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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]

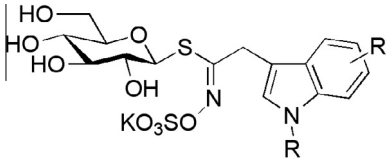

Figure 1. A general structure of indole glucosinolates.

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.

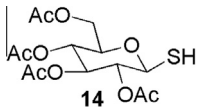
To synthesize 3-(nitroethenyl)indole, the starting material 1-methoxyindole-3-carbaldehyde **1** was synthesized. The compound can be formed by several methods. Selvakumar and Rajulu reported the synthesis of indole **1** from 1-fluoro-2-nitrobenzene.[9] However, the limitation of this method is a low 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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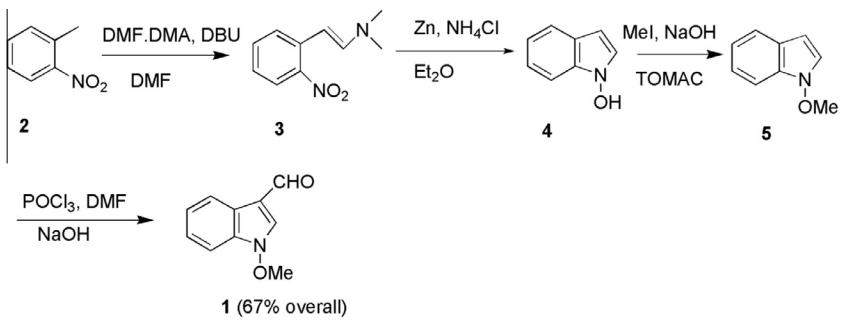
In contrast, for compound **9**, where the nitrogen atom of the indole ring was protected by a methoxy group, the conversion to **16** was higher.

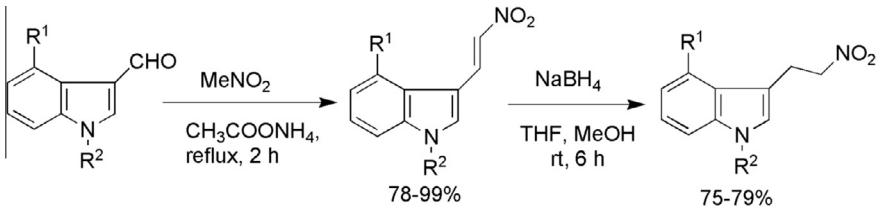
On the other hand, the application of the nitronate method for synthesis of thiohydroxymates **15**, **16** and **17** was much more successful (Scheme 4).

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]

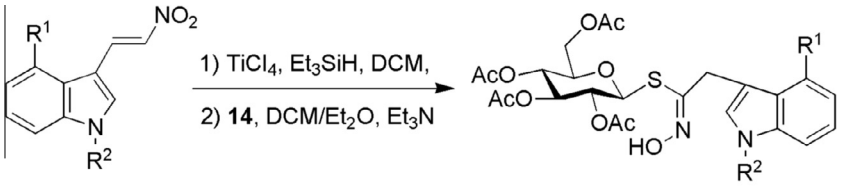


6 $R^1 = R^2 = H$
1 $R^1 = H, R^2 = OMe$
7 $R^1 = OMe, R^2 = H$

8 $R^1 = R^2 = H$
9 $R^1 = H, R^2 = OMe$
10 $R^1 = OMe, R^2 = H$

11 $R^1 = R^2 = H$
12 $R^1 = H, R^2 = OMe$
13 $R^1 = OMe, R^2 = H$

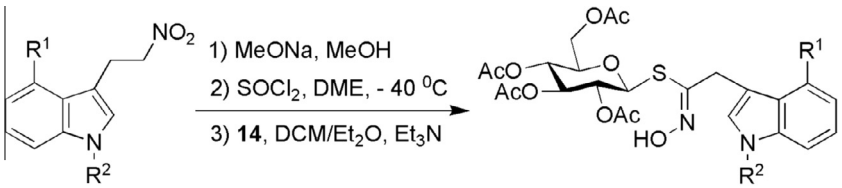
Scheme 2. Synthesis of 3-(nitroethyl)indoles.



8 $R^1 = R^2 = H$
9 $R^1 = H, R^2 = OMe$
10 $R^1 = OMe, R^2 = H$

15 $R^1 = R^2 = H$ (9%)
16 $R^1 = H, R^2 = OMe$ (34%)
17 $R^1 = OMe, R^2 = H$ (10%)

Scheme 3. Coupling of **14** with 3-(nitrovinyl)indoles.



11 $R^1 = R^2 = H$
12 $R^1 = H, R^2 = OMe$
13 $R^1 = OMe, R^2 = H$

15 $R^1 = R^2 = H$ (42%)
16 $R^1 = H, R^2 = OMe$ (45%)
17 $R^1 = OMe, R^2 = H$ (46%)

Scheme 4. Coupling of **14** with 3-(nitroethyl)indoles.

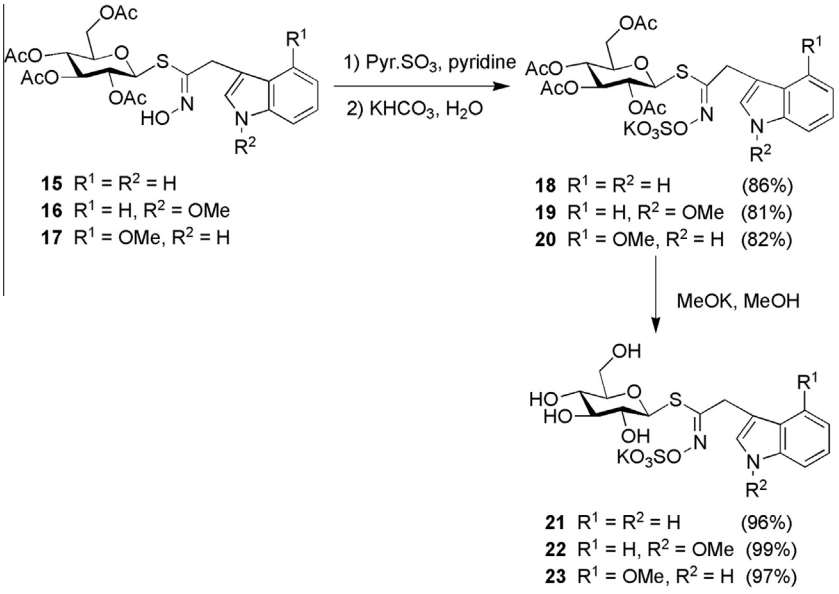
2.1.3. Synthesis of glucosinolates

2.1.3.1. Sulfation of indolylthiohydroximates. Sulfation of the indole thiohydroxymates **15**, **16** and **17** was carried out by the typical method using pyridine–sulfur trioxide complex in DCM or pyridine as the solvent and then treatment with aqueous potassium bicarbonate (Scheme 5).[18–21] It was found that an increase in the temperature of the reaction mixture formed side products so the reaction temperature was controlled at room temperature (22–27 °C). DCM can be used as the solvent for the reaction,[22] but the converted yield of the sulfation products was limited (around 45–55% yield). This may be because the reaction mixture is largely insoluble in DCM, resulting in reducing the yield of the sulfation reaction. Thus, pyridine was used as a convenient solvent for the reaction.[21] However, the study also noted that in the work-up removing the pyridine solvent in the presence of aqueous potassium bicarbonate resulted in deterioration of the product by deacetylation and/or desulfation.[20] The problem was solved by quick extraction of the organic phases with chloroform and then 80% $CHCl_3$/MeOH. As a result, the products **18**, **19** and **20** were obtained in 81–86% yield after purification by flash chromatography on silica gel.

