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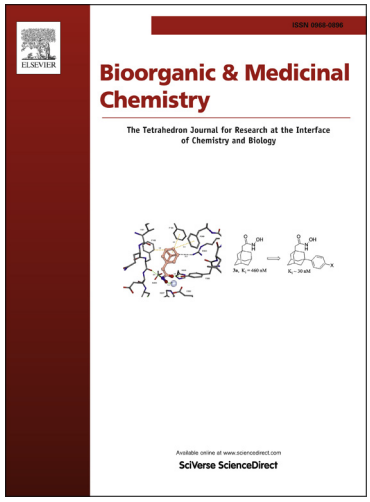

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Graphical Abstract

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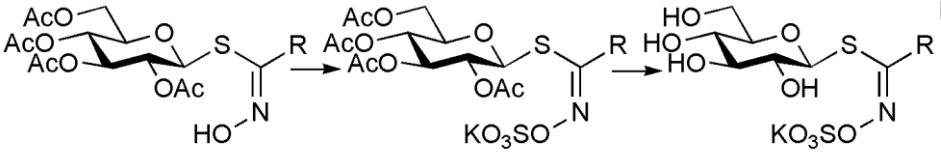

R = Ph, Bn, $PhCH_2CH_2$-, PhCH=CH-, 3,4-dimethoxy-Ph, 4-AcO-Ph, 2,3-dichloro-Ph, 4-bromo-Ph

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Synthesis and anti-inflammatory activity of aromatic glucosinolates

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ABSTRACT

Aromatic GLs are important members of the glucosinolate family of compounds because of their potential biological activity and medicinal properties. This study has shown success in the high yielding synthesis of some important aromatic GLs as well as the results of testing for anti-inflammatory properties of the synthetic GLs. 3,4-Dimethoxyphenylglucosinolate was found to be the most active anti-inflammatory of the seven glucosinolates assayed.

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1. Introduction

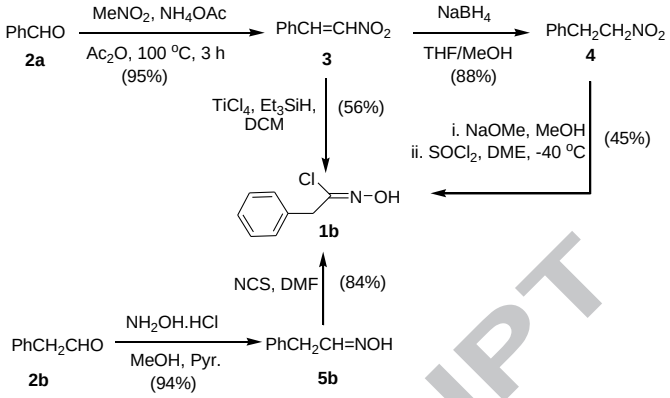
Glucosinolates (GLs) are *β*-thioglucoside *N*-hydroxysulfates with a side chain (R) and a sulfur-linked *β*-D-glucopyranose moiety. These are natural compounds, which are found in a large number of *Brassica* species such as cabbage, broccoli and canola. There are around 120 different GLs that have been identified in these plants. Of the sixteen identified families of natural GLs, aromatic GLs contribute around seventeen different compounds.[1] Studies of aromatic GLs such as the natural products (arylalkyl GLs, glucosinalbin)[2,3] or some unnatural products,[4] indicate that these compounds have high bioactivity and potential applications in biochemistry, genetics as well as medicinal chemistry. The synthesis of both natural and unnatural aromatic GLs has been studied in some detail.[4-12] However, most of the studies have focused on synthesis of GLs or applications of glucosinolate hydrolysis products for biochemistry. Thus, investigation of the synthesis and bio-assay of aromatic GLs needs more attention. Here we report a novel, versatile synthetic approach for the synthesis of both natural and unnatural aromatic GLs and the anti-inflammatory properties of these compounds.

2. Results

2.1. Synthesis of Aromatic Glucosinolates.

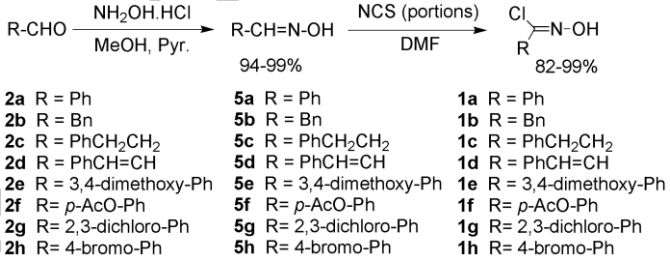
2.1.1. Synthesis of Aromatic Hydroxymoyl Chlorides.

From previous studies,[10] aromatic hydroxymoyl chlorides can be created following three pathways (aldoxime, nitronate and nitrovinyl pathways). The current study targeted synthesis of phenylacetohydroxymoyl chloride **1b** (Scheme 1). Firstly, benzaldehyde **2a** was treated with nitromethane in acetic anhydride in the presence of ammonium acetate at 100 ˚C for 3 hours to generate *β*-nitrostyrene **3** (95% yield).[13] In the vinylnitronate pathway, **1b** was synthesized by Cassel's method.[7] The compound **3** was reacted directly with triethylsilane and titanium (IV) chloride in DCM to form **1b** in 56% yield. Using the nitronate pathway, **1b** was synthesized following the literature.[14] The compound **3** was reduced with sodium borohydride ($NaBH_4$) in THF and MeOH to yield (2-nitroethyl)benzene **4**, which was reacted with sodium methoxide in MeOH and then thionyl chloride in DME at -40 ˚C to give the hydroxymoyl chloride **1b** in 37% yield over three steps. Secondly, the chlorooxime **1b** was synthesized following the aldoxime pathway.[4,6,11] Phenylacetaldehyde **2b** was reacted with hydroxylamine hydrochloride in MeOH in the presence of pyridine to yield oxime **5b**, which was treated with *N*-chlorosuccinimide (NCS) in DMF to create, stereospecifically, (*Z*)-phenylacetohydroxymoyl chloride **1b** in an excellent yield (79% yield over two steps). The protocol was applied to synthesize other aromatic hydroxymoyl chlorides such as hydrocinnamohydroxymoyl chloride, cinnamohydroxymoyl chloride, 2,3-dichlorobenzohydroxymoyl chloride and 4-*O*-acetylbenzohydroxymoyl chloride. In almost all cases, the aldoxime pathway gave a high yield and could be applied to a range of aromatic chlorooximes, while with nitronate and vinylnitronate pathways either the reactions gave a low yield of chlorooxime and too many side-products or the methods could not be applied for other reasons such as incompatibility of functional groups with reaction conditions. For example, the vinylnitronate pathway cannot be applied for synthesis of derivatives of benzohydroxymoyl chlorides because there is no vinyl group in the starting materials. It was concluded that, the aldoxime pathway is one of the most convenient methods for synthesis of aromatic chlorooximes.



Scheme 1. Synthesis of phenylacetohydroxymoyl chloride **5b** by three pathways.

The aldoxime pathway was applied to synthesize a series of aromatic hydroxymoyl chlorides (Scheme 2).

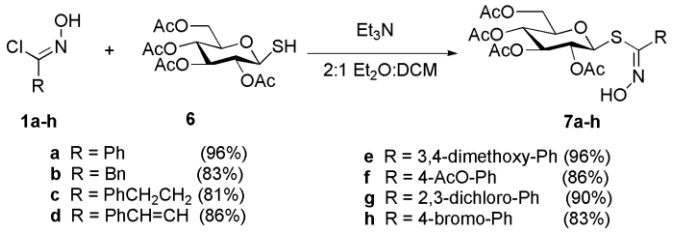


Scheme 2. Synthesis of aromatic hydroxymoyl chlorides.

To increase the yield, in the first step hydroxylamine hydrochloride was added in excess to the solution of reactants, dry MeOH and pyridine. After reaction work-up, the oximes **5a-h** were obtained by flash column chromatography or re-crystallization from hexane and ethyl acetate. In the chlorination step, the yield was affected by the amount of added NCS.[15] This is because reaction of NCS with oximes in DMF exhibits an induction period and can become strongly exothermic for most substrates if the reaction initiates after a considerable portion of the NCS has been added. It was found that it is desirable to initiate the reaction prior to addition of no more than one-fifth of the NCS required.[15] Therefore, to improve the yield, NCS (1.05 eq) was added one-fifth (over 30 minutes) into a cold stirring solution of the oxime **5** and DMF. The reaction mixture was kept at 0 ˚C for the duration of the reaction adding NCS in portions and then it was allowed to increase to rt. After around 4 hours, the reaction was worked-up, and purified by flash column chromatography or re-crystallization to provide chlorooximes **1a-h** in excellent yield (74-97% yield over two steps).

2.1.2. Coupling of Thiol Glucose with Aromatic Hydroxymoyl Chlorides.

The coupling of the thiol **6**[16,17] with hydroxymoyl chlorides **1a-h** was conducted by a general method (Scheme 3).[5,6,12] To guarantee that no thiol material was wasted, the coupling was carried out with an excess of the hydroxymoyl chloride **1** to maximize the conversion of **6**. The thiohydroximates were purified by flash chromatography eluting with DCM/MeOH in excellent yield (81-96%). In all cases the coupling was stereospecific in that only the (*Z*)-isomers were formed.[18]



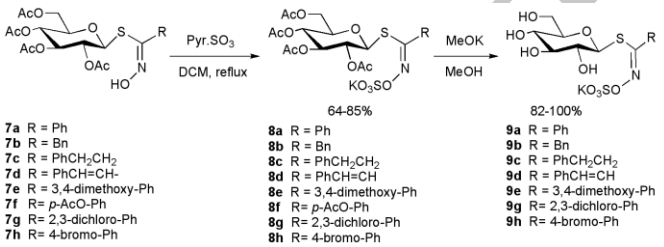
Scheme 3. Coupling of thiol glucose **6** with hydroxymoyl chlorides.

The reaction can be carried out in diethyl ether solvent,[5,6] however the results have indicated that the coupling yield is lower than the mixture of Et_2O:DCM (2:1). In this reaction, triethylamine was used as a base to form the nitrile oxide by 1,3-elimination, followed by attack of the thiol **6** on the nitrile oxide to yield the product.[10]

2.1.3. Synthesis of Glucosinolates.

2.1.3.1. Sulfation of Aromatic Thiohydroximates.

The *O*-sulfation proved to be a tricky step as evidenced by the previous work.[19-21] Firstly, the sulfation was conducted in the normal way by reaction of thiohydroximates 7a-h with pyridine-sulfur trioxide reagent in pyridine followed by displacement of the pyridine with a potassium salt.[6] However, the yield of products **8** was not high (<70%). Monitoring the reaction closely by TLC has shown that this appears to be a clean reaction but in the work-up, removing the pyridine solvent in the presence of aqueous potassium hydrogen carbonate resulted in deterioration of the product by deacetylation and/or desulfation.[22] This problem was solved by using DCM as the solvent, while keeping the pyridine to as small an amount as possible (Scheme 4).



