates **15**, **16** and **17** was much more successful (Scheme 4).

The nitroalkanes **11**, **12** and **13** were reacted with sodium methoxide in MeOH to make sodium nitronate derivatives which were treated with thionyl chloride in DME at −40 °C to convert to (indol-3-yl)acetohydroxymoyl chlorides. The coupling of the hydroxymoyl chlorides and thiol **14** was carried out by a general method in DCM/Et_2O in the presence of Et_3N to make indole thiohydroxymates **15**, **16** and **17** (42–46% overall yield from the nitroalkanes).

Comparison of the two methods showed that the overall yields of the thiohydroxymates following the nitronate method (25–36%) were higher than those by the nitrovinyl method (7–27%). Thus, findings in this study demonstrate that the nitronate pathway should be applied to synthesize indole thiohydroxymates rather than the nitrovinyl pathway.



Scheme 1. Synthesis of 1-methoxyindole-3-carbaldehyde **1** following Somei's method.[11]