Glucosinolates With Their Hydrolysis Products from Two Cruciferous Plants with Study of Antidiabetic Activity Based on Molecular Docking

Khaled Abdelshafeek

Al-Bha univeristy

Walid E. Abdallah

NRC Egypt

Ahmed elhenawy (✉ elhenawy_sci@hotmail.com)

Al-Azhar University Faculty of Science <https://orcid.org/0000-0003-2893-0376>

Abeer Alothami

Taif University

Lila Alharby

ubd Alaziz univeristy

Abdulaziz Alomari

Al Bhaa univeristy

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Abstract

The glucosinolates (Gls.) are natural bioactive compounds which lead to the formation of different metabolites called isothiocyanates(ITC) having various therapeutic effects. So, this study aim to isolate the glucosinolates of both *Carrichtera annua* L.(DC) (CA) and *Farsetia aegyptia* Turra (FA) belonging to Cruciferae family. The total Gls. were isolated from the aqueous methanolic extract of both plants and further purified on acidic aluminum oxide column. Some of the obtained Gls. was identified as it is using different spectroscopic measurements (UV, NMR and MS) and the rest were hydrolyzed using myrosinase enzyme to the corresponding isothiocyanates (ITC) which were identified by GC/MS. only one glucosinolate was identified in CA as: 4-methylthio-3-butenyl Gls (MTBG). through chromatographic and spectroscopic measurements in addition to 6-methyl sulfonylhexyl isothiocyanates(ITC), while 6-methyl sulfonyl-6-hydroxy hexyl ITC, 4-pentenyl ITC, 3-Methylthio propyl ITC, 5-hydroxy pentyl ITC and 4-methylsulphinyl butyl ITC from FA. The docking study targeted a α -glucosidase and amylase, to examine a mode of action of the 4-methylthio-3-butenylglucosinolate. Molecular docking was performed to identify potency of Gls. against hyperglycemia. The data obtained revealed that the Gls. has high binding activity Via α -glucosidase and amylase. Furthermore, further Drug studies as likeness and ADME/T were performed, which proposed that their ligands may be have a good pharmacokinetic character, with no carcinogenesis effect.

1. Introduction

Many plants of the *Cruciferae* (formerly *Brassicaceae*) contain glucosinolates which are precursors of strongly odorous pungent isothiocyanates formed by the action of enzyme myrosinase on the glucosinolate substrate when the plant tissues are ruptured[1]. The family *Cruciferae* consider as one of the largest angiosperm families. It includes many of economically important plants as vegetable salad plants and crop species. The plants of this family have many biological activities like anticancer, antibacterial, antifungal, antirheumatic and antidiabetic[2]. *Carrichtera* and *Farsetia* are two genera belonging to *Cruciferae* family represented in Egypt by only one species known as *C. annua* L.(DC) for *Carrichtera* genus growing in Sinai peninsula specially at Elarish region, while *Farsetia* genus is represented by three species in which *F. aegyptia* Turra. is more popular and growing at many localities in Egypt [3]. The two plants are known to be used by the native Bedouins as antidiabetic and antispasmodic. Moreover, *F. aegyptia* is used for the relieve of rheumatic pains and taken as a cooling medicine after pounding. *F. aegyptia* are prepared as a decoction for toothache, gingivitis and sore eyes. Also, a decoction of the plant mixed with alum is used as a mouth rinse and for mouth disinfection[2–4]. The chemical structure of glucosinolates (Gls.) was found to contain a thioglucosidic bond to the carbon of a sulphonated oxime which attached to a side chain (R group) which may be aliphatic (e.g. alkyl, alkenyl, hydroxy alkenyl, thioalkyl) aliphatic aromatic (eg. Benzyl, substituted benzyl), or heterocyclic (e.g.indolyl,)as shown in chart 1[3]. Gls. and their hydrolysis products (Isothiocyanates,ITC) have different biological activities, like anticancer, antibacterial, antifungal, antioxidative, and allelopathic properties.

Isothiocyanates such as sulforaphane, iberin, phenylethyl and propenyl, derived from glucoraphanin, glucoiberin, gluconasturtiin and sinigrin,respectively, have been found to induce phase – 2 enzymes promoting antiproliferative activity[5–8]. The glucosinolates and their hydrolysis products in suspension culture following elicitation of *F. aegyptia* were found to contain glucotropaeolin(benzyl Gls.) and gucocheirolin(3-methylsulfonylpropyl-Gls), isobutyl Gls. and gluconasturtiin in addition to identification of 22 hydrolysis products[9, 10]. The total glucosinolates of both *C. annua* and *F. aegyptia* exhibited strong antifeedant effect against the 4th instar larvae of Egyptian cotton leaf worm, *S. littoralis* and proved to be highly potent, with the ovipositional repellent effect reaching 100% [4]. In addition, diabetes mellitus is a chronic endocrine disorder that affects the metabolism of carbohydrates, proteins, fat, electrolytes and water. It includes a group of metabolic diseases characterized by hyperglycemia, in which blood sugar levels are elevated either because the pancreas do not produce enough insulin or cells do not respond to the produced insulin [11].Therefore a therapeutic approach to treat diabetes is to decrease postprandial hyperglycemia [12]. This can be achieved by the inhibition of carbohydrate hydrolyzing enzymes like alpha amylase and alpha glucosidase. Alpha glucosidase and amylase are the important enzymes involved in the digestion of carbohydrates. Alpha Amylase is involved in the breakdown of long chain carbohydrates and alpha glucosidase breaks down starch and disaccharides to glucose. They serve as the major digestive enzymes and help in intestinal absorption. Alpha glucosidase and amylase inhibitors are the potential targets in the development of lead compounds for the treatment of diabetes[11, 12]. Consciously, Docking and DFT simulations are powerful computational tools that have been proven to be smarter, inexpensive and efficient approaches for understanding fundamental characters of Biomolecules[13]. DFT calculations gain the important topographic and molecular characters about molecular system as; optimization geometry, charge-transformation, global and local reactivities nature for the molecule and specific regions, respectively,...etc[14–16]. The molecular docking explores the possible binding mode of the compound via its target protein. Its process involves the prediction of ligand-receptor orientation, that useful to guide us for improve compound features[17]. That leads to investigate in the future the efficiency of biomolecule which effective in drug designing. The present study aims to isolation and identification of glucosinolates and their hydrolysis products of both *C. annua* and *F. aegyptia* growing in Egypt. Also, we combined the DFT, ADMET and the molecular docking analysis which will be useful in the future *In-vivo* studies as antidiabetic agent of this molecule.