Scheme 4. Sulfation and de-*O*-acetylation.

The sulfation products **8** can be lost due to the high polarity of the solvent which promotes hydrolysis. Therefore, in the work-up, the solvent mixture 20% MeOH/chloroform should be used to extract organic phases instead of only chloroform in order to avoid losing the product in the aqueous phase. It was also found that to improve the yield, the reaction should be kept under refluxing temperature (around 40 ˚C) and in an argon atmosphere (Table 1).

Table 1. Sulfation of thiohydroximates **7** in alternative conditions.

Compound	R	Yield (%) in solvent		
		Pyridine, rt	DCM, rt	DCM, reflux
8a	Ph	45	60	64
8b	Bn	56	67	85
8c	$PhCH_2CH_2$	57	70	72
8d	PhCH=CH	67	72	83
8e	3,4-Dimethoxyphenyl	46	65	68
8f	*p*-AcO-Ph	47	70	72
8g	2,3-Dichlorophenyl	69	71	81
8h	4-Bromophenyl	70	76	85

2.1.3.2. De-O-acetylation of Potassium 2,3,4,6-Tetra-O-acetyl-arylalkylglucosinolates.

The de-*O*-acetylation was carried out by a general method,[6,9,10] however purification of the final product **9** was a difficult process.[17] The GLs **9a-h** were obtained in a good yield by straightforward de-*O*-acetylation with a catalytic amount of potassium methoxide in MeOH (Scheme 4). From previous studies, purification of the final products **9** could be conducted by using paper and thin layer chromatography,[23,24] ion exchange chromatography on DEAE-Sephadex A-25 or Sephadex G10 size exclusion chromatography,[25,26] or reversed phase (C-18) solid phase extraction or flash chromatography reversed phase techniques.[27] A disadvantage of this work, however, was that the products rarely had established purity,[28] and the techniques were slow and difficult to carry out. This problem was solved by using normal phase flash column chromatography with silica gel as the solid phase and EtOAc/MeOH/H_2O as the mobile phase. It was surprising that all of the final compounds were purified easily and rapidly by using this method (proven by NMR and LC-UV-MS). It was also found that with a percentage of H_2O higher than 5% and the percentage of MeOH higher than 20% the silica gel may be dissolved, which could affect the purification of final compounds. Therefore, the amount of H_2O and MeOH was controlled under 5% and 20%, respectively. The GLs were obtained in a good overall yield (46-68%) (Table 2).

Table 2: Overall yield of synthetic GLs **9**.

No.	R	Yield (%)					
		5	**1**	**7**	**8**	**9**	Overall
a	Ph	94	89	96	64	90	46
b	Bn	94	84	83	85	82	46
c	$PhCH_2CH_2$	97	87	81	72	96	47
d	PhCH=CH	-	83	86	83	89	47
e	3,4-dimethoxyphenyl	95	84	96	68	93	48
f	4-AcO-Ph	96	82	86	72	-	-
g	2,3-dichlorophenyl	99	87	90	81	99	62
h	4-bromophenyl	98	99	83	85	100	68

It was found that the synthesis of 4-hydroxyphenyl glucosinolate **9f** was unsuccessful. This may be due to transposition of the phenolic proton to nitrogen after de-*O*-acetylation. This would result, in formation of a quinoid intermediate (the reaction solvent changed from colorless to yellow) and decomposition of the compound. The mechanism of the decomposition has been studied; however, the data of MS and NMR confirmed that 4-hydroxyphenyl glucosinolate was not present in the reaction products.

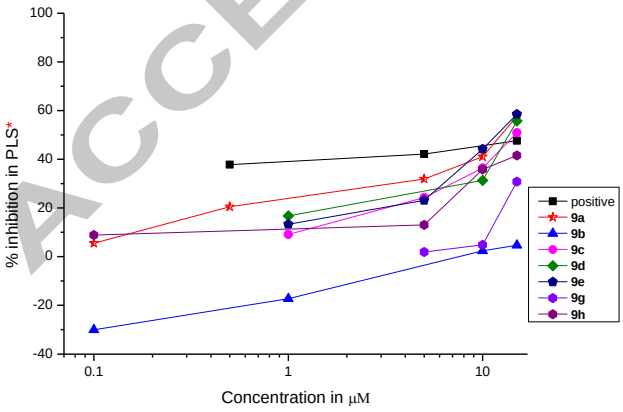
2.1.4. Biological Studies

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 derivatives are responsible for this biological activity and that some of this activity may be due to a reduction of inflammation response e.g. in the cause of heart disease.[29] To test the anti-inflammatory properties of the synthetic GLs an *in vitro* assay was developed based the THP-1 cell line.[30] THP-1 cells are human monocytic leukaemia cell lines[30] that resemble the morphology and differentiation properties of primary monocytes and macrophages.[31] The THP-1 cells are suspension cells that when treated with phorbol-12-myristate-13-acetate (PMA) attach to the culture plate and once adhere begin to differentiate into macrophages.[31]

The innate immune response is the mammalian defence systems' first response to challenge and triggers a cascade of events including induction of the humoral immune system. The innate immune response is involved in inflammation. Activated monocytes and macrophages release large amounts of pro-inflammatory cytokines. One such cytokine released is TNF-α. This cytokine triggers the release of a cascade of other cytokines and recruits activated immune cells, including lymphocytes and macrophages that are instrumental in immune and inflammatory response.[31]

Lipopolysaccharides (LPS), originate from gram negative bacteria where they form the major outer surface membrane. When cells are treated with this bacterial product an innate or natural immune response is activated which leads to the release of cytokines including TNF-α.[32] In the THP-1 cell assay LPS was used to stimulate an immune response and the resultant TNF-α measured by ELISA. An anti-inflammatory response is indicated by an inhibition of the release TNF-α.

The THP-1 cells were individually treated in replicate with compounds **9a-e**, **9g** and **h** for 4 hours at a range of different compound concentrations (0.1, 0.5, 1, 5, 10 or 15 μM). For each plate LPS controls were measured. The results are summarized in Figure 1 and Table 3.



*: 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.[33]

Figure 1: Comparison of TNF-α released by LPS alone and in addition to synthetic GLs at difference concentrations.

Table 3: 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)	244.70 (57.33)[b]
LPS + 15.00 μM **9a**	103.57 (23.60)
LPS + 10.00 μM **9a**	144.15 (24.89)
LPS + 5.00 μM **9a**	166.58 (53.29)
LPS + 0.50 μM **9a**	194.44 (67.74)
LPS + 0.10 μM **9a**	231.15 (55.21)
LPS (50 μg/L)	211.78 (64.51)[b]
LPS + 15.00 μM **9b**	201.87 (22.17)
LPS + 10.00 μM **9b**	206.65 (81.09)
LPS + 1.00 μM **9b**	248.40 (101.56)
LPS + 0.10 μM **9b**	275.48 (84.75)
LPS (50 μg/L)	152.25 (23.27)[b]
LPS + 15.00 μM **9c**	74.76 (9.44)
LPS + 10.00 μM **9c**	96.86 (19.80)
LPS + 5.00 μM **9c**	115.25 (17.18)
LPS + 1.00 μM **9c**	138.43 (23.66)
LPS (50 μg/L)	178.98 (33.50)[b]
LPS + 15.00 μM **9c**	79.23 (29.95)
LPS + 10.00 μM **9c**	122.91 (39.63)
LPS + 1.00 μM **9c**	149.06 (34.75)
LPS (50 μg/L)	166.79 (32.28)[b]
LPS + 15.00 μM **9e**	69.20 (11.32)
LPS + 10.00 μM **9e**	93.10 (12.67)
LPS + 5.00 μM **9e**	128.21 (42.19)
LPS + 1.00 μM **9e**	144.57 (10.49)
LPS + 0.50 μM **9e**	161.76 (44.33)
LPS (50 μg/L)	165.11 (55.40)[b]
LPS + 15.00 μM **9g**	114.17 (34.99)
LPS + 10.00 μM **9g**	157.09 (37.34)
LPS + 5.00 μM **9g**	162.00 (30.37)
LPS (50 μg/L)	190.05 (20.19)[b]
LPS + 15.00 μM **9h**	111.10 (6.13)
LPS + 10.00 μM **9h**	122.04 (18.80)
LPS + 5.00 μM **9h**	173.17 (40.55)
LPS + 0.10 μM **9h**	165.27 (39.50)

a: the results are mean (SD) of 3 different experiments run in duplicate ($P \leq 0.09$)

b: $p \leq 0.07$, compared with control

Catechin is an anti-inflammatory agent and is known for a wide range of protective effects such as cardioprotective and chemoprotective properties.[34,35] Thus catechin was used as a positive control in this experiment.[36] The catechin control had an anti-inflammatory response (37% inhibition) at a concentration of 0.5 μM ($P < 0.05$). The anti-inflammatory effect of the GLs was compared to both LPS and catechin controls. In the presence of aromatic GLs, TNF-α secretion was significantly inhibited at concentrations ≥ 5 μM (≥ 23% inhibition). At a concentration of 15 μM, GLs have a higher percentage inhibition than catechin (47% inhibition) except for **9b**, **g** and **h**. There is no significant effect of R groups in aromatic GLs over anti-inflammatory activity: the TNF-α secretion was inhibited at concentration 5 μM (≈ 23% inhibition) and 15 μM (≈ 50% inhibition). Thus, there is no clear indication of the key structural features responsible for TNF-α inhibition.

The GLs displayed moderate anti-inflammatory activity with the exception of **9b** which was not active at the concentrations tested. Naturally occurring GLs do not occur as single chemical species in plant material but are present as a mixture of different aromatic and aliphatic metabolites. It is possible that the metabolites may act in a synergistic manner when consumed as part of normal diet. They can also be broken down by the enzyme myrosinase which occurs in many brassica species. This enzyme acts on GLs producing compounds including isothiocyanates and in the case of the aliphatic metabolite glucoraphanin, sulforaphane. There is some evidence that these molecules are more biologically active than the intact GLs.[37] Although beyond the scope of this study, it would be interesting to treat the synthetic GLs with myrosinase and assess the biological activity of the resulting metabolized products.