2.1.3.2. De-*O*-acetylation of potassium 2,3,4,6-tetra-*O*-acetyl-indolylglucosinolates. De-*O*-acetylation of potassium 2,3,4,6-tetra-*O*-acetyl-indolyl GLs **18–20** was done using MeOK in MeOH (Scheme 5). The reaction mixture was made neutral (pH 6–7) by the addition of glacial acetic acid. The final indole GLs were successfully purified by flash column chromatography on silica gel.[22] The GB **21**, NGB **22** and MGB **23** were obtained in excellent yield of 96%, 99% and 97% yield, respectively. Consequently, a successful synthesis of the indole GLs was completed in reasonable overall yield (21–29% yield, Table 1), and the first total syntheses of NGB **22** and MGB **23** have been completed.

2.2. Biological studies

There is a growing body of evidence associating the consumption of *Brassica* vegetables with enhanced outcomes for human health, particularly with respect to cancer and heart disease. It has been proposed that the GLs or their metabolites are responsible for this biological activity and that some of this activity may be due to a reduction of the inflammation response e.g. in the cause of heart disease.[23]



Scheme 5. Sulfation and de-*O*-acetylation.

Table 1
Yields of GB, NGB and MGB

R^1, R^2	Yield (%)		
	Sulfates (%)	Indole GLs (%)	Overall (%)
$R^1 = R^2 = H$	**18** 86	**21** 96	21
$R^1 = H$, $R^2 = OMe$	**19** 81	**22** 99	22
$R^1 = OMe$, $R^2 = H$	**20** 82	**23** 97	29

Following the testing method for aromatic GLs,[22] the anti-inflammatory properties of the synthetic indole GLs were tested via an in vitro assay developed based on the THP-1 cell line. Lipopolysaccharides (LPS) were used to trigger an inflammative immune response in a tissue culture. These LPS originate from gram negative bacteria where they cover the outer membrane of the bacteria. When the monocytes are treated with this bacterial product an innate or natural immune response is activated which leads to the release of cytokines including TNF-α.[24] In the THP-1 cell assay LPS were used to stimulate this immune response and the resultant TNF-α was measured by ELISA. Anti-inflammatory activity was measured as the inhibition of the release of TNF-α.

Wells containing THP-1 cells were individually treated in replicate with compounds **21**–**23** at different concentrations for 4 h at an LPS concentration of 50 μg/L. The concentrations used were dependant on available material and were; 0.1, 0.5, 1.0, 5.0, 10 or 15 μM. For each plate LPS controls were measured. The results are summarized in Figure 2 and Table 2. Catechin is an anti-inflammatory agent and is known for a wide range of cardio and chemoprotective effects.[25,26] Thus catechin was used as a positive control in this experiment.[27] The catechin control had an anti-inflammatory response (37% inhibition) at a concentration of 0.5 μM ($P < 0.05$, Fig. 2). The anti-inflammatory effect of the GLs was compared to both LPS and catechin controls.

It was shown that in the presence of indole GLs, TNF-α secretion was significantly inhibited at a concentration of 1 μM (⩾024% inhibition). GB **21** had exhibited higher activity than NGB **22** and MGB **23** in all of testing concentrations. Thus, the presence of methoxy in the indole ring may reduce the anti-inflammatory

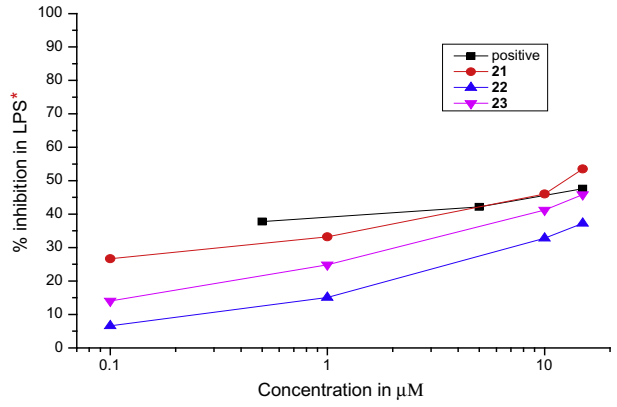


Figure 2. Comparison of TNF-α released by LPS alone and in addition to synthetic GLs at difference concentrations. *: LPS stimulates the release of TNF-α. % Inhibition is calculated from the difference between TNF-α released with LPS alone and in combination with the GLs (from an average of the three replicates). Moderate activity was observed for the majority of the GLs at low micromolar levels.[28]

Table 2
Effects of synthetic GLs on TNF-α secretion in LPS-stimulated THP-1 cells

Treatment	TNF-α secretion pg/mg[a]
LPS (50 μg/L)	488.68 (15.81)[b]
LPS + 15.00 μM catechin	255.94 (33.77)
LPS + 5.00 μM catechin	282.65 (20.53)
LPS + 0.50 μM catechin	303.98 (24.14)
LPS (50 μg/L)	294.37 (16.43)[b]
LPS + 15.00 μM **21**	136.79 (12.83)
LPS + 10.00 μM **21**	158.94 (35.00)
LPS + 1.00 μM **21**	196.65 (46.17)
LPS + 0.10 μM **21**	215.57 (30.94)
LPS (50 μg/L)	269.51 (32.67)[b]
LPS + 15.00 μM **22**	169.06 (11.46)
LPS + 10.00 μM **22**	181.10 (8.46)
LPS + 1.00 μM **22**	228.90 (71.30)
LPS + 0.10 μM **22**	251.69 (72.90)
LPS (50 μg/L)	249.36 (65.53)[b]
LPS + 15.00 μM **23**	135.01 (7.75)
LPS + 10.00 μM **23**	146.53 (49.86)
LPS + 1.00 μM **23**	187.45 (59.36)
LPS + 0.10 μM **23**	214.36 (37.07)

[a] The results are mean (SD) of 3 different experiments run in duplicate ($P \leqslant 0.09$).
[b] $P \leqslant 0.07$, Compared with control.

activity of indole GLs. While MGB **23** rapidly inhibited TNF-α secretion (24%, 41% and 46% inhibition at concentration 1, 10, 15 μM, respectively), NGB **22** has significant inhibition at only concentration ⩾10 μM (⩾32%). Comparison with the positive control (catechin), showed that at concentrations lower than 10 μM, most of the indole GLs had lower inhibition than catechin, but at a concentration 15 μM, GB exhibited higher activity than catechin (54% inhibition of GB compared with 48% inhibition of catechin). Thus, it clearly demonstrates that the synthetic indole GLs have significant anti-inflammatory activity at low concentration (⩾24% inhibition at concentration of ≈1 μM).