2. Results And Discussion

2.1. Isolation and identification:

The total GIs of both plants were isolated and further purified to afford two compounds (CA1 and CA2) from CA and five compounds (FA1-FA5) from FA which were identified as follow:

CA1- 4-methylthio-3-butenyl Gls: The compound was isolated as an off-white amorphous powder and appear as a brown spot ($R_f = 0.52$) after spraying with modified AgNO_3 reagent. The UV absorption spectrum of the compound in methanol showed λ_{max} at 208 nm which shifted to 217 nm by addition of NaOH. When silver nitrate solution (1%) was added a new maximum appeared at 260 nm in addition to the first peak at 215 nm with increase in intensity, indicates the glucosinolate nature of the compound [18].

The ^1H -NMR spectrum (DMSO) showed signals at δ in ppm 2.13(3H, s, $\text{CH}_3\text{-S}$), 2.35(2H, t, H-1), 2.7(2H, q, H-2), 4.75(1H, d, $J = 8.2$ Hz with a small t, H-3), 5.8(1H, d, $J = 8.2$ Hz, H-4), the anomeric proton of glucose displayed as a doublet at 5.1 ppm. The complex group of signals between 3.1 and 3.85 are characteristic for the rest proton of the glucose moiety. The ^{13}C -NMR spectrum gave the anomeric carbon of the glucose unit at 82.16, the two carbon atoms (C-3 and C-4) of the double bond appeared at 137 and 115.6 ppm respectively and the central carbon of the GIs was assigned at 156.21 ppm. The other data were summarized in table-1. These findings were coincided with that reported by Manuela et al in 1992 [11]. The GC/MS of the isolated aglucone of this compound after the enzymatic hydrolysis gave only one peak for the corresponding isothiocyanate which identified through its fragmentation pattern as follow: the mass spectrum displayed a mass to charge ratio (m/z) of 159 due to the molecular ion peak M^+ which correspond to the molecular formula $\text{C}_5\text{H}_7\text{S}_2\text{N}$, in addition to the fragment ion peaks at m/z 144($\text{M}^+ - \text{CH}_3$), 101($\text{M}^+ - \text{NCS}$) and 73($\text{M}^+ - \text{CH}_2\text{NCS}$). By comparing these data with the reported data, it was found that, the fragmentation pattern is the same that reported for 4-methylthio-3-butenyl ITC, so, the chromatographic and spectroscopic measurements could be identified compound CA1 as 4-methylthio-3-butenyl glucosinolate.

Table 1: ^{13}C -nmr data of compound CA1:

Carbon no.	δ in ppm
C1	31.75
C2	30.44
C3	137.01
C4	115.6
S-C5	24.35
C=N	156.21
C1'	82.16
C2'	72.94
C3'	78.15
C4'	69.79
C5'	81.15
C6'	60.93

CA2- 6-methylsulfonylhexyl Gls: this compound was isolated in a small amount about 3 mg as a white yellowish powder which is not enough for nmr measurements. so, it subjected to enzymatic hydrolysis and identification the resultant ITC as follow: the mass spectrum exhibited M^+ at m/z of 221(20.5%) which fit to the molecular formula $\text{C}_8\text{H}_{15}\text{O}_2\text{NS}_2$. The other fragments at $m/z = 220(\text{M}^+ - 1, 23\%)$, $205(\text{M}^+ - \text{CH}_3, 100\%)$, $163(\text{M}^+ - \text{NCS}, 40\%)$ and $142(\text{M}^+ - \text{CH}_3\text{SO}_2, 35\%)$. This fragmentation pattern confirm the identification of the aglucone as 6-methylsulfonylhexyl ITC [$\text{CH}_3\text{SO}_2\text{-CH}_2\text{-(CH}_2)_4\text{-CH}_2\text{-NCS}$] and accordingly the compound was identified as: 6-methylsulfonylhexyl Gls. This is the first report about the isolation of these compounds from *C. annua* growing in Egypt.

The GC/MS data of the GIs. Hydrolysis products from FA was matched with the previously published data [18, 19], it was found that, the GIs, hydrolysis products included only isothiocyanates which were identified in an increasing of their retention manner as follow:

FA1 - 6-methylsulfonyl6-hydroxy-hexyl ITC. R_t : 15.3 min., the mass spectrum displayed a molecular ion peak M^+ at m/z (% relative abundance), =237(5%) correspond to the molecular formula $\text{C}_8\text{H}_{15}\text{O}_3\text{NS}_2$. Other fragments at $236(\text{M}^+ - 1, 12.3\%)$, $221(\text{M}^+ - \text{CH}_4, 28.6\%)$, $204(\text{M}^+ -$

SH, 17.5%), 180(M^+ - NCS, 74%), 165(M^+ - CH₂NCS, 100%), 137(M^+ - (CH₂)₃NCS, 32%), 123(M^+ - (CH₂)₄NCS, 12.5%), 109(M^+ - (CH₂)₅NCS, 18%) and 91(M^+ - [H₂O +(CH₂)₅NCS], 11.8%) or (M^+ -[CH₃SO₂-CH-1]). The latter two fragments (m/z 91 and 109) showed the presence of the OH group at C-6 and the fragmentation pattern proved the identification of the aglucone as *6-methylsulfonyl-6-hydroxy hexyl* ITC [CH₃SO₂-CH(OH)-(CH₂)₄-CH₂- NCS] (Chart 2).

FA2- 4-pentenyl ITC. R_t : 14.4 min., the molecular ion peak M^+ was present at m/z = 127(14.3%) which correspond to the molecular formula C₆H₉NS. Another important fragments at m/z 100(M^+ - (CH₂ = CH⁺, 18%), 85(M^+ -(CH₂)₃, 76%) and 57(M^+ - (CH₂ = CH-(CH₂)₃, 100%). These data substantiate the identification of the compound as *4-pentenyl ITC* CH₂ = CH-(CH₂)₃-NCS(Chart 2).