3. Conclusion

In conclusion, a series of aromatic GLs were successfully synthesized with high overall yield (46-68%). Four novel GLs (**9e**, **d**, **f**, **g**) were synthesized for the first time. The study has also demonstrated the utility of silica flash chromatography as a rapid, high throughput alternative to HPLC for the purification of GLs. This can be applied for GLs and other similar families of compounds in medicinal and chemical fields. These methods will allow the production of the described, and chemically related GLs, to facilitate biochemical and medicinal studies into the biological activity of this fascinating class of molecules.

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} degcm^{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 recorded in $CDCl_3$, 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*/second 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 μm (230–400 mesh).

*4.2. General procedure for the preparation of oximes (**5a-h**)*

Hydroxylamine hydrochloride (1.2 eq.) was added to a solution of the aldehyde **2** (1 eq.) in MeOH (60 ml) followed by pyridine (1 eq.). The reaction was stirred at rt for 2.5 h. After the MeOH was removed *in vacuo*, the residue was suspended in DCM (120 ml) and washed with 1 M HCl solution (3 × 30 ml), H_2O (3 × 30 ml) and brine solution. The organic phase was dried (Na_2SO_4) and then concentrated at reduced pressure. The oximes **5** were purified by silica gel column chromatography eluting with hexane–EtOAc or recrystallization.

*4.2.1. Benzaldehyde oxime **5a**.*

Pure **5a** was obtained as a colorless crystal (2.42 g, 94%). R_f = 0.29 in hexane/ethyl acetate (4:1); mp = 34-35 ˚C (lit.[38] 35-36 ˚C); ^{1}H-NMR (300 MHz, $CDCl_3$) (300 K): δ 9.38 (s, 1H, OH), 8.22 (s, 1H, CH=N), 7.62-7.59 (m, 2H, H2 and H6), 7.46-7.38 (m, 3H, H3, H4 and H5); ^{13}C-NMR (75 MHz, $CDCl_3$) (300 K): δ 150.2 (CH=N), 131.5 (C-4), 129.5 (C-1), 128.5 (C-3 and C-5), 126.8 (C-4 and C-6).

*4.2.2. Phenylacetaldehyde oxime **5b**.*

Pure **5b** was obtained as a colorless solid and a 1.2:1 mixture of geometric isomers **a** and **b** (7.65 g, 94%). R_f = 0.63 in hexane/ethyl acetate (3:7); mp = 67-69 ˚C (lit.[39] 66-68 ˚C); IR (NaCl) ν_{max} = 3192 (OH), 1801, 1716, 1452, 1419, 1055 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) (300 K): 8.34 (br s, 2H, OH isomers **a** and **b**), 7.57 (t, *J* = 6.3 Hz, 1H, CH=N, isomer **a**), 7.33-7.20 (m, 10H, Ar-H isomers **a** and **b**), 6.93 (t, *J* = 5.1 Hz, 1H, CH=N, isomer **b**), 3.77 (d, *J* = 5.1 Hz, 2H, CH_2 isomer **b**), 3.56 (d, *J* = 6.3 Hz, 2H, CH_2 isomer **a**); ^{13}C-NMR (75 MHz, $CDCl_3$) (300 K): δ 150.5, 150.3 (CH=N isomers **a** and **b**), 136.2, 135.6, 128.5, 128.4, 126.6, 126.4, 35.6 and 31.4 (CH_2 isomers **a** and **b**).

*4.2.3. Hydrocinnamaldehyde oxime **5c**.*

Pure **5c** was obtained as a white solid and a 1.12:1 mixture of geometric isomers **a** and **b** (4.32 g, 97%). R_f = 0.42 in hexane/ethyl acetate (7:3); mp = 75-76 ˚C (lit.[40] 74 ˚C); IR (NaCl) ν_{max} = 3326 (OH), 2934, 2826, 1600 (C=N) cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) (300 K): δ 8.06 (br s, 2H, OH of mixture isomers **a** and **b**), 7.46 (t, *J* = 5.7 Hz, 1H, CH=N isomer **a**), 7.32-7.18 (m, 10H, Ar-H isomers **a** and **b**), 6.77 (t, J = 5.4 Hz, 1H, CH=N isomer **b**), 2.86-2.49 (m, 8H, CH_2CH_2 isomers **a** and **b**); ^{13}C-NMR (75 MHz, $CDCl_3$) (300 K): δ 151.3 and 151.1 (CH=N isomers **a** and **b**), 140.2, 140.1, 128.2, 128.0, 127.9, 125.9, 32.4, 31.5, 30.8, 26.0.

*4.2.4. 3,4-Dimethoxybenzaldehyde oxime **5e**.*

Pure **5e** was obtained as a white solid (5.12 g, 95%). R_f = 0.43 in hexane/ethyl acetate (7:3); mp = 94-95 ˚C (lit.[38] 93-94 ˚C); IR (NaCl) ν_{max} = 3450 (OH), 1602 (C=N), 1514, 1269 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) (300 K): δ 8.74 (s, 1H, OH), 8.06 (s, 1H, CH=N), 7.19 (d, *J* = 1.8 Hz, 1H, Ar-H), 7.01 (dd, *J* = 2.1 Hz and *J* = 8.4 Hz, 1H, Ar-H), 6.82 (d, *J* = 8.1 Hz, 1H, Ar-H), 3.85 (s, 6H, 2 × CH_3O); ^{13}C-NMR (75 MHz, $CDCl_3$) (300 K): δ 150.5, 149.8, 148.9, 124.3, 121.4, 110.4, 107.8, 55.5 (2 × CH_3O);

*4.2.5. 4-O-Acetylbenzaldehyde oxime **5f**.*

Pure **5f** was obtained as a white solid (6.48 g, 96%). R_f = 0.31 in hexane/ethyl acetate (7:3); mp = 87-88 ˚C; IR (NaCl) ν_{max} = 3379 (OH), 1730 (C=O), 1600 (C=N), 1581, 1558, 1232, 1195 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) (300 K): δ 9.06 (s, 1H, OH), 8.12 (s, 1H, CH=N), 7.58 (d, *J* = 8.7 Hz, 2H, H3 and H5), 7.12 (d, *J* = 8.7 Hz, 2H, H2 and H6), 2.28 (s, 3H, CH_3COO); ^{13}C-NMR (75 MHz, $CDCl_3$) (300 K): δ 169.1 (CH_3*C*OO), 151.5 (C-4), 148.9 (C=N), 129.4 (C-1), 127.8 (C-3, C-5), 121.7 (C-2, C-6), 20.8 (*C*H_3COO); HRMS (ESI) *m/z* for $C_9H_9NO_3$ $[M+H]^+$ calcd 180.0655, found 180.0649.

*4.2.6. 2,3-Dichlorobenzaldehyde oxime **5g**.*

Pure **5g** was obtained as white crystals (5.7 g, 99%). R_f = 0.61 in hexane/ethyl acetate (7:3); mp = 121-122 ˚C; ^{1}H-NMR (300 MHz, $CDCl_3$) (300 K): δ 8.57 (s, 1H, CH=N), 8.13 (s, 1H, OH), 7.73 (dd, $J_{4,5}$ = 8.1 Hz, 1H, H4), 7.49 (dd, $J_{5,6}$ = 7.8 Hz, 1H, H6), 7.20 (t, $J_{5,6}$ = 7.8 Hz, $J_{4,5}$ = 8.1 Hz, 1H, H5); ^{13}C-NMR (75 MHz, $CDCl_3$) (300 K): δ = 147.2 (C=N), 133.4 (C-3), 131.8 (C-2), 131.5 (C-4), 131.2 (C-1), 127.1 (C-6), 125.0 (C-5); HRMS (ESI) *m/z* for $C_7H_4Cl_2NO$ $[M+H]^+$, calcd 189.9821, found 189.9813.

*4.2.7. 4-Bromobenzaldehyde oxime **5h**.*

Pure **5h** was obtained as white crystals (5.93 g, 98%). R_f = 0.49 in hexane/ethyl acetate (3:2); mp = 112-113 ˚C (lit.[41] 112 ˚C); ^{1}H-NMR (300 MHz, $CDCl_3$) (300 K): δ 8.35 (s, 1H, OH), 8.08 (s, 1H, CH=N), 7.52-7.41 (m, 5H, ArH); ^{13}C-NMR (75 MHz, $CDCl_3$) (300 K): δ 149.0 (CH=N), 131.7 (C-3 and C-5), 130.5 (C-4), 128.1 (C-2 and C-6), 123.9 (C-1).

*4.3. General procedure for the preparation of hydroxymoyl chloride (**1a-h**).*

One-fifth of *N*-chlorosuccinimide (1.05 eq.) (CAUTION: induction period) was added initially to a solution of the oxime 5 in DMF (30 ml). The reaction was cooled and stirred in an ice bath (for about 30 minutes) for the reaction happened (as indicated by a slight temperature rise). The reaction temperature was kept under 35 ˚C, while the addition of the NCS was repeated until all the rest of NCS was added. The reaction mixture was allowed to reach rt over 4 h. After that, the reaction mixture was poured into ice-water and extracted with Et_2O (3 × 20 ml). The organic layers were washed with water, dried over $MgSO_4$ and concentrated under reduced pressure. The pure hydroxymoyl chlorides 1 were obtained by chromatography eluting with hexane\EtOAc.

*4.3.1. N-Hydroxybenzimidoyl chloride **1a**.*

Pure **1a** was obtained as slight yellow solid (2.89 g, 89%). R_f = 0.41 in 80% hexane/ethyl acetate; mp = 50-51 ˚C (lit.[42] 48-49 ˚C); ^{1}H-NMR (300 MHz, $CDCl_3$) (300 K): δ 9.31 (s, 1H, OH), 7.85-7.82 (m, 2H, H2 and H6), 7.46-7.36 (m, 3H, H3, H4 and H5); ^{13}C-NMR (75 MHz, $CDCl_3$) (300 K): δ 140.2 (C=N), 132.0 (C-4), 130.5 (C-1), 128.2 (C-3 and C-5), 126.9 (C-3 and C-7).