3. Conclusion

Two methods to synthesize indole GLs have been investigated; it was shown that the nitronate pathway is the simplest and most convenient method to create indole thiohydroxymates. The method can be applied to synthesize both natural and unnatural indole GLs in moderate overall yield. Two indole GLs, MGB and NGB, were successfully synthesized with reasonable overall yield (21% and 29%) for the first time. An anti-inflammatory response of the synthetic indole GLs, which is indicated by an inhibition of TNF-α secretion in LPS-stimulated THP-1 cells, showed that the synthetic indole GLs have significant anti-inflammatory activity at low concentration.

4. Experimental section

4.1. General procedures

Melting points (mp) were recorded on a Reichert 'thermopan' hot stage apparatus and are uncorrected. Optical rotations were measured at the stated temperatures in the stated solvent on a Perkin Elmer 141 polarimeter at the sodium d-line (589 nm); $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Infrared spectra (ν_{max}) were recorded on a Bruker Vector 22 Fourier-Transform Spectrometer or a Perkin Elmer 1720-X FT-IR Spectrometer. Samples were analyzed using KBr Diffuse Reflectance Fourier Transform (DRIFT) spectra (for solids) or as thin films on NaCl plates (for liquids/oils). Unless otherwise specified, proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer operating at 300 MHz for proton and 75 MHz for carbon nuclei. Chemical shifts are recorded as δ values in parts per million (ppm). Spectra were acquired in deuterated chloroform ($CDCl_3$) at 300 K unless otherwise stated. For ^{1}H NMR spectra were recorded in $CDCl_3$ and the peak due to residual $CHCl_3$ (δ_H 7.24) was used as the internal reference, while the central peak (δ_C 77.0) of the $CDCl_3$ triplet was used as the reference for proton-decoupled ^{13}C NMR spectra. Low-resolution mass spectra were measured on a Brüker Daltonics Esquire 6000 mass spectrometer at 300 °C and scan rate of 5500 *m*/*z*/*s* using either water/methanol/acetic acid in a ratio of 0/99/1 or 50/50/1 as a mobile phase. Accurate mass measurement was by mass spectrometry utilising a LTQ Orbitrap Velos instrument (Thermo Scientific, Waltham, MA, USA; Bremen, Germany) with a heated electrospray ionisation (HESI) source. The mass spectrometer was operated with full scan (50–1000 amu) in positive or negative FT mode (at a resolution of 100,000). The analyte was dissolved in water/methanol/acetic acid in a ratio of 0/99/1 or 50/50/1 and infused via syringe pump at a rate of 5 μl/min. The heated capillary was maintained at 320 °C with a source heater temperature of 350 °C and the sheath, auxiliary and sweep gases were at 40, 15 and 8 units, respectively. Source voltage was set to 4.2 kV. Solvents were dried over standard drying agents and freshly distilled before use. Ethyl acetate and hexane used for chromatography were distilled prior to use. All solvents were purified by distillation. Reactions were monitored by TLC on silica gel 60 F254 plates with detection by UV fluorescence or charring with a basic potassium permanganate stain. Flash column chromatography was performed on silica gel 60 particle size 0.040–0.063 m (230–400 mesh).

4.2. 1-Methoxyindole 5

A mixture of 2-nitrotoluene **2** (6.00 g, 43.7 mmol), DMF.DMA (10.95 g, 91.87 mmol), DBU (682 mg, 4.3 mmol), and absolute DMF (50 ml) was refluxed for 35 h with stirring. After evaporation of the solvent in vacuo, the red colored residue (crude amine **3**) was dissolved in diethyl ether (300 ml). To this ether solution, zinc powder (31.6 g, 483.3 mmol) and a solution of NH_4Cl (8.6 g, 160.80 mmol) in H_2O (60 ml) was added with vigorous stirring. After additional stirring of the mixture for 4 h at rt, insoluble zinc was filtered off through silica gel and the filtrate was washed with satd aq $NaHCO_3$ (3 × 50 ml). To the ether solution containing 1-hydroxyindole **4**, MeI (14.4 ml, 232 mmol), 10% aq NaOH (300 ml), and tri(*n*-octyl)methyl ammonium chloride (2.0 g, 2.3 mmol) were added. The whole mixture was stirred for 21 h at rt then the organic layer was separated. The aqueous layer was extracted with ether (3 × 30 ml) and the combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduce pressure to leave an oil. The product **5** was obtained by flash column chromatography on silica gel eluting with 70% hexane–ethyl acetate as colorless oil (4.50 g, 70%). R_f = 0.44 in 70% hexane/EtOAc; ^{1}H NMR (300 MHz, $CDCl_3$) (300 K) δ 7.66 (d, *J* = 7.8 Hz, 1H, H4), 7.52 (dd, *J* = 0.9 Hz, *J* = 8.1 Hz, 1H, H7), 7.36–7.28 (m, H2 and H6), 7.17 (t, *J* = 8.1 Hz, H5), 6.42 (dd, *J* = 0.9 Hz, *J* = 3.6 Hz, 1H, H3), 4.11 (s, 3H, CH_3O); ^{13}C NMR (75 MHz, $CDCl_3$) (300 K) δ 131.5 (C-8), 123.9 (C-9), 122.6 (C-2), 121.9 (C-5), 120.9 (C-6), 119.6 (C-4), 107.9 (C-7), 97.7 (C-3), 65.5 (CH_3O).

4.3. 1-Methoxy-3-indolecarbaldehyde 1

Phosphorus oxychloride (3.5 ml, 36.4 mmol) was added to ice-cooled dry DMF (20 ml) with stirring. A solution of **5** (3.42 g, 23.2 mmol) in dry DMF (10 ml) was added to the resultant viscous solution and stirring was continued for 3 h at rt. Then crushed ice and 16% aq NaOH (100 ml) were added to the reaction mixture and the whole mixture was extracted with ether (3 × 30 ml). The extract was washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure to give a crystalline solid. The product **1** was obtained by crystallization from ether–hexane as colorless prisms (3.92 g, 96%). R_f = 0.14 in 70% hexane/EtOAc; mp = 51–52 °C (Lit. 50–51 °C)[11]; ^{1}H NMR (300 MHz, $CDCl_3$) (300 K) δ 9.89 (s, 1H, CHO), 8.29 (dd, *J* = 1.5 Hz, *J* = 6.9 Hz, 1H, H4), 7.85 (s, 1H, H2), 7.45–7.26 (m, 3H, H5, H6 and H7), 4.13 (s, 3H, CH_3O); ^{13}C NMR (75 MHz, $CDCl_3$) (300 K) δ 183.8 (CHO), 132.3 (C-8), 131.6 (C-6), 124.3 (C-5), 123.1 (C-4), 121.7 (C-2), 121.2 (C-9), 113.6 (C-3), 108.4 (C-7), 66.5 (CH_3O).