FA3- 3-Methylthio propyl ITC. R_t : 16.9 min., the mass spectrum gave M^+ at m/z of 147(12%) which fit with the molecular formula C₅H₉NS₂. The peaks at m/z 101 (M^+ - CH₃S, 100%), 86 (M^+ - CH₃S-CH₂, 2%), 72 (M^+ - CH₃S-CH₂-CH₂, 50%) and 61 (M^+ - -CH₂-CH₂-NCS, 44%) confirmed the presence of a methylthio unit attached to a propyl group and the compound identified as *3-Methylthio propyl ITC* CH₃S-CH₂-CH₂-CH₂-NCS (Chart 2).

FA4- 5-hydroxypentyl ITC. R_t : 19.2 min., the mass spectrum of this compound showed M^+ at m/z = 145(3%) correspond to the molecular formula C₆H₁₁ONS. The fragments at 101(M^+ -(CH₂-OH), 22.5%), 87(M^+ - NCS, 20.6%), 73(M^+ - CH₂-NCS, 67.8%) and 59(M^+ - [CH₂-(OH)CH₂CH₂, 100%) confirm the identification of the aglucone as *5-hydroxypentyl ITC* [CH₂ (OH)-(CH₂)₃-CH₂- NCS] (Chart 2).

FA5- sulphoraphane (4-methylsulphinyl butyl ITC): R_t : 28.7 min., the MS of the compound revealed the presence of M^+ at m/z = 177 corresponding to C₆H₁₁ONS₂ as a molecular formula. The fragmentation giving rise to 114 (M^+ - CH₃SO-, 6%), 86 (M^+ - CH₃SO-CH₂, 5%) and 72 (M^+ - CH₂NCS, 100%). So the compound was identified as *4-methylsulphinyl butyl ITC*, CH₃SO-(CH₂)₄NCS(Chart 2).

The variation of glucosinolate hydrolysis products between previous studies and the present work may be due to geographical and seasonal differences, environment, soil type, stress and plant part examined, or due to the differences in the experimental conditions.

2.2. Computational studies:

2.2.1. Frontier orbital analysis:

"FMOs" frontier molecular orbitals through two types HOMO (donating electron) and LUMO (accepting electron) are a crucial orbitals for molecule. This orbitals able to display the binding manner of biomolecule with receptor. FMOs gap was characterized the chemical reactivity and kinetic stability of the molecule[19, 20].

Figure 1

The HOMO was localized over methyl(prop-1-en-1-yl)sulfane region with (Fig. 1), while sulfanecarbaldehyde O-((oxidaneyl)dioxo-l6-sulfaneyl) oxime fragment covered by LUMO zone. Thus, the electrons transition (HOMO→LUMO) from sulfonyl to sulfonyl groups. The low ΔG enhance the interaction between HOMO ligand and the LUMO receptor (Table 1), with inversed mode was took place [20]. The charge transfer was represented the reactivity index term " ΔN_{max} ", which was restricted by the stabilization energy during the system was gained charge from the biological media (Table 1). The low softness value may be combined with a high potential against biological environment (Table 2).

Table 2

Calculated energetic global reactivity parameters 4-methylthio-3-butenyl gluconate at DFT with a B3LYP\6-311G* Basics sets

E	-1554.78	E: The total energy (Kcal/mol)., HOMO: Highest Occupied Molecular Orbital (eV), LUMO: Lowest Occupied Molecular Orbital (eV), ΔG : difference between HOMO and LUMO energy levels(eV), I: Ionization potential, A; electron affinity; η : Hardness(eV), S: Softness(eV), χ : Electronegativity (eV); μ^+ : electron accepting chemical potentials, μ^- : electron donating chemical potentials, μ^+ : chemical potential(eV), ω^+ : electron accepting capacity, ω^- : Electrodonating capacity; $\omega^\pm$: :Electrophilicity index in valance state (eV); $\Delta N_{\max}$: maximum number of electrons transfer.
HOMO	-9.23	
LUMO	-1.39	
ΔG	-7.84	
I	9.23	
A	1.39	
η	3.92	
S	0.255	
χ	-5.31	
μ^+	-3.35	
μ^-	-7.027	
ω^-	6.741	
ω^+	3.106	
$\omega^\pm$	9.847	
$\Delta N_{\mu\xi}$	1.354	

The ligand exhibited that (Table 1), high electrophilicity value " $\omega^\pm = 6.741$ ", rising of the electron donating power ($\mu^- = 7.02\text{eV}$) with increasing donating capacity ($\omega^- = 6.74\text{eV}$). The high electrophilicity for ligand may be led to increasing attacking power against a polar residue in the receptor, which increase the potency.

2.2.2. Molecular electrostatic potential (MEP)

The repulsive and attractive forces were figured by the molecular electrostatic potential (MEP) for 4-methylthio-3-butenylglucosinolate (Fig. 1). Orange, yellow, red colors depicted a high electron density area, while the blue color represented the positive potential, and green color represented the intermediate potential value. The negative charge capped the most area of the molecule, while formaldehydoxime area covered by positive charge. The variation of color in MEP surface linked with difference in electrostatic potential. this force accountable for identification of the substrate with receptor binding site, and hence promotion the electrostatic bond between substrate-receptor[21].

2.3. Docking studies:

The docking study have targeted a α -glucosidase and β -amylase, to examine a mode of action of the 4-methylthio-3-butenylglucosinolate. The ligand–protein interaction behavior were estimated based gold score function as implemented in MOE 2015.10[22]. All calculations of docking experiment were represented (Table 2). The crystal structures for α -glucosidase(PDB: 4yvx[23]) and α -amylase(PDB: 4gqr[24]) have been obtained from protein databank, which their complexed with Glimipride and myricetin as reference drugs, respectively. The tested ligand docked into active site for two enzymes.