*4.3.2. Phenylacetohydroximoyl Chloride **1b**.*

Pure **1b** was obtained as slight yellow solid (2.7 g, 84%). R_f = 0.40 in 70% hexane/ethyl acetate; mp = 86-87 ˚C (lit.[43] 87 ˚C); ^{1}H-NMR (300 MHz, $CDCl_3$) (300 K): δ 8.69 (s, 1H, OH), 7.37-7.25 (m, 5H, Ar-H), 3.88 (s, 2H, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$) (300 K): δ 141.0 (C=N), 134.0, 128.6, 128.4, 127.1, 42.5 (CH_2).

*4.3.3. Hydrocinnamohydroxymoyl chloride **1c**.*

Pure **1b** was obtained as a colorless liquid (4.62 g, 87%). R_f = 0.42 in 70% hexane/EtOAc; ^{1}H NMR (300 MHz, $CDCl_3$) (300 K) δ 8.29 (s, 1H, NOH), 7.18–7.33 (m, 5H, PhH), 2.95–2.99 (m, 2H, $PhCH_2CH_2$), 2.79–2.84 (m, 2H, $PhCH_2CH_2$); ^{13}C NMR (75 MHz, $CDCl_3$) (300 K) δ 141.2 (C=N), 139.2 (C-1 of Ph), 128.2 (C-3, C-5 of Ph), 128.1 (C-2, C-6 of Ph), 126.5 (C-4 of Ph), 38.1 ($PhCH_2CH_2$), 32.2 ($PhCH_2CH_2$); HRMS-FTMS (ESI) *m/z* for $C_9H_{10}ClON$ $[M+H]^+$, calcd 184.0524, found 184.0515.

*4.3.4. Cinnamohydroxymoyl chloride **1d**.*

Pure **1d** was obtained as yellow solid (2.08 g, 83%). R_f = 0.42 in 70% hexane/ethyl acetate; ^{1}H-NMR (300 MHz, $CDCl_3$) (300 K): δ 8.29 (s, 1H, OH), 7.33-7.18 (m, 6H, 5 × ArH and PhC*H*=CH), 6.88 (d, *J* = 15.6 Hz, 1H, Ph-CH=C*H*); ^{13}C-NMR (75 MHz, $CDCl_3$) (300 K): δ 140.8 (C=N), 137.8 (Ph*C*H=CH), 134.8 (C-1 of Ph), 128.9 (C-4 of Ph), 128.5 (C-3, C-5 of Ph), 127.0 (C-2, C-6 of Ph), 120.1 (PhCH=*C*H); HRMS (ESI) *m/z* for C_9H_8ClNO $[M+H]^+$, calcd 182.0367, found 182.0361.

*4.3.5. 3,4-Dimethoxybenzohydroxymoyl chloride **1e**.*

Pure **1e** was obtained as a yellow solid (4.94 g, 84%). R_f = 0.55 in 40% hexane/ethyl acetate; mp = 151-152 ˚C; IR (NaCl) ν_{max} = 3381 (OH), 1600 (C=N), 1581, 1541, 1265, 1143, 1004 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) (300 K): δ 8.13 (s, 1H, OH), 7.44 (d, $J_{5',6'}$ = 8.4 Hz, 1H, H6), 7.38 (s, 1H, H2), 6.87 (d, $J_{5',6'}$ = 8.4 Hz, 1H, H5), 3.90 (s, 6H, 2 × CH_3O); ^{13}C-NMR (75 MHz, $CDCl_3$) (300 K): δ 150.9 (C-4), 148.4 (C-3), 139.5 (C=N), 124.7 (C-1), 120.5 (C-2), 110.2 (C-5), 109.2 (C-6), 55.6 (2 × CH_3O); HRMS (ESI) *m/z* for $C_9H_{10}ClNO_3$ $[M+H]^+$, calcd 216.0422, found 216.0414.

*4.3.6. 4-O-Acetylbenzohydroxymoyl chloride **1f**.*

Pure **1f** was obtained as a yellow solid (7.06 g, 82%). R_f = 0.53 in 40% hexane/ethyl acetate; mp = 116-117 ˚C; IR (NaCl) ν_{max} = 3311 (OH), 1732 (C=O), 1606 (C=N), 1502, 1236 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) (300 K): δ 8.61 (s, 1H, OH), 7.84 (d, *J* = 9.0 Hz, 2H, H3 and H5), 7.14 (d, *J* = 8.7 Hz, 2H, H2 and H6), 2.39 (s, 3H, CH_3COO); ^{13}C-NMR (75 MHz, $CDCl_3$) (300 K): δ 169.2 (CH_3COO), 152.0 (C-4), 138.6 (C=N), 129.8 (C-1), 128.1 (C-3, C-5), 121.3 (C-2, C-6), 20.8 (CH_3COO); HRMS (ESI) *m/z* for $C_9H_8ClNO_3$ $[M+H]^+$, calcd 214.0265, found 214.0257.

4.3.7. 2,3-Dichlorobenzohydroxymoyl chloride ***1g***.

Pure **1g** was obtained as white crystals (5.9 g, 87%). R_f = 0.63 in hexane/ethyl acetate (2:3); mp = 112-113 ˚C; IR (NaCl) ν_{max} = 3392 (OH), 1629 (C=N), 1462, 1409, 1242 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) (300 K): δ 8.76 (s, 1H, OH), 7.55 (d, *J* = 8.1 Hz, 1H, H4), 7.36 (d, *J* = 7.8 Hz, 1H, H6), 7.27 (dd, $J_{4,5}$ = 8.1 Hz, $J_{5,6}$ = 7.8 Hz, 1H, H5); ^{13}C-NMR (75 MHz, $CDCl_3$) (300 K): δ 136.4 (C=N), 134.2 (C-3), 133.8 (C-2), 131.7 (C-4), 131.6 (C-1), 128.8 (C-6), 127.1 (C-5); HRMS (ESI) *m/z* for $C_7H_4Cl_3NO$ $[M+H]^+$, calcd 223.9431, found 223.9421.

4.3.8. 4-Bromobenzohydroxymoyl chloride ***1h***.

Pure **1h** was obtained as white crystals (7.03 g, 100%). R_f = 0.61 in 60% hexane/ethyl acetate; mp = 77-78 ˚C; ^{1}H-NMR (300 MHz, $CDCl_3$) (300 K): δ 8.69 (s, 1H, OH), 7.68-7.65 (m, 2H, H3 and H5), 7.53-7.50 (m, 2H, H2 and H6); ^{13}C-NMR (75 MHz, $CDCl_3$) (300 K): δ 139.2 (C=N), 131.4 (C-3, C-5), 130.9 (C-4), 128.3 (C-2, C-6), 125.1 (C-1); HRMS (ESI) *m/z* for $C_7H_5BrClON$ $[M+H]^+$, calcd 233.9316, found 233.9305.

4.4. General procedure for the preparation of hydroxymates ***7a-h***.

To stirred a solution of hydroxymoyl chloride **1** (1.5 eq.) in dry Et_2O:DCM (2:1, 45 ml) was added a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylthiol **6** (1 eq.) in dry DCM (6 ml). The resulting mixture was treated with Et_3N (6 eq.) in Et_2O (12 ml). The reaction mixture was stirred for 2 h at rt under N_2 then acidified with 1 M H_2SO_4 (7 ml/mmol of sugar). The mixture was left to stand for 10 min and then separated. The aqueous phase was extracted with DCM (3 × 30 ml). The combined organic layers were dried over $MgSO_4$, filtered and the filtrate was concentrated under reduced pressure. The thiohydroxymate **7** was obtained by flash chromatography eluting with 0-3% MeOH/DCM.

4.4.1. S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-phenylthiohydroxymate ***7a***.

Pure **7a** was obtained as a white solid (2.79 g, 96%). R_f = 0.60 in 90% DCM/MeOH; mp = 147-149 ˚C; IR (NaCl) ν_{max} = 3329 (OH), 1755 (C=O), 1598 (C=N), 1226, 1041 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) (300 K): δ 7.50-7.38 (m, 5H, ArH), 5.07-4.94 (m, 3H, H2, H3 and H4), 4.44 (d, $J_{1,2}$ = 9.6 Hz, 1H, H1), 4.09 (dd, $J_{5,6b}$ = 4.8 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6b), 3.94 (dd, $J_{5,6a}$ = 1.8 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6a), 3.02-2.99 (m, 1H, H5), 1.92, 1.94, 2.02 (3 × s, 12H, CH_3COO); ^{13}C-NMR (75 MHz, $CDCl_3$) (300 K): δ 170.3, 169.9, 168.9(2) (4 × CH_3COO), 152.4 (C=N), 131.9 (C-1 of Ph), 129.7 (p-Ph-C), 128.6 (m-Ph-C), 128.0 (o-Ph-C), 80.9 (C-1), 75.3 (C-5), 73.4 (C-3), 69.5 (C-2), 67.5 (C-4), 61.4 (C-6), 20.3, 20.5, 20.14, 20.08 (4 × CH_3COO).

4.4.2. S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl) phenylacetothiohydroximate ***7b***.

Pure 7b was obtained as a white solid (2.50 g, 83%). R_f = 0.54 in 40% hexane/EtOAc; mp = 163–164 ˚C; $[\alpha]_D^{18}$ = –10 (*c* = 1.0, $CHCl_3$); IR (NaCl) ν_{max} 3356 (NOH), 1751 (C=O), 1596 (C=N), 1367, 1226, 1039 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) (325 K) δ 8.62 (s, 1H, NOH), 7.36–7.23 (m, 5H, ArH), 5.02–4.90 (m, 3H, H2, H3 and H4), 4.79 (d, $J_{1,2}$ = 9.6 Hz, 1H, H1), 4.15 (dd, $J_{5,6b}$ = 5.7 Hz, $J_{6a,6b}$ = 12.6 Hz, H6b), 3.99–3.88 (m, 3H, H6a and CH_2Ph), 3.48–3.44 (m, 1H, H5), 2.05, 1.98, 1.94(2) (4 × s, 12H, CH_3COO); ^{13}C NMR (75 MHz, $CDCl_3$) (300 K) δ 170.2, 169.9, 168.9, 168.8 (4 × CH_3COO), 150.9 (C=N), 135.3 (C-1 of Ph), 128.6, 127.7 (m- + o-Ph-C), 127.1 (p-Ph-C), 79.0 (C-1), 75.3 (C-5), 73.4 (C-3), 69.6 (C-2), 67.6 (C-4), 61.8 (C-6), 38.5 (CH_2-Ph), 20.5(2), 20.3(2) (4 × CH_3COO); HRMS-FTMS (ESI) *m/z* for $C_{22}H_{27}O_{10}NS$ $[M+Na]^+$, calcd 520.1248, found 520.1226.