4.4. General procedure for the preparation of 3-(2-nitrovinyl) indoles (8–10)

Ammonium acetate (0.5 equiv) was added to indole-3-carboxaldehyde **1**, **6** or **7** (1 equiv) in nitromethane (15 ml) and the mixture was stirred vigorously while heating under reflux for 2 h. The reaction was followed by TLC (silica, 60% ethyl acetate–hexane). The resulting solution was concentrated under reduced pressure and the remaining bright-red solid was purified by column chromatography on silica gel eluting with 60% ethyl acetate–hexane to give the pure nitroalkenes **8**–**10**.

4.4.1. 3-(2-Nitrovinyl)indole 8

Pure **8** was obtained as a light brown solid (510 mg, 79%). R_f = 0.3 in 60% hexane/EtOAc; mp = 168–170 °C (Lit. 169–172 °C)[7]; IR (KBr drift) ν_{max} 3284, 1610, 1300, 1264, 981 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) (300 K) δ 8.39 (d, J = 13.2 Hz, 1H, C*H*=CHNO$_2$), 8.20 (s, 1H, H2), 7.99 (d, J = 13.2 Hz, 1H, CH=C*H*NO$_2$), 7.92 (dd, J = 1.5 Hz, J = 6.9 Hz, 1H, H4), 7.50 (dd, J = 1.2 Hz, J = 6.6 Hz, 1H, H7), 7.27–7.17 (m, 2H, H5 and H6); ^{13}C NMR (75 MHz, DMSO-d_6) (300 K) δ 137.8 (C-8), 136.4 (CHNO$_2$), 134.8 (*C*H=CHNO$_2$), 131.2 (C-2), 124.7 (C-9), 123.5 (C-6), 122.0 (C-5), 120.6 (C-4), 112.9 (C-7), 108.3 (C-3); HRMS (ESI) m/z for $C_{10}H_8O_2N_2Na$ $[M+Na]^+$, calcd 211.0478, found 211.0425.

4.4.2. 3-(2-Nitrovinyl)-1-methoxyindole 9

Pure **9** was obtained as a bright yellow solid (3.83 g, 78%). R_f = 0.44 in 60% hexane/EtOAc; mp = 83–85 °C (dec); IR (NaCl) ν_{max} 2936, 1611, 1300, 1264, 981, 950 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) (300 K) δ 8.21 (d, J = 13.5 Hz, 1H, C*H*=CHNO$_2$), 7.76–7.70 (m, 3H, H2, H4 and CHNO$_2$), 7.52 (d, J = 7.5 Hz, 1H, H7), 7.41 (t, $J_{5,6}$ = $J_{6,7}$ = 7.5 Hz, 1H, H6), 7.34 (t, $J_{4,5}$ = $J_{5,6}$ = 7.5 Hz, 1H, H5), 4.16 (s, 3H, CH_3O); ^{13}C NMR (75 MHz, $CDCl_3$) (300 K) δ 132.9 (C-8), 132.7 (CHNO$_2$), 132.4 (*C*H=CHNO$_2$), 128.7 (C-2), 124.1 (C-6), 122.6 (C-5), 121.6 (C-9), 120.3 (C-4), 109.1 (C-7), 104.2 (C-3), 66.5 (CH_3O); HRMS (ESI) m/z for $C_{11}H_{10}O_3N_2Na$ $[M+Na]^+$, calcd 241.0584, found 241.0579.

4.4.3. 3-(2-Nitrovinyl)-4-methoxyindole 10

Pure **10** was obtained as a bright red solid (680 mg, 99%). R_f = 0.34 in 60% hexane/EtOAc; mp > 110 °C (dec.) IR (KBr drift) ν_{max} 3276, 3104, 3053, 1611, 1264, 981 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) (300 K) δ 8.54 (d, J = 13.5 Hz, 1H, C*H*=CHNO$_2$), 8.22 (s, 1H, H2), 8.08 (d, J = 13.5 Hz, 1H, CH=C*H*NO$_2$), 7.14 (t, $J_{5,6}$ = $J_{6,7}$ = 7.8 Hz, 1H, H6), 7.07 (d, $J_{6,7}$ = 7.8 Hz, 1H, H7), 6.72 (d, $J_{5,6}$ = 7.8 Hz, 1H, H5), 3.91 (s, 3H, CH_3O); ^{13}C NMR (75 MHz, DMSO-d_6) (300 K) δ 153.7 (C-4), 138.8 (C-8), 135.5 (CHNO$_2$), 132.4 (*C*H=CHNO$_2$), 132.3 (C-2), 124.3 (C-6), 115.2 (C-9), 108.0 (C-3), 106.0 (C-7), 102.6 (C-5), 55.4 (CH_3O); HRMS (ESI) m/z for $C_{11}H_{10}O_3N_2Na$ $[M+Na]^+$, calcd 241.0584, found 241.0575.

4.5. General procedure for the preparation of 3-(2-nitroethyl)indoles (11–13)

To a stirred mixture of $NaBH_4$ (3 equiv) in THF (5 ml) and MeOH (1.3 ml), a solution of the nitroalkene **8**–**10** (1 equiv) in THF (5 ml) was added in portions over a period of 15 min. After 6 h, the remaining $NaBH_4$ was quenched with aq HCl (1 M). The reaction mixture was filtered, the filtrate was extracted with EtOAc (3 × 30 ml), dried over $MgSO_4$ and concentrated under reduced pressure. The nitroalkane was obtained by flash chromatography eluting with 80% hexane–ethyl acetate to yield the pure nitroalkanes **11**–**13**.

4.5.1. 3-(2-Nitroethyl)indole 11

Pure **11** was obtained as a yellow solid (1.35 g, 75%). R_f = 0.47 in 60% hexane/EtOAc;; IR (KBr drift) ν_{max} 3371, 2983, 2839, 1584, 1261, 1083 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) (300 K) δ 8.09 (s, 1H, NH), 7.57 (d, J = 7.8 Hz, 1H, H4), 7.37 (d, J = 8.1 Hz, 1H, H7), 7.24–7.12 (m, 2H, H5 and H6), 7.04 (s, 1H, H2), 4.65 (t, J = 7.2 Hz, 2H, CH_2NO_2), 3.48 (t, J = 7.2 Hz, 2H, C*H*$_2$CH$_2$NO$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) (300 K) δ 135.8 (C-8), 126.2 (C-9), 122.1 (C-6 and C-2), 119.5 (C-5), 117.7(C-4), 109.6 (C-7), 107.3 (C-3), 75.3 (CH_2NO_2), 23.2 (*C*H$_2$CH$_2$NO$_2$); HRMS (ESI) m/z for $C_{10}H_{12}O_2N_2$ $[M+H]^+$, calcd 191.0815, found 191.0824.