Figure 2 :

The active site of these enzymes were defined to include residues within a 3.5 Å radius around reference drugs atoms. The molecular dynamic (MD) using (MMFF94) force field to 0.05 Kcal/mol gradient convergence range, which their was applied to minimize energy for obtained ligand-enzyme complexes. The binding affinity for the tested ligand was determined by highest MOE scoring function (Table 3). The premier ligands revealed MOE score (-7.511 and - 6.157 Kcal/mol), respectively, for α -glucosidase and β -amylase. In α -glucosidase binding site, Glimipride make important strong hydrogen bond with His117 and Tyr55, at the time this ligand forms π - π bonds with Tyr24 and Trp22. The analysis binding site of β -amylase-Myricetin pose exhibited that, the ligand interacted with important amino acid backbone as Asp197, which contacted with 2-H-bonds. The tested compound exhibited a higher binding affinity with MOE scores (-6.611 Kcal/mol.) than reference drug (-6.115 Kcal/mol.). This ligand was formed an important hydrogen bond with important Asp amino acid (Fig. 3). Our compound arranged in binding pock at parallel mode with Asp (Fig. 3).

Table 3

Docking energy scores (Kcal/mol) derived from the MOE for 4-methylthio-3-butenyl gluconate.

Enzyme	4yvx		4gqr	
	Glimipride	Tested Ligand	myricetin	Tested Ligand
E_{score1}	-7.75118	-7.11842	-101.157	-131.364
$E_{d_{GE}}$	1.62884	1.122716	-5.19	-7.209
E_{conf}	-263.798	85.79013	-101.987	-117.44
E_{place}	-49.6922	-104.664	-367.2	-566.262
E_{int}	-13.9234	-14.7619	-101.987	-117.44
$E_{\text{H.B.}}$	-42.0326	-39.5142	-37.74	-56.14
Eele	-7.75118	-7.11842	-3.07	-3.6
Evdw	-7.49118	-7.28842	2.59	3.24
RMS	1.62884	1.122716	0.9652	0.865
E_{score1} : Initial free binding energy of the ligand from a given pose. $E_{d_{GE}}$: Final free binding energy of the ligand from a given pose, E_{conf} : Free binding energy of the ligand from a given conformer. E_{place} : Free binding energy of the ligand from a receptor. E_{int} : Affinity binding energy of ligand with receptor, H.B.: Hydrogen bonding energy between protein and ligand. Eele: Electrostatic interaction with the receptor, Evdw: Van der Waals energies between the ligand and the receptor. RMSD; The root mean square deviation of the pose of the docking pose compared to the co-crystal ligand position.				

The hydrophilicity of binding pocket is postulated that, the hydrophobicity and membrane permeability are an important pharmacokinetic character for absorption molecule in biological system. The results clearly revealed that, the amino acid residues close to the reference molecules are mostly the same as observed in the tested compound.

Figure 3 :

2.4. In silico Toxicological study

2.4.1. *In silico* pharmacokinetic Profile:

Cytotoxicity screening in silico through ADMET parameters acting a fundamental route in therapeutic bioactive molecule. The calculated descriptors for 4-methylthio-3-butenylglucosinolate and Glimipride were calculated by MOE, SwissADME [25] and admet-SAR model[26], which these disclosed in (Table 4). The physicochemical and ADME parameters for 4-methylthio-3-butenyl glucosinolate Glimipride (reference drug), which stated that this ligand suitable for Lipinski's rule with one violation related to molecular weight. Furthermore, when applied Ghose Veber, Egan and Muegge rules, the tested compounds don't passed these rules, except Glimipride drug is qualified for Muegge rule. Consequently, the tested compound exhibited bioavailability Scores (0.11 and 0.55) with biodegradation values (0.6362 and 0.4523). These data confirmed that the investigated compound have good oral bioavailability. The lead-likeness profile plotted the Bioavailability Radar planner for tested compound (Fig. 4). The reader figured relation between polarity, size, lipophilicity, solubility, saturation and flexibility[20], the optimal range for each parameters represented in pink color. From the first glance for the reader, 4-methylthio-3-butenyl glucosinolate and Glimipride exhibited deviation for polarity and flexibility, respectively, (Fig. 4).

Table 4

ADMET(absorption, distribution, metabolism, and excretion - toxicity) for 4-methylthio-3-butenyl gluconate and Glimipride.

ADME-T (absorption, distribution, metabolism, and excretion - toxicity) for 4-methyl-2,5-butenyl glucuronate and Glimipride.								
	Ligand	Glimipride		Ligand	Glimipride			
Molecular weight	434.48	490.62	Physicochemical Properties	Log Po/w (iLOGP)	2.8	2.42	Lipophilicity parameters	
Num. heavy atoms	26	34		Log Po/w (XLOGP3)	1.38	3.85		
Num. arom. heavy atoms	0	6		Log Po/w (WLOGP)	0.42	3.77		
Fraction Csp3	0.75	0.54		Log Po/w (MLOGP)	2.37	1.88		
Num. rotatable bonds	9	11		^a Consensus Log P _{o/w}	1.84	2.76		
Num. H-bond acceptors, donner	10,5	5,3		Lipinski	Yes; violation = 1; No rO < 10	Yes; 0 violation	Drug likeness	
TPSA (Topological surface area)	186.32A ²	133.06A ²		Ghose	No; violation = 1: WLOGP>0.4	No; 2 violations: MW > 480, MR > 130		
Absorption percentage	44.48	63.09		Veber	No; violation = 1: TPSA140<	No; 1 violation: Rotors > 10		
Mutagenic	none	none	Pharmacokinetics parameters	Egan	No; violation = 1: TPSA131.6<	No; 1 violation: TPSA > 131.6		
Tumorigenic	none	none		Muegge	No violation = 2: TPSA < 150 H, acc < 10	Yes		
Carcinogens	None	None		Bioavailability Score	0.11	0.55		
Reproductive Effective	high	high		PAINS	0 alert	0 alert		Medicinal chemistry
Irritant	low	low		^b Brenk	0 alerts	0 alert		
HIA (Human Intestinal Absorption)	0.9893	0.6412		Leadlikeness	2 violations: MW > 350, Rotors > 7	3 violations: MW > 350, Rotors > 7, XLOGP3 > 3.5		
Caco2-Permeability	0.5734	0.68523		Synthetic accessibility	5.66	4.71		
hERG_inhibition	ambiguous	ambiguous		Log S (ESOL)	-1.07	-4.71		water
BBB(blood brain barrier Permeability)	No	No		Solubility	0.36 mg/ml; 0.85 × 10 ⁻² mol/l	9.52 × 10 ⁻³ mg/ml ; 1.94 × 10 ⁻⁵ mol/l		solubility
Biodegradation	0.6362	0.4523		Qualitative solubility	Very soluble	Poorly soluble		