4.4.3. S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-(2-phenylethyl)thiohydroximate ***7c***.

Pure **7c** was obtained as a white solid (2.72 g, 81%). R_f = 0.17 in 60% hexane/EtOAc; mp = 198–199 ˚C; $[\alpha]_D^{20}$ = –11.3 (*c* = 2.0, $CHCl_3$); IR (NaCl) ν_{max} 3345 (NOH), 1751 (C=O), 1600 (C=N), 1485, 1367, 1226, 1041 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) (300 K) δ 7.32–7.16 (m, 5H, ArH), 5.24–4.95 (m, 4H, H1, H2, H3 and H4), 4.12–4.09 (m, 2H, H6a and H6b), 3.69–3.64 (m, 1H, H5), 2.99–2.76 (m, 4H, $PhCH_2CH_2$), 2.04, 2.02, 2.01, 1.98 (4 × s, 12H, CH_3COO); ^{13}C NMR (75 MHz, $CDCl_3$) (300 K) δ 170.2, 169.8, 168.9, 168.8 (4 × CH_3COO), 151.8 (C=N), 140.0 (C-1 of Ph), 128.3 (C-3, C-5 of Ph), 127.9 (C-2, C-6 of Ph), 126.2 (C-4 of Ph), 79.5 (C-1), 75.7 (C-5), 73.3 (C-3), 69.7 (C-2), 67.7 (C-4), 61.8 (C-6), 33.9 ($PhCH_2CH_2$), 33.9 ($PhCH_2CH_2$), 20.3(2), 20.2(2) (4 × CH_3COO); HRMS-FTMS (ESI) *m/z* for $C_{23}H_{29}O_{10}NS$ $[M+Na]^+$, calcd 534.1404, found 534.1377.

4.4.4. S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-(2-phenylethenyl)thiohydroxymate ***7d***.

Pure **7d** was obtained as a yellow solid (2.2 g, 86%). R_f = 0.14 in 60% hexane/EtOAc; mp = 159-160 ˚C; IR (NaCl) ν_{max} = 3361 (OH), 2951, 1751 (C=O), 16027 (C=N), 1448, 1367, 1224, 1041 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) (300 K) δ 7.48-7.23 (m, 6H, ArH and PhC*H*=CH), 6.85 (d, *J* = 15.9 Hz, 1H, PhCH=C*H*), 5.20-5.04 (m, 4H, H1, H2, H3 and H4), 4.25 (dd, $J_{5,6b}$ = 5.7 Hz, $J_{6a,6b}$ = 12.3 Hz, 1H, H6b), 4.14 (dd, $J_{5,6a}$ = 2.4 Hz, $J_{6a,6b}$ = 12.3 Hz, 1H, H6a), 3.73-3.69 (m, 1H, H5), 2.03, 2.02, 1.99, 1.98 (4 × s, 12H, CH_3COO); ^{13}C-NMR (75 MHz, $CDCl_3$) (300 K): δ 170.3, 169.8, 168.9(2) (4 × CH_3COO), 151.2 (C=N), 137.6 (Ph*C*H=CH), 135.1 (C-1 of Ph), 128.9 (C-4 of Ph), 128.6 (C-3, C-5 of Ph), 126.9 (C-2, C-6 of Ph), 120.3 (PhCH=*C*H), 81.4 (C-1), 75.7 (C-5), 73.4 (C-3), 10.1 (C-2), 67.8 (C-4), 61.9 (C-6), 20.3, 20.2(3) (4 × *C*H_3COO); HRMS (ESI) *m/z* for $C_{23}H_{27}O_{10}NS$ $[M+H]^+$, calcd 510.1428, found 510.1402.

4.4.5. S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-3,4-dimethoxyphenylacetothiohydroxymate ***7e***.

Pure **7e** was obtained as a yellow solid (3.13 g, 96%). R_f = 0.54 in 40% hexane/EtOAc; mp = 60-61 ˚C; $[\alpha]_D^{19}$ = +26.6 (*c* = 1.0, $CHCl_3$); IR (NaCl) ν_{max} = 3419 (OH), 2956 (CH_3OPh), 1751 (C=O), 1600 (C=N), 1367, 1226, 1041 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) (300 K): δ 9.49 (s, 1H, OH), 7.08 (d, $J_{5',6'}$ = 8.1 Hz, 1H, H6'-Ph-H), 6.98 (s, 1H, H2'-Ph-H), 6.88 (d, $J_{5',6'}$ = 8.1

Hz, 1H, H5'-Ph-H), 5.00-4.95 (m, 3H, H2, H3 and H4), 4.58-4.55 (m, 1H, H1), 4.07-4.01 (m, 1H, H6b), 3.93-3.79 (m, 7H, H6a and 2 × CH_3OPh), 3.10-3.09 (m, 1H, H5), 2.01, 2.00, 1.95, 1.94 (4 × s, 12H, CH_3COO); ^{13}C-NMR (75 MHz, $CDCl_3$) (300 K): δ 170.3, 169.9, 168.9, 168.8 (4 × CH_3COO), 151.3 (C=N), 150.1 (C-4 of Ph), 148.5 (C-3 of Ph), 124.6 (C-1 of Ph), 121.5 (C-2 of Ph), 111.6 (C-5 of Ph), 110.3 (C-6 of Ph), 81.3 (C-1), 75.3 (C-5), 73.4 (C-3), 69.7 (C-2), 67.5 (C-4), 61.3 (C-6), 55.8, 55.6 (CH_3O), 20.6, 20.2, 20.1, 20.0 (4 × CH_3COO); HRMS (ESI) *m/z* for $C_{23}H_{29}O_{12}NS$ $[M-H]^-$, calcd 542.1332, found 542.1330.

4.4.6. S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-4-acetyl-O-phenylthiohydroxymate ***7f***.

Pure **7f** was obtained as a yellow solid (2.8 g, 86%). R_f = 0.27 in 40% hexane/EtOAc; mp = 93-94 ˚C; $[\alpha]_D^{19}$ = +22.5 (*c* = 2.0, $CHCl_3$); IR (NaCl) ν_{max} = 3369 (OH), 1751 (C=O), 1600 (C=N), 1504, 1369, 1291, 1041 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) (300 K): δ 7.55 (d, *J* = 8.1 Hz, 2H, H3' and H5'-Ph-H), 7.19 (d, *J* = 8.7 Hz, 2H, H2' and H6'-Ph-H), 5.04-4.97 (m, 3H, H2, H3 and H4), 4.38 (d, *J* = 9.6 Hz, 1H, H1), 4.10 (dd, $J_{5,6b}$ = 4.5 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6b), 3.92 (dd, $J_{5,6a}$ = 5.7 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6a), 3.01-2.97 (m, 1H, H5), 2.30 (s, 3H, C*H*$_3$COOPh), 2.08, 2.04, 1.94, 1.93 (4 × s, 12H, CH_3COO); ^{13}C-NMR (75 MHz, $CDCl_3$) (300 K): δ 170.0, 169.6, 168.8, 168.4 (4 × CH_3*C*OO), 168.9 (CH_3COOPh), 160.6 (C=N), 152.1 (C-4 of Ph), 130.0 (C-3, C-5 of Ph), 127.8 (C-1 of Ph), 121.6 (C-2, C-6 of Ph), 81.2 (C-1), 75.5 (C-5), 73.2 (C-3), 69.0 (C-2), 67.1 (C-4), 61.2 (C-6), 20.8 (*C*H_3COOPh), 20.3, 20.2, 20.1, 18.9 (4 × *C*H_3COO); HRMS (ESI) *m/z* for $C_{23}H_{27}O_{12}NS$ $[M+Na]^+$, calcd 564.1146, found 564.1118.

4.4.7. S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-2,3-dichlorophenylacetothiohydroxymate ***7g***.

Pure **7g** was obtained as a yellow solid (3.55 g, 90%). R_f = 0.36 in 40% hexane/EtOAc; mp = 172-173 ˚C; $[\alpha]_D^{19}$ = +22 (*c* = 2.0, $CHCl_3$); IR (NaCl) ν_{max} = 3319 (OH), 1749 (C=O), 1602 (C=N), 1597, 1411, 1367, 1222, 1053 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) (300 K): δ 7.59-7.56 (m, 1H, H4'-Ph-H), 7.34-7.25 (m, 2H, H5' and H6'-Ph-H), 5.02-4.97 (m, 3H, H2, H3 and H4), 4.14-4.03 (m, 2H, H1 and H6b), 3.89-3.84 (m, 1H, H6a), 2.86-2.84 (m, 1H, H5), 2.07, 2.03, 1.95, 1.93 (4 × s, 12H, CH_3COO); ^{13}C-NMR (75 MHz, $CDCl_3$) (300 K): δ 170.2, 169.9, 168.9, 168.8 (4 × CH_3*C*OO), 150.3 (C=N), 133.4 (C-3 of Ph), 132.5 (C-2 of Ph), 132.4 (C-4 of Ph), 131.7 (C-6 of Ph), 129.6 (C-1 of Ph), 126.7 (C-5 of Ph); 80.8 (C-1), 75.3 (C-5), 73.2 (C-3), 68.9 (C-2), 67.3 (C-4), 60.9 (C-6), 20.3, 20.2, 20.14, 20.08 (4 × *C*H_3COO); HRMS (ESI) *m/z* for $C_{21}H_{23}O_{10}Cl_2NNaS$ $[M+Na]^+$, calcd 574.0312, found 574.0283.

4.4.8. S-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-4-bromophenylthiohydroxymate ***7h***.

Pure **7h** was obtained as a white solid (2.81 g, 83%). R_f = 0.56 in 40% hexane/EtOAc; mp = 147-148 ˚C; $[\alpha]_D^{20}$ = +31.4 (*c* = 1.0, $CHCl_3$); IR (NaCl) ν_{max} = 3358 (OH), 1751 (C=O), 1587 (C=N), 1485, 1367, 1226, 1041 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) (300 K): δ 8.86 (s, 1H, OH), 7.58-7.54 (m, 2H, H3' and H5'-Ph-H), 7.44-7.40 (m, 2H, H2' and H6'-Ph-H), 5.05-4.94 (m, 3H, H2, H3 and H4), 5.53-4.48 (m, 1H, H1), 4.12 (dd, $J_{5,6b}$ = 4.8 Hz, $J_{6a,6b}$ = 12.3 Hz, 1H, H6b), 3.99 (dd, $J_{5,6a}$ = 2.4 Hz, $J_{6a,6b}$ = 12.3 Hz, 1H, H6a), 3.18-3.13 (m, 1H, H5), 2.08, 2.03, 1.96, 1.96 (4 × s, 12H, CH_3COO); ^{13}C-NMR (75 MHz, $CDCl_3$) (300 K): δ 170.2, 169.9, 168.9(2) (4 × CH_3*C*OO), 151.0 (C=N), 131.4 (C-3, C-5 of Ph), 131.1 (C-4 of Ph), 130.1 (C-2, C-6 of Ph), 124.3 (C-1 of Ph), 81.0 (C-1), 75.4 (C-5), 73.2 (C-3), 69.6 (C-2), 67.5 (C-4), 61.5 (C-6), 20.4, 20.3, 20.2, 20.1 (4 × *C*H_3COO); HRMS (ESI) *m/z* for $C_{21}H_{24}O_{10}BrNNaS$ $[M+Na]^+$, calcd 584.0197, found 584.0170.