4.5.2. 3-(2-Nitroethyl)-1-methoxyindole 12

Pure **12** was obtained as a pale yellow oil (2.95 g, 77%). R_f = 0.3 in 80% hexane/EtOAc; IR (NaCl) ν_{max} 2971, 2945, 2832, 1586, 1302, 1262, 1083 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) (300 K) δ 7.54 (d, J = 7.8 Hz, 1H, H4), 7.44 (d, J = 7.8 Hz, 1H, H7), 7.26 (t, J = 7.8 Hz, 1H, H6), 7.16–7.11 (m, 2H, H2 and H5), 4.63 (t, J = 7.2 Hz, 2H, CH_2NO_2), 4.05 (s, 3H, CH_3O), 3.44 (t, J = 7.2 Hz, 2H, C*H*$_2$CH$_2$NO$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) (300 K) δ 132.0 (C-8), 122.7 (C-9), 122.5 (C-6), 121.2 (C-2), 119.7 (C-5), 11.0 (C-4), 108.2 (C-7), 105.4 (C-3), 75.3 (CH_2NO_2), 65.5 (CH_3O), 23.0 (*C*H$_2$CH$_2$NO$_2$); HRMS (ESI) m/z for $C_{11}H_{13}O_3N_2$ $[M+H]^+$, calcd 221.0921, found 221.0914.

4.5.3. 3-(2-Nitroethyl)-4-methoxyindole 13

Pure **13** was obtained as a white solid (540 mg, 79%). R_f = 0.45 in 60% hexane/EtOAc; mp = 86–87 °C (dec.); IR (KBr drift) ν_{max} 3371, 2971, 2943, 2839, 1586, 1261, 1083 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) (300 K) δ 7.97 (s, 1H, NH), 7.10 (t, $J_{5,6}$ = $J_{6,7}$ = 8.1 Hz, 1H, H6), 6.96 (d, $J_{6,7}$ = 8.1 Hz, 1H, H7), 6.90 (d, J = 2.1 Hz, 1H, H2), 6.51 (d, $J_{5,6}$ = 8.1 Hz, 1H, H5), 4.71 (t, J = 7.2 Hz, 2H, CH_2NO_2), 3.92 (s, 3H, CH_3O), 3.53 (t, J = 7.2 Hz, 2H, C*H*$_2$CH$_2$NO$_2$); ^{13}C NMR (75 MHz, $CDCl_3$) (300 K) δ 153.8 (C-4), 137.7 (C-8), 122.9 (C-6), 121.3 (C-2), 116.3 (C-9), 110.0 (C-3), 104.3 (C-7), 99.2 (C-5), 76.6 (CH_3O), 54.7 (CH_2NO_2), 25.1 (*C*H$_2$CH$_2$NO$_2$); HRMS (ESI) m/z for $C_{11}H_{13}O_3N_2$ $[M+H]^+$, calcd 221.0921, found 221.0913.

4.6. General procedure for the preparation of indole thiohydroxymates (15–17)

Procedure **A**: the nitronate pathway

To a stirred solution of the indole nitroalkane **11**–**13** (1.0 equiv - mol) in dry MeOH (15 ml) under a nitrogen atmosphere was added sodium methoxide (2.0 equiv mol). After 20 min the reaction was concentrated at reduced pressure giving the nitronate as a white solid which was dried under a high vacuum for 15 min. The nitronate was cooled to −40 °C under nitrogen and then it was treated with dry DME (20 ml) at −40 °C. A solution of thionyl chloride (2.6 equiv mol) in dry DME (5 ml) at −40 °C was added to the nitronate dropwise to give a burgundy colored solution. After 30 min at −40 °C, water (30 ml) was added and the solution was extracted with DCM (3 × 50 ml). The organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure to give an indol-3-yl acetohydroxymoyl chloride which was left under a high vacuum for 15 min and then reacted directly in the next step. Indol-3-yl acethydroxymoyl chloride in dry Et_2O/DCM (2:1, 30 ml) was treated with a solution of 2,3,4,6-tetra-*O*-acetyl-l-thio-β-D-glucopyranose **14** (1.0 equiv mol) and dry triethylamine (6.0 equiv mol) in dry DCM (10 ml). The reaction was stirred for 2 h giving an orange solution. The reaction mixture was acidified with 1 M H_2SO_4 (7 ml/mmol sugar) then extracted using DCM (3 × 30 ml). The organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The indole thiohydroxymates **15**–**17** were obtained by flash column chromatography on silica gel eluting with 2–5% MeOH/DCM.

Procedure **B**: the nitrovinyl pathway[7]

To a stirred solution of indole nitroalkene **8**–**10** (1 equiv) in DCM (20 ml) under argon, Et_3SiH (2.0 equiv) was added. The solution was cooled in an ice bath and $TiCl_4$ (2.4 equiv) was added dropwise. The mixture was stirred under an argon atmosphere for 1 h, and then it was poured onto ice and extracted three times with DCM. The organic layer was washed with water, dried over $MgSO_4$, filtered and the filtrate was concentrated under reduced pressure to give indol-3-yl acethydroxymoyl chloride, which was left under a high vacuum for 15 min and then it was reacted directly in the next step. The indol-3-yl acethydroxymoyl chloride in dry Et_2O:DCM (2:1, 30 ml) was treated with a solution of 2,3,4,6-tetra-*O*-acetyl-l-thio-β-D-glucopyranose **14** (1.0 equiv mol) and dry triethylamine (6.0 equiv mol) in dry DCM (10 ml). The reaction was stirred for 2 h giving an orange solution. The reaction mixture was acidified with 1 M H_2SO_4 (7 ml/mmol sugar) then extracted using DCM (3 × 30 ml). The organic extracts were dried

($MgSO_4$), and concentrated under reduced pressure. The indole thiohydroximates **15**–**17** were obtained by flash column chromatography on silica gel eluting with 2–5% MeOH/DCM.

4.6.1. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl-((indol-3-yl) methyl)thiohydroximate 15

Pure **15** was obtained as a cream colored solid (1.27 g, 42%(**A**)). R_f = 0.2 in 60% hexane/EtOAc; mp = 72–74 °C (dec); $[\alpha]_D^{20}$ = +4 (*c* 1, $CHCl_3$); IR (NaCl) ν_{max} 3371, 1751, 1600, 1405, 1041 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) (300 K) δ 8.54 (s, 1H, NH), 7.54 (d, $J_{4i,5i}$ = 7.5 Hz, 1H, H4i), 7.33 (d, $J_{6i,7i}$ = 8.1 Hz, 1H, H7i), 7.17–7.05 (m, 2H, H5i and H6i), 7.01 (s, 1H, H2i), 4.92–4.86 (m, 4H, H1, H2, H3 and H4), 4.10 (d, J_{AB} = 16.5 Hz, 1H, C*H*HC=N), 4.03 (d, J_{AB} = 16.5 Hz, 1H, CH*H*C=N), 3.99–3.83 (m, 2H, H6a and H6b), 3.19–3.18 (m, 1H, H5), 2.02, 1.96, 1.89, 1.80 (4 × s, 12H, CH_3COO); ^{13}C NMR (75 MHz, $CDCl_3$) (300 K) δ 170.3, 169.8, 169.0, 168.9 (4 × CH_3COO), 151.1 (C=N), 135.9 (C-8i), 126.2 (C-9i), 122.4 (C-2i), 121.9 (C-6i), 119.3 (C-5i), 117.8 (C-4i), 111.2 (C-7i), 109.5 (C-3i), 79.1 (C-1), 75.1 (C-5), 73.3 (C-3), 69.7 (C-2), 67.5 (C-4), 61.6 (C-6), 28.8 (CH_2C=N), 20.6, 20.3, 20.2, 20.1 (4 × CH_3COO); HRMS (ESI) *m/z* for $C_{24}H_{28}O_{10}N_2NaS$ $[M+Na]^+$, calcd 559.1362, found 559.1352.