^a Consensus Log P_{o/w}: average four prediction of partition coefficient between n-octanol- water.^bhet-C-het_not_in_ring, imine_1, imine_2, oxygen-nitrogen_single_bond^c In vitro Ames test result for each TA100 strain (Metabolic activation by rat liver homogenate), TA100 strain (No metabolic activation), TA1535 strain (Metabolic activation by rat liver homogenate) and TA1535 strain (No metabolic activation)[9].^d Toxicity Class ranging from 1 to 6 according to the Global Harmony System (GHS) [8].

	Ligand		Glimipride		
GI absorption	Low	Low	°AMES Toxicity	None	toxicity prediction
P-gp substrate	Yes	Yes	Acute Oral Toxicity	None	
CYP1A2 inhibitor	No	No	LD50	16 mg/kg	
CYP2C19 inhibitor	No	No	^d Predicted Toxicity Class	2	
CYP2C9 inhibitor	No	Yes	Average similarity%	34.27	
CYP2D6 inhibitor	No	No	Prediction accuracy%	23	
CYP3A4 inhibitor	No	Yes	Binding to Toxicity	No binding targets	
Log Kp (skin permeation)	-9.93 cm/s	-6.56 cm/s	Toxic fragments	Nil.	
a Consensus Log P _{o/w} : average four prediction of partition coefficient between n-octanol- water.					
^b het-C-het_not_in_ring, imine_1, imine_2, oxygen-nitrogen_single_bond					
^c In vitro Ames test result for each TA100 strain (Metabolic activation by rat liver homogenate), TA100 strain (No metabolic activation), TA1535 strain (Metabolic activation by rat liver homogenate) and TA1535 strain (No metabolic activation)[9].					
^d Toxicity Class ranging from 1 to 6 according to the Global Harmony System (GHS) [8].					

Both compounds showed high saturation degree(Fraction Csp3 = 0.75,0.45, respectively), which pass the filter of Fraction Csp3 < 0.25[27]. The solubility parameters are important key for absorption, the 4-methylthio-3-butenyl glucosinolate exhibited strong soluble in H₂O when applied ESOL topological model with absorption% (%ABS = 44.48)[28], in contrary Glimipride showed poor solubility in this model (Table 4). Also, lipophilicity parameters in medicinal chemistry incorporated as lead-likeness filter, which identified problematic fragments in bioactive molecules based on two Structural alerts as PAINS and Brenk's filters [20]. The tested compounds exhibited no structural alerts against PAINS and Brenk's filters.

Figure 4

Mutagenic, Tumorigenic, Reproductive Effective, Irritant, Human Intestinal absorption, were investigated *in silico* using admet-sar pharmacokinetic parameters[29]. Furthermore, the other (SVM) algorithm was applied to identify substrate or non-substrate of the permeability for skin permeation (Log Kp), Caco-2, blood brain barrier (BBB) and p-glycoprotein (P-gp), as well as, to recognize inhibition effect against the chief cytochromes P450 isoenzymes (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4). The results obtained from (Table 3) exhibited that, Glimipride drug inhibited both *CYP2C9* and *CYP3A4*, but our tested ligand has no inhibition action for all types of P450. The BOILED-Egg model is represented relation for WLOGP vs TPSA in (Fig. 4), which demonstrated that, High BBB permeability, high GI absorption, high brain penetration, ambiguous inhibition action for against human *Ether-à-go-go*-Related Gene (hERG). In addition, the Glimipride showed activation action against **P-gp** (multidrug resistance protein 1), but our compound act as P-gp inhibitor. Furthermore, skin permeability (Kp) was predicted for our compounds, which described that, the higher negative value combined with low skin permeability for compounds. The data in (Table 4) showed that, the Glimipride drug is higher skin permeability than our ligand. The carcinogen behavior was investigated by admet-sar for the investigated compounds, through compared with 981 various carcinogenic chemical structures, that obtained from "Carcinogenic Potency Database (CPDB [30])", the results exhibited no carcinogenicity, mutagenicity and tumorigenicity effects for tested compounds.

2.4.2. Oral toxicity prediction:

The possible toxicity was predicted[31], through calculate estimate rodent oral toxicity based on extracted from the Chemical European Biology Laboratory (ChEMBL) database[26]. Also, median lethal doses (LD₅₀) values was estimated in rodents, which our ligand (LD₅₀ = 16 mg/Kg) showed lower value than reference drug (LD₅₀ = 3250 mg/Kg). The calculated toxicity using this database depend on highest endpoints including of 33 models (Table 4). The calculated schematic is divided into several stages of toxicity as "toxicity, toxicological endpoints (mutagenicity, carcinotoxicity, organ toxicity (hepatotoxicity), cytotoxicity and immunotoxicity), toxicological pathways (AOPs) and toxicity targets thereby"[25], which provided a suitable visions into the possible molecular mechanism as well as toxic response (Table 5). The Glimipride located in lower toxicity classes 5 than 4-methylthio-3-butenyl glucosinolate, which sited in Class (No. 4), while both compounds do

not exhibited any toxic fragments without non-binding to any toxicity targets (Table 5). In general, the tested compounds have been a good oral bioavailability, high ability *BBB* transport, and no marked health effects observed for rodent toxicity profiles.

Table 5: Toxicity Model Report for ligand which extracted from admet-SAR.