*4.5. General procedure for the preparation of potassium sulfate salts of thiohydroxymates (****8a-h****).*

To a stirred solution of the thiohydroxymate **7** (1 eq.) in dry DCM (40 ml) was added pyridine sulfur trioxide complex (2.5 eq.). After stirring and refluxing under Ar for 18 h, an additional portion of the complex (0.3 eq.) was added and stirring was continued for 2 h. After that, a solution of $KHCO_3$ (4 eq.) 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 (2 × 30 ml) and then with 80% $CHCl_3$/MeOH (3 × 30 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 **8a-h** were obtained by flash chromatography eluting with DCM/MeOH.

4.5.1. Potassium 2,3,4,6-Tetra-O-acetylphenylglucosinolate ***8a***.

Pure **8a** was obtained as a white solid (1.3 g, 64%). R_f = 0.38 in 15% MeOH/EtOAc; mp = 125-126 ˚C (dec.); $[\alpha]_D^{21}$ = –8.8 (*c* = 2.0, MeOH); IR (KBr DRIFT) ν_{max} = 1747 (C=O), 1600 (C=N), 1431, 1369, 1232, 1061 cm^{-1}; ^{1}H-NMR (300 MHz, [D_6]DMSO) (300 K): δ 7.53-7.41 (m, 5H, ArH), 5.20 (t, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, 1H, H3), 4.89-4.78 (m, 2H, H2 and H4), 4.68 (d, $J_{1,2}$ = 10.2 Hz, 1H, H1), 4.01 (dd, $J_{5,6b}$ = 5.7 Hz, $J_{6a,6b}$ = 12.3 Hz, 1H, H6b), 3.85 (dd, $J_{5,6a}$ = 1.8 Hz, $J_{6a,6b}$ = 12.5 Hz, 1H, H6a), 3.32-3.22 (m, 1H, H5), 2.04, 2.00, 1.89 1.88 (4 × s, 12H, CH_3COO); ^{13}C-NMR (75 MHz, [D_6]DMSO) (300 K): δ 170.1, 169.5, 169.3(2) (4 × CH_3*C*OO), 153.1 (C=N), 131.7 (C-1 of Ph), 130.1 (p-Ph-C), 128.9 (m-Ph-C), 128.5 (o-Ph-C), 80.1 (C-1), 74.5 (C-5), 72.8 (C-3), 69.6 (C-2), 67.8 (C-4), 61.7 (C-6), 20.6, 20.5, 20.4, 20.3 (4 × *C*H_3COO); HRMS (ESI) *m/z* for $C_{21}H_{24}O_{13}NS_2K$ $[M-K]^-$, calcd 562.0689, found 562.0670.

4.5.2. Potassium 2,3,4,6-tetra-O-acetylglucotropaeolin ***8b***.

Pure **8b** was obtained as a white solid (0.85 g, 83%). R_f = 0.25 in 10% MeOH/EtOAc; mp = 196–198 ˚C (dec.) (Lit. 197–199 ˚C)[6]; $[\alpha]_D^{20}$ = –18.5 (*c* = 1.0, H_2O) (Lit. $[\alpha]_D^{20}$ = –19 (*c* = 1, H_2O))[6]; IR (KBr DRIFT) ν_{max} 1757 (C=O), 1579 (C=N), 1500, 1434, 1234, 1044 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) (300 K) δ 7.39–7.26 (m, 5H, Ph-H), 5.08-4.99 (m, 3H, H2, H3 and H4), 4.79 (t, $J_{1,2}$ = 9.3 Hz, 1H, H1), 4.17 (dd, $J_{5,6b}$ = 4.8 Hz, $J_{6a,6b}$ = 12.3 Hz, 1H, H6b), 4.04–3.93 (m, 3H, H6a and CH_2Ph), 3.70–3.66 (m, 1H, H5), 2.03, 1.96, 1.90, 1.88 (4 × s, 12H, CH_3COO); ^{13}C NMR (75 MHz, CD_3OD) (325 K) δ 170.5, 169.7, 169.3, 169.0 (4 × CH_3COO), 155.5 (C=N), 135.3 (C-1 of Ph), 128.1, 127.7 (*m*- + *o*-Ph-C), 126.5 (*p*-Ph-C), 79.2 (C-1), 75.1 (C-5), 73.5 (C-3), 69.7 (C-2), 67.8 (C-4), 61.5 (C-6), 38.0 (CH_2Ph), 18.(2), 18.6(2) (4 × CH_3COO); HRMS-ESI) *m/z* for $C_{22}H_{26}O_{13}NS_2$ $[M-K]^-$, calcd 576.0846, found 576.0825.

4.5.3. Potassium 2,3,4,6-Tetra-O-acetylgluconasturtiin ***8c***.

Pure **8c** was obtained as a white solid (1.1 g, 72%). R_f = 0.34 in 15% MeOH/DCM; mp = 199-201 ˚C (dec.); $[\alpha]_D^{20}$ = –11.6 (*c* = 1.0, MeOH); IR (KBr DRIFT) ν_{max} = 1750 (C=O), 1585 (C=N), 1430, 1368, 1235, 1061 cm^{-1}; ^{1}H-NMR (300 MHz, CD_3OD) (300 K): δ 7.28-7.17 (m, 5H, ArH), 5.32 (t, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, 1H, H3), 5.27 (d, $J_{1,2}$ = 10.2 Hz, 1H, H1), 5.02-4.92 (m, 2H, H2 and H4), 4.20-4.04 (m, 2H, H6a and H6b), 3.94-3.88 (m, 1H, H5), 3.09-2.89 (m, 4H, $PhCH_2CH_2$), 2.01, 1.99, 1.95, 1.81 (4 × s, 12H, CH_3COO); ^{13}C-NMR (75 MHz, CD_3OD) (300 K): δ 170.4, 169.7, 169.5, 169.1 (4 × CH_3COO), 156.9 (C=N), 140.4 (C-1 of Ph), 127.9 (C-3, C-5 of Ph), 127.9 (C-2, C-6 of Ph), 125.6 (C-4 of Ph), 78.9 (C-1), 75.1 (C-5), 73.2 (C-3), 69.5 (C-2), 67.8 (C-4), 61.7 (C-6), 33.6 ($PhCH_2CH_2$), 32.5 ($PhCH_2CH_2$), 18.8(2), 18.6(2) (4 × CH_3COO); HRMS-FTMS (ESI) *m/z* for $C_{23}H_{28}O_{13}NS_2$ [M-K]$^-$, calcd 590.1008 found 590.0989.

4.5.4. Potassium 2,3,4,6-Tetra-O-acetyl-(2-phenylethenyl)glucosinolate ***8d***.

Pure **8d** was obtained as a white solid (1.13 g, 83%). R_f = 0.29 in 15% MeOH/DCM ; mp = 124-125 ˚C (dec.); $[\alpha]_D^{20}$ = –13.8 (*c* = 1.0, MeOH); IR (KBr DRIFT) ν_{max} = 2942, 1749 (C=O), 1627, 1539 (C=N), 1447, 1370, 1230, 1058 cm^{-1}; ^{1}H-NMR (300 MHz, CD_3OD) (300 K): δ 7.58-7.55 (m, 2H, H2' and H6'-Ph-H), 7.41-7.32 (m, 4H, H3', H4', H5'-Ph-H and PhC*H*=CH), 6.93 (d, *J* = 15.9 Hz, 1H, PhCH=C*H*), 5.38-5.31 (m, 2H, H1 and H3), 5.08-5.00 (m, 2H, H2 and H4), 4.26 (dd, $J_{5,6b}$ = 5.4 Hz, $J_{6a,6b}$ = 12.3 Hz, 1H, H6b), 4.12 (dd, $J_{5,6a}$ = 2.1 Hz, $J_{6a,6b}$ = 12.3 Hz, 1H, H6a), 4.00-3.94 (m, 1H, H5), 2.02, 1.99, 1.95, 1.90 (4 × s, 12H, CH_3COO); ^{13}C-NMR (75 MHz, CD_3OD) (300 K): δ 170.5, 169.7, 169.5, 169.4 (4 × CH_3COO), 154.1 (C=N), 137.9 (Ph*C*H=CH), 135.1 (C-1 of Ph), 128.8 (C-4 of Ph), 128.3 (C-3, C-5 of Ph), 126.9 (C-2, C-6 of Ph), 118.8 (PhCH=*C*H), 80.3 (C-1), 75.1 (C-5), 73.3 (C-3), 69.9 (C-2), 67.9 (C-4), 61.7 (C-6), 18.8(2), 18.7(2) (4 × CH_3COO); HRMS-FTMS (ESI) *m/z* for $C_{23}H_{26}O_{13}NS_2$ [M-K]$^-$, calcd 588.0851 found 588.0835.