4.6.2. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl-((1-methoxyindol-3-yl)methyl)thiohydroximate 16

Pure **16** was obtained as a foam solid (520 mg, 45% (**A**)). R_f = 0.19 in 60% hexane/EtOAc; mp = 65–67 °C; $[\alpha]_D^{20}$ = +10.0 (*c* 1, $CHCl_3$); IR (KBr drift) ν_{max} 3306, 2942, 2832, 1750, 1600, 1232, 1045 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) (300 K) δ 11.35 (s, 1H, OH), 7.63 (s, 1H, H2i), 7.53 (d, *J* = 7.5 Hz, 1H, H4i), 7.39 (d, *J* = 7.5 Hz, 1H, H7i), 7.16 (t, *J* = 7.5 Hz, 1H, H6i), 7.01 (t, *J* = 7.5 Hz, 1H, H5i), 5.55 (d, *J* = 10.2 Hz, 1H, H1), 5.34 (t, *J* = 9.6 Hz, 1H, H3), 4.91 (t, *J* = 9.6 Hz, 1H, H4), 4.82 (t, *J* = 9.6 Hz, 1H, H2), 4.13–3.72 (m, 8H, H5, H6a, H6b, CH_3O and CH_2CN), 1.96, 1.91, 1.89. 1.86 (4 × s, 12H, CH_3COO); ^{13}C NMR (75 MHz, DMSO-d_6) (300 K) δ 170.1, 169.6, 169.3, 169.1 (4 × CH_3COO), 148.3 (C=N), 132.2 (C-8i), 123.6 (C-2i), 122.9 (C-6i), 122.4 (C-9i), 119.5(2) (C-4i and C-5i), 108.3 (C-7i), 106.4 (C-3i), 78.2 (C-1), 74.5 (C-5), 72.9 (C-3), 69.9 (C-2), 68.0 (C-4), 65.8 (CH_3O), 62.0 (C-6), 28.1 (CH_2C=N), 20.8, 20.5, 20.3, 20.2 (4 × CH_3COO); HRMS (ESI) *m/z* for $C_{25}H_{30}O_{11}N_2NaS$ $[M+Na]^+$, calcd 589.1468, found 589.1460.

4.6.3. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl-((4-methoxyindol-3-yl)methyl)thiohydroximate 17

Pure **17** was obtained as a foam solid (350 mg, 46% (**A**)). R_f = 0.37 in 40% hexane/EtOAc; mp = 93–95 °C; $[\alpha]_D^{20}$ = −3 (*c* 1, $CHCl_3$); IR (KBr drift) ν_{max} 3337, 2940, 2838, 1754, 1650, 1231, 1046 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) (300 K) δ 7.04 (t, $J_{6i,7i}$ = $J_{5i,6i}$ = 8.1 Hz, 1H, H6i), 6.99 (d, $J_{6i,7i}$ = 8.1 Hz, 1H, H7i), 6.89 (s, 1H, H2i), 6.55 (d, $J_{5i,6i}$ = 8.1 Hz, 1H, H5i), 5.13 (d, $J_{1,2}$ = 9.6 Hz, 1H, H1), 4.87–4.77 (m, 3H, H2, H3 and H4), 4.24 (d, J_{AB} = 18.3 Hz, 1H, C*H*HC = N), 4.21 (d, J_{AB} = 18.3 Hz, 1H, CH*H*C=N), 4.05 (dd, $J_{5,6a}$ = 5.4 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6a), 3.94 (s, 3H, CH_3O), 3.75 (dd, $J_{5,6b}$ = 2.4 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6b), 3.05–3.00 (m, 1H, H5), 1.98, 1.93, 1.90, 1.89 (4 × s, 12H, CH_3COO); ^{13}C NMR (75 MHz, CD_3OD) (300 K) δ 169.6, 169.2, 169.1, 169.0 (4 × CH_3COO), 153.8 (C-4i), 151.3 (C=N), 137.8 (C-8i), 121.8 (C-6i), 121.2 (C-2i), 115.8 (C-9i) 109.5 (C-3i), 104.5 (C-7i), 98.6 (C-5i), 79.1 (C-1), 74.7 (C-5), 73.4 (C-3), 69.6 (C-2), 67.3 (C-4), 60.8 (C-6), 53.9 (CH_3O), 29.4 (CH_2C=N), 18.7(2), 18.6(2) (4 × CH_3COO); HRMS (ESI) *m/z* for $C_{25}H_{30}O_{11}N_2NaS$ $[M+Na]^+$, calcd 589.1468, found 589.1444.

4.7. General procedure for the preparation of potassium sulfate salts of thiohydroximates (18–20)

To a stirred solution of the thiohydroximate **15**–**17** (1 equiv) in dry pyridine (40 ml) was added pyridine sulfur trioxide complex (2.5 equiv). After stirring under N_2 for 18 h, an additional portion of the complex (0.3 equiv) was added and stirring was continued for 2 h. After that, a solution of $KHCO_3$ (4 equiv) in water (40 ml) was added and the mixture was stirred for 30 min and then it was concentrated under reduced pressure. The residue was dissolved in water and extracted with chloroform (3 × 40 ml). The organic layers were dried ($MgSO_4$), filtered and concentrated under reduce pressure. To remove excess pyridine, the mixture was co-distilled several times with toluene. The compounds **18**–**20** were obtained by flash chromatography eluting with 80–85% DCM/MeOH.

4.7.1. Potassium 2,3,4,6-tetra-*O*-acetylglucobrassicin 18

Pure **18** was obtained as a white solid (1.13 g, 86%). R_f = 0.4 in DCM/MeOH (20%); mp = 120–122 °C (dec); $[\alpha]_D^{20}$ = −5 (*c* 1.0, MeOH); IR (KBr drift) ν_{max} 3376, 1750, 1650, 1237, 1042 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) (300 K) δ 10.48 (s, 1H, NH), 7.68 (d, $J_{4i,5i}$ = 7.8 Hz, 1H, H4i), 7.39 (d, $J_{6i,7i}$ = Hz, 1H, H7i), 7.23 (s, 1H, H2i), 7.16–7.03 (m, 2H, H5i and H6i), 5.09 (d, $J_{1,2}$ = 9.9 Hz, 1H, H1), 4.90–4.81 (m, 3H, H2, H3 and H4), 4.17–4.04 (m, 3H, CH_2C=N and H6a), 3.87 (dd, $J_{5,6b}$ = 2.4 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6b), 3.43–3.36 (m, 1H, H5), 2.03, 1.92, 1.87, 1.80 (4 × s, 12H, CH_3COO); ^{13}C NMR (75 MHz, CD_3OD) (300 K) δ 170.4, 169.6, 169.3, 169.0 (4 × CH_3COO), 157.1 (C=N), 136.4 (C-8i), 126.1 (C-9i), 122.9 (C-2i), 121.1 (C-6i), 118.5 (C-5i), 117.5 (C-4i), 110.8 (C-7i), 108.1 (C-3i), 78.9 (C-1), 75.0 (C-5), 73.2 (C-3), 69.3 (C-2), 67.4 (C-4), 61.3 (C-6), 28.8 (CH_2C=N), 18.8, 18.6(2), 18.5 (4 × CH_3COO); HRMS (ESI) *m/z* for $C_{24}H_{27}O_{13}N_2S_2$ [M-K], calcd 615.0960, found 615.0956.