Classification	Target	Shorthand	Prediction	Probability	Prediction	Probability
				Glucosanate	Glimipride	
Organ toxicity	Hepatotoxicity	dili	Inactive	0.62	Inactive	0.71
Toxicity end points	Carcinogenicity	carcino	Inactive	0.56	Inactive	0.72
Toxicity end points	Immunotoxicity	immuno	Inactive	0.84	Inactive	0.99
Toxicity end points	Mutagenicity	mutagen	Inactive	0.58	Inactive	0.76
Toxicity end points	Cytotoxicity	cyto	Inactive	0.7	Inactive	0.64
Tox21-Nuclear receptor signaling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	0.91	Inactive	0.97
Tox21-Nuclear receptor signaling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.9	Inactive	0.98
Tox21-Nuclear receptor signaling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	0.96	Inactive	0.96
Tox21-Nuclear receptor signaling pathways	Aromatase	nr_aromatase	Inactive	0.92	Inactive	0.97
Tox21-Nuclear receptor signaling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.83	Inactive	0.96
Tox21-Nuclear receptor signaling pathways	Estrogen Receptor Ligand Binding Domain (ER-LBD)	nr_er_lbd	Inactive	0.91	Inactive	0.99
Tox21-Nuclear receptor signaling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.96	Inactive	0.95
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/ antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.96	Inactive	0.99
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	0.96	Inactive	0.99
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.92	Inactive	0.85
Tox21-Stress response pathways	Phosphoprotein (Tumor Suppressor) p53	sr_p53	Inactive	0.93	Inactive	0.96
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	Inactive	0.98	Inactive	0.99

3. Material And Methods

3.1. Plant Materials:-

The aerial parts of both plants (*Carrichtera annua* (L.) DC. and *Farsetia aegyptia* Turra) were collected from Alariesh airport region (North Saini governerate) and wadi Hagol (at Cairo-Sues road, km-30), respectively through the flowering stage at the end of winter season (25-march). The plants were kindly identified by prof. Dr. Ibrahim Elgarf, prof. of taxonomy at botany dept., faculty of science, Cairo univ. and the voucher specimens were deposited at NRC herbarium. Each plant was air dried and grounded to a fine powder.

3.2. Instruments and Reagents:

^1H and ^{13}C NMR spectra were measured using Joel JNM EX270FTNMR system at 270 MHz for ^1H and 70 MHz for ^{13}C at the central lab. For services, NRC, Cairo, Egypt. The sample was dissolved in DMSO and the chemical shifts are given in δ (ppm) relative to tetramethylsilane (Me_4Si). UV spectra were recorded on Shimadzu model UV-240 and 2401 PC spectrophotometer (Shimadzu Inc., Tokyo, Japan) in methanol in the range 200–450 nm.

3.3. Conditions for GC/MS analysis of the isothiocyanates:

3.3.1. Apparatus:

- GC/MS Finning an SSQ7000, Digital DEC3000 with **column**: DB5 capillary (I.d.0.25 mm), ionization mode: EI at 70ev, **temperature program** start at : 50 °C up to 250 °C with a rate of 4 °C/min., **Detector**: MS, sample volume 2 μl and the mass from 40–400.
- modified silver nitrate reagent [12] in which the GlS. appears as brownish spots on a yellowish background.
- Solvent systems: S1, Butanol: acetic acid : water 4:1:5 upper layer(which contain the organic solvent saturated with water) and S2, Butanol: ethanol: water 4:1:3. Acidic aluminium oxide (anionotropic 1.5 kg, activity grade I, type WA-I acidic, Sigma chemical Co.), Cellulose for column (Merck, 64271 Darmstadt, Germany) and DEAE Sephadex A-25 (Germany). myrosinase enzyme (Thioglucosidase, Sigma, T – 4528).

Extraction and Isolation of Glucosinolates:

- About 2.0 kg of air dried powdered herb of both *C. annua* and *F. aegyptia* were defatted, separately, with petroleum ether(br. 40 – 60 °C) in a Soxhlet apparatus for three days (operate 12 hrs. daily). The defatted powders were air dried and extracted (separately) with methyl

alcohol (80%, 3 × 3L, 3 days each), the combined methanol extract of each plant was evaporated *in vacuo* at 40°C and the residues were dissolved in hot distilled water (750 ml), left in the refrigerator for 24 hrs. and filtered the gummy ppt.. The aqueous filtrates were allowed to pass slowly through an acidic aluminium oxide column (Aluminium oxide, for chromatography, acidic, Brockmann I, 50–200 µm, 60A, Acros Organics, Fluca, 7.5 × 60) to remove the coloring matters, then, the total GIs were eluted with potassium sulfate solution (2%, 2.5L) till the brown zone(containing the total glucosinolates) reached the bottom of the column, the eluate containing the brown zone was evaporated under reduced pressure till dryness. The residue for each plant was dissolved with hot methanol, filtered through centered glass funnel (G3) and evaporated *in vacuo* at 40 °C till dryness to give a faint brownish residue (2.1 g for CA and 500 mg for FA)

3.4. Purification of GIs :

The obtained brownish residue was further purified using cellulose column eluted with the upper layer of the solvent S2, after the complete elution, the eluate was concentrated and subjected to preparative paper chromatography (PPC) using chromatographic paper Whatmann 3 mm developed in the upper layer of solvent S1 (two runs) applying the descending technique. Two main zones were determined with pencil cut into small pieces and eluted with methanol (90%). Each zone was further purified over DEAE Sephadex A-25 column eluted with 0.5% ammonium acetate soln., to afford two compounds (CA1 and CA2, R_f 0.52 and 0.65, relative to benzyl GIs, 17 mg and 3 mg respectively).

3.5. Enzymatic hydrolysis of GIs:

Due to the small amounts of the compound A2 and the residue of total GIs from FA, so, compound A2 and the total GIs from FA were subjected, separately, to enzymatic hydrolysis using myrosinase enzyme. The aqueous acidic solution after separation of the aglucones was neutralized with barium carbonate and filtered. The clear filtrate was evaporated till dryness. The residue was dissolved in 10% isopropanol and subjected to paper chromatography using ethyl acetate : pyridine: water (12: 5: 4) as developing solvent and different authentic sugars. The chromatogram was visualized by spraying with aniline phthalate, and heated at 105 °C for few minutes. Only glucose was detected as a sugar and the presence of sulfate ions in the aqueous solution were detected by addition of few drops of barium chloride soln., where a white ppt. was noticed proving the presence of sulphate ions.