4.5.5. Potassium 2,3,4,6-Tetra-O-acetyl-3,4-dimethoxyphenylglucosinolate ***8e***.

Pure **8e** was obtained as a white solid (1.24 g, 68%). R_f = 0.72 in 10% MeOH/DCM; mp = 114-115 ˚C (dec.); $[\alpha]_D^{21}$ = +5 (*c* = 1.0, MeOH); IR (KBr DRIFT) ν_{max} = 2944 (CH_3), 1746 (C=O), 1599 (C=N), 1514, 1247, 1062, cm^{-1}; ^{1}H-NMR (300 MHz, CD_3OD) (300 K): δ 7.26-7.21 (m, 2H, H2' and H6'- Ph-H), 7.05 (d, $J_{5',6}$ = 5.4 Hz, 1H, H5'-Ph-H), 5.14 (t, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, 1H, H3), 5.00-4.94 (m, 2H, H2 and H4), 4.87 (d, $J_{1,2}$ = 10.2 Hz, 1H, H1), 4.19 (dd, $J_{5,6b}$ = 4.2 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6b), 4.03 (dd, $J_{5,6a}$ = 2.4 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6a), 3.87 (s, 6H, 2 × CH_3OPh), 3.42-3.37 (m, 1H, H5), 2.04, 2.03, 1.94 1.93 (4 × s, 12H, CH_3COO); ^{13}C-NMR (75 MHz, CD_3OD) (300 K): δ 170.5, 169.7, 169.4, 169.3 (4 × CH_3COO), 155.9 (C=N), 150.9 (C-4 of Ph), 148.6 (C-3 of Ph), 124.1 (C-1 of Ph), 121.9 (C-2 of Ph), 112.2 (C-5 of Ph), 110.5 (C-6 of Ph), 81.5 (C-1), 75.2 (C-5), 73.3 (C-3), 69.9 (C-2), 67.5 (C-4), 61.1 (C-6), 55.0, 54.8 (2 × CH_3OPh), 18.9(2), 18.7(2) (4 × CH_3COO); HRMS (ESI) *m/z* for $C_{23}H_{28}O_{15}NS_2$ [M-K]$^-$, calcd 622.0906, found 622.0998.

4.5.6. Potassium 2,3,4,6-Tetra-O-acetyl-4-acetyl-O-phenylglucosinolate ***8f***.

Pure **8f** was obtained as a white solid (0.76 g, 72%). R_f = 0.48 in 20% MeOH/DCM; mp = 129-131 ˚C (dec.); $[\alpha]_D^{21}$ = –20 (*c* = 1.0, MeOH); IR (KBr DRIFT) ν_{max} = 2939 (CH_3), 1747 (C=O), 1599 (C=N), 1504, 1433, 1371, 1230, 1061 cm^{-1}; ^{1}H-NMR (300 MHz, CD_3OD) (300 K): δ 7.66 (d, *J* = 8.7 Hz, 2H, H3' and H5'-Ph-H), 7.25 (d, *J* = 8.7 Hz, 2H, H2' and H6'-Ph-H), 5.12 (t, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, 1H, H3), 4.99-4.92 (m, 2H, H2 and H4), 4.69 (d, $J_{1,2}$ = 10.2 Hz, 1H, H1), 4.19 (dd, $J_{5,6b}$ = 9.3 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6b), 3.98 (dd, $J_{5,6a}$ = 2.1 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6a), 3.26-3.24 (m, 1H, H5), 2.30 (s, 3H, CH_3CO_2Ph), 2.06, 2.02, 1.94, 1.92 (4 × s, 12H, CH_3COO); ^{13}C-NMR (75 MHz, CD_3OD) (300 K): δ 169.4 (CH_3COOPh), 168.5, 168.3, 168.2, 168.1 (4 × CH_3COO), 155.0 (C=N), 150.9 (C-4 of Ph), 128.9 (C-3, C-5 of Ph), 127.9 (C-1 of Ph), 120.2 (C-2, C-6 of Ph), 79.7 (C-1), 73.9 (C-5), 72.1 (C-3), 68.4 (C-2), 66.3 (C-4), 59.9 (C-6), 18.0 (CH_3COOPh), 17.8, 17.7, 17.6(2) (4 × CH_3COO); HRMS (ESI) *m/z* for $C_{23}H_{26}O_{15}NS_2$ [M-K]$^-$, calcd 620.0749 found 620.0728.

4.5.7. Potassium 2,3,4,6-Tetra-O-acetyl-2,3-dichlorophenylglucosinolate ***8g***.

Pure **8g** was obtained as a white solid (0.78 g, 81%). R_f = 0.31 in 15% MeOH/DCM; mp = 142-144 ˚C (dec.); $[\alpha]_D^{20}$ = +15 (*c* = 1.0, MeOH); IR (KBr DRIFT) ν_{max} = 2942, 2884, 1754 (C=O), 1650, 1572, 1505, 1413, 1396, 1217, 1062 cm^{-1}; ^{1}H-NMR (300 MHz, CD_3OD) (300 K): δ 7.73-7.69 (m, 1H, H4'-Ph-H), 7.49-7.43 (m, 2H, H5' and H6'-Ph-H), 5.12 (t, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, 1H, H3), 4.97-4.91 (m, 2H, H2 and H4), 4.37 (d, $J_{1,2}$ = 10.2 Hz, 1H, H1), 4.11 (dd, $J_{5,6b}$ = 4.2 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6b), 3.90 (dd, $J_{5,6a}$ = 2.4 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6a), 3.06-3.01 (m, 1H, H5), 2.06, 2.02, 1.92, 1.92 (4 × s, 12H, CH_3COO); ^{13}C-NMR (75 MHz, CD_3OD) (300 K): δ 170.4, 169.7, 169.3, 169.1 (4 × CH_3COO), 154.7 (C=N), 132.8 (C-3 of Ph), 131.8 (C-4 of Ph), 131.8 (C-1 of Ph), 131.7 (C-2 of Ph), 129.8 (C-6 of Ph), 127.2 (C-5 of Ph), 80.5 (C-1), 74.9 (C-5), 73.0 (C-3), 69.0 (C-2), 67.3 (C-4), 60.8 (C-6), 18.9, 18.8, 18.7(2) (4 × CH_3COO); HRMS (ESI) *m/z* for $C_{21}H_{22}O_{13}Cl_2NS_2$ [M-K]$^-$, calcd 629.9915 found 629.9971.

4.5.8. Potassium 2,3,4,6-Tetra-O-acetyl-4-bromophenylglucosinolate ***8h***.

Pure **8h** was obtained as a white solid (1.27 g, 85%). R_f = 0.26 in 15% MeOH/DCM mp = 135-136 ˚C (dec.); $[\alpha]_D^{20}$ = +28.3 (*c* = 1.0, MeOH); IR (KBr DRIFT) ν_{max} = 2937, 2873, 1750 (C=O), 1585 (C=N), 1485, 1430, 1368, 1235, 1063 cm^{-1}; ^{1}H-NMR (300 MHz, CD_3OD) (300 K): δ 7.67-7.64 (m, 2H, H3' and H5'-Ph-H), 7.59-7.54 (m, 2H, H2' and H6'-Ph-H), 5.16 (t, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, 1H, H3), 5.00-4.92 (m, 2H, H2 and H4), 4.78 (d, $J_{1,2}$ = 10.2 Hz, 1H, H1), 4.18 (dd, $J_{5,6b}$ = 4.8 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6b), 3.98 (dd, $J_{5,6a}$ = 2.4 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6a), 3.39-3.34 (m, 1H, H5), 2.07, 2.03, 1.95, 1.92 (4 × s, 12H, CH_3COO); ^{13}C-NMR (75 MHz, CD_3OD) (300 K): δ 170.4, 169.6, 169.4, 169.2 (4 × CH_3COO), 155.3 (C=N), 131.1 (C-3, C-5 of Ph), 130.9 (C-4 of Ph), 130.4 (C-2, C-6 of Ph), 124.2 (C-1 of Ph), 80.9 (C-1), 75.1 (C-5), 73.2 (C-3), 69.6 (C-2), 67.5 (C-4), 61.1 (C-6), 18.9, 18.8, 18.7(2) (4 × CH_3COO); HRMS (ESI) *m/z* for $C_{21}H_{23}O_{13}BrNS_2$ [M-K]$^-$, calcd 639.9800 found 639.9937.

*4.6. General procedure for the preparation of glucosinolates (**9a-h**).*

To a solution of *O*-acetylglucosinolate **8** in anhydrous MeOH (20 ml) under a N_2 atmosphere was added dry MeOK (0.4 eq.) until pH = 8-9 was reached. After stirring for 3 h at rt, the solution was made neutral by the addition of glacial acetic acid then the solution was concentrated under reduced pressure. The GLs **9a-h** were obtained by flash chromatography eluting with EtOAc:MeOH:H_2O (16:4:1).

*4.6.1. Phenylglucosinolate **9a**.*

Pure **9a** was obtained as a white solid (99 mg, 90%). R_f = 0.26 in EtOAc:MeOH:H_2O 16:4:1; mp = 119-121 ˚C (dec.); $[\alpha]_D^{21}$ = –12.5 (*c* = 1.8, H_2O); IR (KBr DRIFT) ν_{max} = 3322 (OH), 1632 (C=N), 1555, 1444, 1252, 1062 cm^{-1}; ^{1}H-NMR (300 MHz, D_2O) (300 K): δ 7.55-7.43 (m, 5H, ArH), 4.19 (d, $J_{1,2}$ = 10.2 Hz, 1H, H1), 3.60 (dd, $J_{5,6a}$ = 2.4 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6a), 3.52 (dd, $J_{5,6b}$ = 4.2 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6b), 3.39 (dd, $J_{2,3}$ = 8.7 Hz, $J_{1,2}$ = 10.2 Hz, 1H, H2), 3.29 (dd, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 6.6 Hz, 1H, H4), 3.17 (dd, $J_{2,3}$ = 8.7 Hz, $J_{3,4}$ = 9.0 Hz, 1H, H3), 2.64-2.59 (m, 1H, H5); ^{13}C-NMR (75 MHz, D_2O) (300 K): δ 163.6 (C=N), 130.6 (C-1 of Ph), 129.9 (p-Ph-C), 128.6 (m-Ph-C), 128.4 (o-Ph-C), 83.1 (C-1), 79.5 (C-5), 76.6 (C-3), 71.2 (C-2), 68.4 (C-4), 59.8 (C-6); HRMS (ESI) *m/z* for $C_{13}H_{16}O_9NS_2K$ [M-K]$^-$, calcd 394.0266, found 394.0256.

*4.6.2. Glucotropaeolin **9b**.*

Pure **9b** was obtained as a white solid (101 mg, 82%). R_f = 0.14 in 15% EtOAc/MeOH; mp = 132-134 ˚C (dec.); $[\alpha]_D^{18}$ = +5 (*c* = 1.0, H_2O); IR (KBr DRIFT) ν_{max} = 3317 (OH), 1650 (C=N), 1567, 1495, 1265, 1061 cm^{-1}; ^{1}H-NMR (300 MHz, D_2O) (300 K): δ 7.37-7.26 (m, 5H, ArH), 4.61-4.59 (m, 1H, H1), 4.05 (s, 2H, CH_2Ph), 3.60-3.46 (m, 2H, H6a and H6b), 3.33-3.20 (m, 4H, H2, H3, H4 and H5); ^{13}C-NMR (75 MHz, D_2O) (300 K): δ 162.3 (C=N), 134.7 (C-1 of Ph), 128.8, 127.7 (m- + o-Ph-C), 127.2 (p-Ph-C), 80.9 (C-1), 79.5 (C-5), 76.6 (C-3), 71.4 (C-2), 68.4 (C-4), 59.9 (C-6), 37.8 (CH_2Ph); HRMS (ESI) *m/z* for $C_{14}H_{18}O_9NS_2$ [M-K]$^-$, calcd 408.0423, found 408.0414.