4.7.2. Potassium 2,3,4,6-tetra-*O*-acetylneoglucobrassicin 19

Pure **19** was obtained as a slight yellow solid (360 mg, 81%). R_f = 0.2 in 20% DCM/MeOH; mp = 124–126 °C (dec); $[\alpha]_D^{20}$ = −5 (*c* 1.0, MeOH); IR (KBr drift) ν_{max} 2940, 1754, 1582, 1441, 1370, 1231, 1059 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) (300 K) δ 7.72 (d, *J* = 7.5 Hz, 1H, H4i), 7.47 (s, 1H, H2i), 7.44 (d, *J* = 7.5 Hz, 1H, H7i), 7.23 (t, *J* = 7.5 Hz, 1H, H6i), 7.11 (t, *J* = 7.5 Hz, 1H, H5i), 5.18 (d, *J* = 10.2 Hz, 1H, H1), 5.00–4.77 (m, 3H, H2, H3 and H4), 4.13–4.07 (m, 6H, H6b, CH_3O and CH_2CN), 3.85 (dd, $J_{5,6b}$ = 2.1 Hz, $J_{6a,6b}$ = 12.6 Hz, H6a), 3.54–3.52 (m, 1H, H5), 2.02, 1.94, 1.89, 1.78 (4 × s, 12H, CH_3COO); ^{13}C NMR (75 MHz, CD_3OD) (300 K) δ 170.4, 169.6, 169.2, 169.0 (4 × CH_3COO), 156.3 (C=N), 132.2 (C-8i), 122.8 (C-9i), 122.1 (C-6i), 121.8 (C-2i), 119.5, 119.3 (C-4i and C-5i), 107.7 (C-7i), 105.3 (C-3i), 79.1 (C-1), 75.1 (C-5), 73.2 (C-3), 69.4 (C-2), 67.4 (C-4), 64.7 (CH_3O), 61.3 (C-6), 28.6 (CH_2C=N), 18.8, 18.7, 18.6, 18.5 (4 × CH_3COO); HRMS (ESI) *m/z* for $C_{25}H_{29}O_{14}N_2S_2$ $[M-K]^-$, calcd 645.1066, found 645.1055.

4.7.3. Potassium 2,3,4,6-tetra-*O*-acetyl-4-methoxyglucobrassicin 20

Pure 20 was obtained as a white solid (340 mg, 82%). R_f = 0.17 in DCM/MeOH (20%); mp = 126–127 °C (dec.); $[\alpha]_D^{20}$ = −26 (*c* 0.58, MeOH); IR (KBr drift) ν_{max} 3325, 2940, 2839, 1746, 1584, 1236, 1058 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) (300 K) δ 10.53 (s, 1H, NH), 7.06–6.97 (m, 3H, H2i, H6i and H7i), 6.57 (d, $J_{5i,6i}$ = 7.2 Hz, 1H, H5i), 5.15 (d, $J_{1,2}$ = 9.9 Hz, 1H, H1), 4.90–4.78 (m, 3H, H2, H3 and H4), 4.38 (d, J_{AB} = 16.6 Hz, 1H, C*H*HC=N), 4.33 (d, J_{AB} = 16.6 Hz, 1H, CH*H*C=N), 4.04 (dd, $J_{5,6a}$ = 4.2 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6a), 3.96 (s, 3H, CH_3O), 3.72 (dd, $J_{5,6b}$ = 2.1 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6b), 3.01–2.98 (m, 1H, H5), 1.99, 1.90(2), 1.88 (4 × s, 12H, CH_3COO); ^{13}C NMR (75 MHz, CD_3OD) (300 K) δ 170.4 169.6, 169.2, 169.0 (4 × CH_3COO), 158.2 (C=N), 153.7 (C-4i), 137.6 (C-8i), 122.0 (C-6i), 121.9 (C-2i), 115.6 (C-9i), 108.3 (C-3i), 104.6 (C-7i), 98.7 (C-5i), 79.4 (C-1), 74.8 (C-5), 73.3 (C-3), 69.2 (C-2), 67.1 (C-4), 60.7 (C-6), 53.9 (CH_3O), 29.6 (CH_2C=N), 18.7(2), 18.6(2) (4 × CH_3COO); HRMS (ESI) *m/z* for $C_{25}H_{29}O_{14}N_2S_2$ $[M-K]^-$, calcd 645.1066, found 645.1058.

4.8. General procedure for the preparation of GLs (21–23)

To a solution of *O*-acetylglucosinolate **21**–**23** in anhydrous MeOH (20 ml) under a N_2 atmosphere was added dry MeOK (0.8 equiv) until pH = 8–9 was reached. After stirring for 18 h at rt, the solution was made neutral by the addition of glacial acetic acid and then the solution was concentrated under reduced pressure. The GLs **21**–**23** were obtained by flash chromatography eluting with EtOAc:MeOH:H_2O (16:4:1).[22]

4.8.1. Glucobrassicin 21

Pure **21** was obtained as a purple solid (390 mg, 96%). R_f = 0.14 in EtOAc/MeOH/H_2O (16:4:1); mp = 124–126 °C (dec); $[\alpha]_D^{20} = -12$ (*c* 1.0, H_2O); IR (KBr drift) ν_{max} 3378, 1600, 1236, 1041 cm^{-1}; ^1H NMR (300 MHz, D_2O) (300 K) δ 7.66 (d, $J_{4i,5i}$ = 7.8 Hz, 1H, H4i), 7.44 (d, $J_{6i,7i}$ = 8.1 Hz, 1H, H7i), 7.22 (s, 1H, H2i), 7.19–7.06 (m, 2H, H5i and H6i), 4.72 (d, $J_{1,2}$ = 9.6 Hz, 1H, H1), 4.21 (d, J_{AB} = 16.2 Hz, 1H, C*H*HC=N), 4.13 (d, J_{AB} = 16.2 Hz, 1H, CH*H*C=N), 3.46–3.45 (m, 2H, H6a and H6b), 3.30–3.06 (m, 3H, H2, H3 and H4), 2.85–2.81 (m, 1H, H5); ^{13}C NMR (75 MHz, D_2O) (300 K) δ 162.7 (C=N), 135.8 (C-8i), 125.7 (C-9i), 123.6 (C-2i), 121.8 (C-6i), 119.2 (C-5i), 118.0 (C-4i), 111.6 (C-7i), 107.7 (C-3i), 81.0 (C-1), 79.4 (C-5), 76.5 (C-3), 71.3 (C-2), 68.2 (C-4), 59.8 (C-6), 28.9 (*C*H_2C=N); HRMS (ESI) *m/z* for $C_{16}H_{19}O_9N_2S_2$ $[M\text{-}K]^-$, calcd 447.0537, found 447.0537.