Also, the total GIs. were extracted from *F. aegyptia* using the same method and they were purified using PPC with different solvents and repeated Sephadex LH-20 columns to afford five compounds in small amounts so, they subjected to enzymatic hydrolysis as before, and the hydrolysis products were extracted with ether and identified by GC/MS. The aqueous layer was found to contain glucose and sulphate ions.

3.6. Computational study:

3.6.1. Preparation of Small Molecule:

The target compound was built and minimized using the PM3 semi-empirical Hamiltonian molecular orbital calculation MOPAC16 package[32], then employing density function theory in Gaussian 09 program package[28] with the Becke3-Lee-Yang-parr (B3LYP) level using 6-311G* basis as implemented in MOE 2015 package[17]. The optimization Geometry for molecular structures were carried out, for improve knowledge of chemical structures. Our compounds were introduced into the binding sites according to the published crystal structures. The global chemical reactivity descriptors for molecules have been computed (Table 1), like; S ; softness (measures stability of molecules and chemical reactivity with direct proportional, η ; hardness (reciprocal of softness), μ ; chemical potential, χ ; electronegativity strength catching electrons[33], the μ^- ; electron donating power, μ^+ ; potency for catching electron, ω^- ; electro donating capacity, ω^+ ; electro accepting capacity, $\omega^\pm$; net electrophilicity (measuring a relative powers between electron accepting and electron donating)[15], ω_i ; Electrophilicity index in ground state (determining decreasing energy obtained from maximal electrons current movement between donor and acceptor media), ω_i^{VS} ; Electrophilicity index in valance state. These parameters are represented in terms I ; ionization potential and A ; electron affinity[15], the previous terms represented in.

3.6.2. Selection of proteins structures.

Docking experiment was carried out for the target active site into α -glucosidase(PDB: 4yvx) and β -amylase(PDB: 4gqr) using MOE 2015[17]. The errors of active sites were corrected by the structure preparation process in MOE. After the correction, hydrogens were added and partial charges (Amber12:EHT) were calculated. Energy minimization (AMBER12:EHT, root mean square gradient: 0.100) was performed.

3.6.3. Binding site analysis

The binding site of each receptors were identified through the MOE Site Finder program, which uses a geometric approach to calculate putative binding sites in a protein, starting from its tridimensional structure. This method is not based on energy models, but only on alpha spheres, which are a generalization of convex hulls. The prediction of the binding sites, performed by the MOE Site Finder module, confirmed the binding sites defined by the co-crystallized ligands in the holo-forms of the investigated proteins.

3.6.4. MOE Stepwise Docking Method:

The crystal structures of the of enzymes were obtained. Water and inhibitors molecule were removed, and hydrogen atoms were added. The parameters and charges were assigned with MMFF94x force field. After alpha-site spheres were generated using the site finder module of MOE. The optimized 3D structures of molecules were subjected to generate different poses of ligands using triangular matcher placement method, which generating poses by aligning ligand triplets of atoms on triplets of alpha spheres represented in the receptor site points, a random triplet of alpha sphere center was used to determine the pose during each iteration. The pose generated was rescored using London dG. scoring function. The poses generated refined with MMFF94x forcefield, also, the solvation effects were treated. The Born solvation model (GB/VI) was used to calculate the final energy, and the finally assigned poses were assigned a score based on the free energy in Kcal/mol.

2.1.1. ADMET predictions

The ADMET in silico profile was applied using “MOE” and “admetSAR” tools to predication of pharmacokinetic and ADMET characters (absorption, distribution, metabolism, excretion, and toxicity).

Conclusions

The glucosinolates of both Carrichtera annua and Farsetia aegyptia (Crucifereae family) were identified. Some of the obtained Gls. were identified as it is and the others were enzymatically hydrolyzed to the corresponding isothiocyanates. The identified compounds are : 4-methylthio-3-butenyl Gls. and 6-methyl sulfonylhexyl Gls. from CA while 6-methylsulfonyl-6-hydroxy hexyl ITC., 4-pentenyl Gls., 3-Methylthio propyl ITC.,5-hydroxy pentyl ITC. and 4-methylsulphinylyl butyl ITC. from FA. the future experiments will be conducted to isolate the glucosinolates of Farsetia aegyptia in reasonable quantity to be able to identify them by different spectroscopic measurements and to investigate their activity as antitumor agents. The obtained Docking data showed that the ability interaction for Gls via. α-glucosidase and amylase. We predict that this Gls. may to be a suitable inhibitor, and these can be used as antidiabetic agent. The ADMET *in silico* showed that, these compounds have a good oral bioavailability and high ability transport through “BBB”. Furthermore, when applied these compounds showed via the “rodent toxicity profile” no observed any marked carcinogenic and health effect.

Abbreviations

E: The total energy (Kcal/mol).	I: Ionization potential,
LUMO: Lowest Occupied Molecular Orbital (eV)	A: electron affinity;
HOMO: Highest Occupied Molecular Orbital (eV)	η: Hardness(eV),
ΔG: difference between HOMO and LUMO energy levels(eV)	S: Softness(eV),
ΔNmax: maximum number of electrons transfer.	χ: Electronegativity (eV);
E_{score1}: Initial free binding energy of the ligand from a given pose.	μ⁺: electron accepting chemical potentials ,
ΔE: Final free binding energy of the ligand from a given pose,	μ⁻: electron donating chemical potentials,
E_{conf}: Free binding energy of the ligand from a given conformer	ω⁺: electron accepting capacity,
E_{place}: Free binding energy of the ligand from a receptor.	ω⁻: Electrodonating capacity;
E_{Int.}: Affinity binding energy of ligand with receptor,	ω±: :Electrophilicity index in valance state (eV);
H.B.: Hydrogen bonding energy between protein and ligand.	Evdw: Van der Waals energies between the ligand and the receptor.
Eele: Electrostatic interaction with the receptor,	RMSD: The root mean square deviation of the pose of the docking pose compared to the co-crystal ligand position.