*4.6.3. Gluconasturtiin **9c**.*

Pure **9c** was obtained as a white solid (651 mg, 96%). R_f = 0.17 in EtOAc/MeOH/H_2O (16:4:1); mp = 169-171 ˚C (dec.); $[\alpha]_D^{20}$ = –22.5 (*c* = 1.0, H_2O); IR (KBr DRIFT) ν_{max} = 3335 (OH), 1568 (C=N), 1484, 1412, 1257, 1061 cm^{-1}; ^{1}H-NMR (300 MHz, D_2O) (300 K): δ 7.32-7.18 (m, 5H, ArH), 4.80 (d, $J_{1,2}$ = 9.3 Hz, 1H, H1), 3.76 (dd, $J_{5,6a}$ = 2.1 Hz, $J_{6a,6b}$ = 12.3 Hz, 1H, H6a), 3.44-3.01 (m, 4H, H2, H3, H4 and H5), 3.01-2.86 (m, 4H, $PhCH_2CH_2$); ^{13}C-NMR (75 MHz, D_2O) (300 K): δ 163.0 (C=N), 140.2 (C-1 of Ph), 128.4 (C-3 and C-5 of Ph), 128.4 (C-2 and C-6 of Ph), 126.3 (C-4 of Ph), 81.3 (C-1), 79.7 (C-5), 76.7 (C-3), 71.5 (C-2), 68.7 (C-4), 60.2 (C-6), 33.5 (Ph*C*H_2CH_2), 32.2 ($PhCH_2$*C*H_2); HRMS (ESI) *m/z* for $C_{15}H_{20}O_9NS_2$ [M-K]$^-$, calcd 422.0585 found 422.0570.

*4.6.4. 2-Phenylethenylglucosinolate **9d**.*

Pure **9d** was obtained as a yellow solid (206 mg, 89%). R_f = 0.17 in EtOAc/MeOH/H_2O (16:4:1); mp = 117-119 ˚C (dec.); $[\alpha]_D^{20}$ = –40.2 (*c* = 1.0, H_2O); IR (KBr DRIFT) ν_{max} = 3374 (OH), 2887, 1628 (C=N), 1575, 1537, 1494, 1448, 1271, 1060 cm^{-1}; ^{1}H-NMR (300 MHz, D_2O) (300 K): δ 7.54-7.51(m, 2H, H2' and H6'-Ph-H), 7.38-7.34 (m, 3H, H3', H4' and H5'-Ph-H), 7.26 (d, *J* = 15.9 Hz, 1H, Ph*CH*=CH), 6.85 (d, *J* = 15.9 Hz, 1H, PhCH=*CH*), 4.92 (d, *J* = 9.6 Hz, 1H, H1), 3.78-3.55 (m, 2H, H6a and H6b), 3.42-3.33 (m, 4H, H2, H3, H4 and H5); ^{13}C-NMR (75 MHz, D_2O) (300 K): δ 160.7 (C=N), 138.9 (Ph*C*H=CH), 134.5 (C-1 of Ph), 129.6 (C-4 of Ph), 128.8 (C-3, C-5 of Ph), 127.3 (C-2, C-6 of Ph), 117.5 (PhCH=*C*H), 82.4 (C-1), 80.1 (C-5), 76.7 (C-3), 71.7 (C-2), 68.8 (C-4), 60.3 (C-6) HRMS (ESI) *m/z* for $C_{15}H_{18}O_9NS_2$ [M-K]$^-$, calcd 420.0428 found 420.0415.

*4.6.5. 3,4-Dimethoxyphenylglucosinolate **9e**.*

Pure **9e** was obtained as a white solid (110 mg, 93%). R_f = 0.11 in EtOAc/MeOH/H_2O (16:4:1); mp = 104-106 ˚C (dec.); $[\alpha]_D^{20}$ = +12.3 (*c* = 1.0, H_2O); IR (KBr DRIFT) ν_{max} = 3317 (OH), 1650 (C=N), 1567, 1495, 1265, 1061 cm^{-1}; ^{1}H-NMR (300 MHz, D_2O) (300 K): δ 7.13-7.11 (m, 2H, H2' and H6'-Ph-H), 7.07-7.04 (m, 1H, H5'-Ph-H), 4.27 (d, $J_{1,2}$ = 9.9 Hz, 1H, H1), 3.81 (s, 6H, 2 × CH_3O), 3.58-3.46 (m, 2H, H6a and H6b), 3.38-3.14 (m, 3H, H2, H3 and H4), 2.67-2.63 (m, 1H, H5); ^{13}C-NMR (75 MHz, D_2O) (300 K): δ 162.9 (C=N), 149.9 (C-4 of Ph), 147.8 (C-3 of Ph), 122.8 (C-1 of Ph), 122.4 (C-2 of Ph), 112.0 (C-5 of Ph), 111.2 (C-6 of Ph), 83.5 (C-1), 79.6 (C-5), 76.7 (C-3), 71.3 (C-2), 68.4 (C-4), 59.8 (C-6), 35.6, 55.5 (CH_3OPh); HRMS (ESI) *m/z* for $C_{15}H_{20}O_{11}NS_2$ [M-K]$^-$, calcd 454.0483 found 454.0470.

*4.6.6. 2,3-Dichlorophenylglucosinolate **9g**.*

Pure **9g** was obtained as a white solid (145 mg, 99 %). R_f = 0.14 in EtOAc/MeOH/H_2O (16:4:1); mp = 117-119 ˚C (dec.); $[\alpha]_D^{18}$ = +60 (*c* = 1.0, H_2O); IR (KBr DRIFT) ν_{max} = 3354 (OH), 2882, 1566 (C=N), 1412, 1276, 1265, 1064 cm^{-1}; ^{1}H-NMR (300 MHz, D_2O) (300 K): δ 7.62-7.57 (m, 1H, H4'-Ph-H), 7.38-7.29 (m, 2H, H5'and H6'-Ph-H), 4.01 (d, $J_{1,2}$ = 9.9 Hz, 1H, H1), 3.47-3.41 (m, 2H, H6a and H6b), 3.31-3.18 (m, 2H, H2 and H4), 3.10 (t, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, 1H, H3), 2.43-2.38 (m, 1H, H5); ^{13}C-NMR (75 MHz, D_2O) (300 K): δ 160.5 (C=N), 132.7 (C-3 of Ph), 132.4 (C-4 of Ph), 131.1 (C-1 of Ph), 130.3 (C-2 of Ph), 129.3 (C-6 of Ph), 127.7 (C-5 of Ph), 82.7 (C-1), 79.4 (C-5), 76.5 (C-3), 70.9 (C-2), 68.0 (C-4), 59.1 (C-6); HRMS (ESI) *m/z* for $C_{13}H_{14}O_9Cl_2NS_2$ [M-K]$^-$, calcd 461.9493 found 461.9470.

*4.6.7. 4-Bromophenylglucosinolate **9h**.*

Pure **9h** was obtained as a white solid (223 mg, 100%). R_f = 0.2 in EtOAc/MeOH/H_2O (16:4:1); mp = 140-142 ˚C (dec.); $[\alpha]_D^{24}$ = +10.2 (*c* = 1.0, H_2O); IR (KBr DRIFT) ν_{max} = 3337 (OH), 2875, 1568 (C=N), 1485, 1410, 1257, 1066 cm^{-1}; ^{1}H-NMR (300 MHz, D_2O) (300 K): δ 7.66 (d, *J* = 8.4 Hz, 2H, H3' and H5'-Ph-H), 7.44 (d, *J* = 8.4 Hz, H2' and H6'-Ph-H), 4.22 (d, $J_{1,2}$ = 9.9 Hz, 1H, H1), 3.62 (dd, $J_{5,6a}$ = 2.4 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6a), 3.53 (dd, $J_{5,6b}$ = 4.8 Hz, $J_{6a,6b}$ = 12.6 Hz, 1H, H6b), 3.36 (dd, $J_{2,3}$ = 9.0 Hz, $J_{1,2}$ = 9.9 Hz, 1H, H2), 3.28 (dd, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 9.3 Hz, 1H, H4), 3.18 (dd, $J_{2,3}$ = 9.0 Hz, $J_{3,4}$ = 9.0 Hz, 1H, H3), 2.69-2.66 (m, 1H, H5); ^{13}C-NMR (75 MHz, D_2O) (300 K): δ 162.5 (C=N), 131.6 (C-3 and C-5 of Ph), 130.4 (C-2 and C-6 of Ph), 129.1 (C-4 of Ph), 124.7 (C-1 of Ph), 83.1 (C-1), 79.6 (C-5), 76.7 (C-3), 71.3 (C-2), 68.4 (C-4), 59.8 (C-6); HRMS (ESI) *m/z* for $C_{13}H_{15}O_9NS_2$ [M-K]$^-$, calcd 471.9377 found 471.9365.

4.7. 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 x 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 x 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 µM-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 ^{o}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 x PBST (0.05% Tween-20) before adding 200 µl/well assay diluent incubated at room temperature for 1 hour. Standards were prepared by 2 fold serial dilution to range from 500 pg/ml-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 but were soaked for 30 sec 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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5. Abbreviations

DCM, dichloromethane; DME, 1,2-dimethoxyethane; DMF, *N*,*N*-dimethylformamide; ESI, electrospray ionization; FTMS, Fourier transform mass spectrometry; GLs, Glucosinolates; HESI, heated electrospray ionization; HRMS, high resolution mass spectrometry; IR, infra-red; MS, mass spectrometry; NCS, *N*-chlorosuccinimide; NMR, nuclear magnetic resonance; THF, tetrahydrofuran; TLC, thin layer chromatography; UV, ultra-violet; Z, benzyloxycarbonyl; ELISA, enzyme-linked immunosorbent assay; TNF- α, tumor necrosis factor alpha; PMA, phorbol-12-myristate-13-acetate; LPS, lipopolysaccharides.

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