4.8.2. Neoglucobrassicin 22

Pure **22** was obtained as a purple solid (77.0 mg, 99%). R_f = 0.17 in EtOAc/MeOH/H_2O (16:4:1); mp = 109–111 °C (dec.); $[\alpha]_D^{20} = -2.5$ (*c* 1, H_2O); IR (KBr drift) ν_{max} 3126, 2936, 1713, 1573, 1453, 1242, 1060 cm^{-1}; ^1H NMR (300 MHz, D_2O) (300 K) δ 7.64 (d, *J* = 7.5 Hz, 1H, H4i), 7.45 (d, *J* = 7.5 Hz, 1H, H7i), 7.39 (s, 1H, H2i), 7.22 (t, *J* = 7.5 Hz, 1H, H6i), 7.09 (t, *J* = 7.5 Hz, 1H, H5i), 4.65 (d, *J* = 10.2 Hz, H1), 4.09 (d, J_{AB} = 16.7 Hz, 1H, C*H*HC=N), 4.02 (d, J_{AB} = 16.7 Hz, 1H, CH*H*C=N), 3.96 (s, 3H, CH_3O), 3.47–3.41 (m, 2H, H6a and H6b), 3.25 (t, *J* = 9.0 Hz, 1H, H4), 3.22 (t, *J* = 9.0 Hz, 1H, H2), 3.09 (t, *J* = 9.0 Hz, 1H, H3), 2.87–2.83 (m, 1H, H5); ^{13}C NMR (75 MHz, D_2O) (300 K) δ 162.3 (C=N), 131.9 (C-8i), 122.8 (C-9i), 122.4 (C-6i), 122.3 (C-2i), 120.1 (C-5i), 118.6 (C-4i), 108.4(C-7i), 105.0 (C-3i), 81.2 (C-1), 79.6 (C-5), 76.5 (C-3), 71.4 (C-2), 68.3 (C-4), 65.7 (CH_3O), 59.8 (C-6), 28.7 (*C*H_2C=N); HRMS (ESI) *m/z* for $C_{17}H_{21}O_{10}N_2S_2$ $[M\text{-}K]^-$, calcd 477.0643, found 477.0643.

4.8.3. 4-Methoxyglucobrassicin 23

Pure **23** was obtained as a dark-green solid (145 mg, 97%). R_f = 0.17 in EtOAc/MeOH/H_2O (16:4:1); mp = 104–106 °C (dec.); $[\alpha]_D^{20} = -51$ (*c* 1, H_2O); IR (KBr drift) ν_{max} 3452, 3265, 2908, 2841, 1259, 1060 cm^{-1}; ^1H NMR (300 MHz, D_2O) (300 K) δ 7.09–7.01 (m, 3H, H2i, H6i and H7i), 6.57 (d, $J_{5i,6i}$ = 7.2.Hz, 1H, H5i), 4.77 (d, $J_{1,2}$ = 9.9 Hz, 1H, H1), 4.32 (d, J_{AB} = 16.8 Hz, 1H, C*H*HC=N), 4.28 (d, J_{AB} = 16.8 Hz, 1H, CH*H*C=N), 3.85 (s, 3H, CH_3O), 3.47 (dd, $J_{5,6a}$ = 3.9 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6a), 3.35 (dd, $J_{5,6b}$ = 2.3 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6b), 3.27–3.16 (m, 2H, H2 and H4), 3.05 (t, $J_{2,3} = J_{3,4}$ = 9.0 Hz, 1H, H3), 2.67–2.64 (m, 1H, H5); ^{13}C NMR (75 MHz, D_2O) (300 K) δ 163.6 (C=N), 153.4 (C-4i), 137.4 (C-8i), 122.7 (C-6i), 122.5 (C-2i), 115.4 (C-9i), 108.0 (C-3i), 105.1 (C-7i), 99.7 (C-5i), 81.5 (C-1), 79.2 (C-5), 76.6 (C-3), 71.2 (C-2), 67.9 (C-4), 59.5 (C-6), 54.8 (CH_3O), 29.7 (*C*H_2C=N); HRMS (ESI) *m/z* for $C_{17}H_{21}O_{10}N_2S_2$ $[M\text{-}K]^-$, calcd 477.0643, found 477.0624.

4.9. Anti-inflammatory assays

The Human monocytic leukaemia THP-1 cells were obtained from the American-Type Culture Collection (ATCC). The cells were grown in 10% heated-inactivated fetal bovine serum and Invitrogen RPMI-1640 containing 2 mM L-glutamine. The cytokine (TNF-α) Elisa Kit including the reagents, were obtained from BD Bioscience (R&D systems).

All compounds were dissolved in sterile distilled water then further diluted in Invitrogen DMEM (Dulbecco's Modified Eagle Medium).

The cells were grown in a 75 ml flask and maintained at 37 °C in humidified 5% CO_2 atmosphere. The experiments were carried out once the cells had reached 1×10^5 cells/ml. The PMA was dissolved in DMSO to a concentration of 1 mg/ml then further diluted before use. The cells were plated out to a cell density of 10×10^4 cell/ml, at 100 µl/well in a 96-well plate then treated with PMA to a final concentration of 50 nM for 24 h at 37% in humidified 5% CO_2 atmosphere.

The LPS was dissolved in sterile water to a concentration of 5 mg/ml then further diluted to working stock of 10 µg/ml. The THP-1 cells were challenged with various compounds ranging from 15 to 0.1 µM. They were stimulated with LPS at a final concentration of 50 ng/ml. Supernatants were collected after 4 h incubation and stored at −20 °C until ELISA analysis.

A sandwich ELISA was used to screen supernatants for the release of cytokine TNF-α.

The ELISA plates were coated with a capture antibody (1:250) which was diluted in coating buffer and left at 4 °C overnight. The ELISA plates were aspirated and washed 3 times with 1 × PBST (0.05% Tween-20) before adding 200 µl/well assay diluent incubated at room temperature for 1 h. Standards were prepared by 2-fold serial dilution to range from 500 pg/ml to 7.8 pg/ml in assay buffer diluent. Standards and sample were added in quadruplicate into appropriate wells and incubated at room temperature for 2 h. After the 2 h incubation the plates were aspirated and washed with a total of 5 washes. The detection antibody and HRP reagent was added (100 µl/well) and incubated at room temperature for 1 h. The plates were aspirated and washed again, this time with a total of 7 washes and were soaked for 30 s between each wash. The substrate solutions were added (100 µl/well) and incubated at room temperature for 30 min in the dark. The reaction was stopped by adding 50 µl/well of kit stop solution then read at 450 nm in a plate reader within 30 min with λ correction at 570 nm.

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Supplementary data

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