Declarations

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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Author information

- Department of chemistry, faculty of sciences and arts, Mukhwah , Albaha university, , Albaha, Saudi Arabia

Khaled A. Abdelshafeek, Ahmed A. Elhenawy, and AbdulAziz Ali Alomari

- Department chemistry of medicinal plants, Pharmaceutical Industries Div., National Research Centre,33 El Buhouth St.(Former El Tahrir St.), 12622-Dokki, Giza, Egypt.

Khaled A. Abdelshafeek, Walid E. Abdallah

- **Chemistry Department, Faculty of Science, Al-Azhar University (Boys Branch),Nasr City, Cairo, Egypt.**

Ahmed A. Elhenawy

- **Chemistry Department, Faculty of Science, Taif University, Saudi Arabia.**

Abeer M. Alosaimi

- **Chemistry Department, King Abdulaziz University, 80203 Jeddah, Saudi Arabia.**

L.M.Al-Harbi

Contributions

K.A.A.: conceived and designed the experiments. W.E.A: performed the experiments and wrote the paper. A.A.E.: have performed evaluation and validation of docking methods and scoring functions and drafted the manuscript. A.A.E. and K.A.A.: analyzed and interpreted the data. K.A.A. and A.A.E.: outlined the research strategy and revised the manuscript. A.A.A. and L.M.A: has completed the literature search. All authors read and approved the final manuscript.

Contributions

Correspondence to [Ahmed Elhenawy](#).

Ethics declarations

Ethics approval and consent to participate

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Figures

Figure 1

The chemical structure of glucosinolates

Glucose

Aglycone

Figure 1

The chemical structure of glucosinolates

R= CH ₃ S-CH=CH-(CH ₂) ₂	4-methylthio-3-butenyl Gls.
= CH ₃ -SO ₂ -CH ₂ -(CH ₂) ₄ CH ₂	6-methylsulfonyl hexyl Gls.
= CH ₃ -SO ₂ -CH(OH)-(CH ₂) ₄ CH ₂	6-methylsulfonyl -6-hydroxyhexyl Gls.
= CH ₂ =CH-(CH ₂) ₃	4-pentenyl Gls.
= CH ₃ -S-CH ₂ -(CH ₂) ₂	3-Methylthio propyl
= CH ₂ (OH)-(CH ₂) ₃ CH ₂	5-hydroxypentyl
= CH ₃ SO-CH ₂ -(CH ₂) ₃	Sulphoraphane

Figure 2

Identified isolated compounds

R= CH ₃ S-CH=CH-(CH ₂) ₂	4-methylthio-3-butenyl Gls.
= CH ₃ -SO ₂ -CH ₂ -(CH ₂) ₄ CH ₂	6-methylsulfonyl hexyl Gls.
= CH ₃ -SO ₂ -CH(OH)-(CH ₂) ₄ CH ₂	6-methylsulfonyl -6-hydroxyhexyl Gls.
= CH ₂ =CH-(CH ₂) ₃	4-pentenyl Gls.
= CH ₃ -S-CH ₂ -(CH ₂) ₂	3-Methylthio propyl
= CH ₂ (OH)-(CH ₂) ₃ CH ₂	5-hydroxypentyl
= CH ₃ SO-CH ₂ -(CH ₂) ₃	Sulphoraphane

Figure 2

Identified isolated compounds

Figure 3

Plotting optimization geometry and molecular orbital for HOMO, LUMO and MEP of 4-methylthio-3-butenyl glucosinolate.

Figure 3

Plotting optimization geometry and molecular orbital for HOMO, LUMO and MEP of 4-methylthio-3-butenyl glucosinolate.

A. Glimipride

B. GLs.

Figure 4

Interaction between A.) reference dug and B.) GLs. with binding site of α- glucosidase .

A. Glimipride

B. GLs.

Figure 4

Interaction between A.) reference dug and B.) GLs. with binding site of α- glucosidase .

A. Reference drug

B. GLs.

Figure 5

Interaction between A.) reference drug and B.) GLs. with binding site of α -amylase.

A. Reference drug

B. GLs.

Figure 5

Interaction between A.) reference drug and B.) GLs. with binding site of α -amylase.

Figure 6

A; Bioavailability Radar plot of 4-methythio-3-butenyl glucosinolate . The pink area shows the optimal range for each properties (Lipophilicity: $-0.7 \leq \text{XLOGP3} \leq +5.0$, size: $150 \leq \text{MW} \leq 500$ g/mol, polarity: $\text{TPSA} \leq 140 \text{ \AA}^2$, solubility: $\log S \leq 6$, saturation: fraction of carbons in the hybridization $\text{sp}^3 \geq 0.25$, and flexibility: rotatable bonds ≤ 9). B; BOILED-Egg plot of the predicted compounds 4-methythio-3-butenyl glucosinolate and Glimipride. White region represented a highly probable HIA (GI) absorption, and the yellow region is for highly probable BBB permeation. The outside grey region stands for molecules are predicted with low absorption and not brain penetration. As well, the point in blue color represented as P-gp substrate (PGP+) and in red as P-gp non-substrate (PGP-).

Figure 6

A; Bioavailability Radar plot of 4-methythio-3-butenyl glucosinolate . The pink area shows the optimal range for each properties (Lipophilicity: $-0.7 \leq \text{XLOGP3} \leq +5.0$, size: $150 \leq \text{MW} \leq 500$ g/mol, polarity: $\text{TPSA} \leq 140 \text{ \AA}^2$, solubility: $\log S \leq 6$, saturation: fraction of carbons in the hybridization $\text{sp}^3 \geq 0.25$, and flexibility: rotatable bonds ≤ 9). B; BOILED-Egg plot of the predicted compounds 4-methythio-3-butenyl glucosinolate and Glimipride. White region represented a highly probable HIA (GI) absorption, and the yellow region is for highly probable BBB

permeation. The outside grey region stands for molecules are predicted with low absorption and not brain penetration. As well, the point in blue color represented as P-gp substrate (PGP+) and in red as P-gp non-substrate (PGP-).