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Review

The Glucosinolates, A Sulphur Glucoside Family of Mustard Phytochemicals With Diverse Biomedical Application

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Abstract: This study reviewed aspects of the biology of two members of the glucosinolate family, namely sinigrin and glucoraphanin and their potential biomedical therapeutic and industrial applications. Sinigrin and glucoraphanin are converted by the ⍵-sulphoglucosidase myrosinase or the gut microbiota into their bioactive forms, allyl isothiocyanate (AITC) and sulphoraphanin (SFN) which constitute part of a sophisticated defence mechanism plants have developed over several hundred million years of evolution to protect them from parasitic attack from aphids, ticks and nematodes. These compounds display biological activities in a number of mammalian physiological processes and potential biotherapeutic application. Glucosinolates may be useful in bio-fumigation and treatment of biofilms which occur on plant equipment and medical implants formed by problematic pathogenic bacteria such as *Pseudomonas aeruginosa*. AITC and SFN display similar antibiotic activity as Vancomycin in the treatment of bacteria listed by the World Health Organization as antibiotic-resistant "priority pathogens".*AITC and SFN* also display bioactivity in cancer chemoprevention through the induction of phase II antioxidant enzymes which inactivate potential carcinogens. The glucosinolates have found application in the prevention of bacterial and fungal spoilage of food substances during processing and in advanced food packaging formats which improve the shelf-life of food products.

Keywords: glucosinolate; sulphopharane; allyl isothiocyanate, phase II detoxification enzymes; anti-tumour agents; anti-bacterials; neutraceutical; sinigrin; glutathione-S-transferase.

Abbreviations

AD	Alzheimers disease
AKT	a serine/threonine-specific protein kinase
EPA	Environment Protection Agency
ESBL	Extended Spectrum Beta-Lactamases
Keap-1-Nrf2-ARE	Kelch-like ECH-Associating protein 1-nuclear factor erythroid 2 related factor-2 -antioxidant response element
AITC	Allyl isothiocyanate
GARDP	Global Antibiotic Research and Development Partnership
GSK	Glycogen Synthase Kinase
GST	Glutathione-S-transferase

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DNDI	Drugs for Neglected Diseases initiative
IACG	Interagency Coordination Group on Antimicrobial Resistance
LPS	Lipopolysaccharide
MAPK	A mitogen-activated protein kinase
NFκB	Nuclear factor kappa light chain enhancer of activated B cells
NSAID	Non Steroidal anti-inflammatory
PDGF	Platelet derived growth factor
ROS	Reactive oxygen species
SMC	Smooth muscle cell
TNFα ⊚	⊚umour necrosis factor-alpha
WHO	World Health Organization

Introduction

Plants produce a myriad of phytochemicals and many of these have valuable nutritive, medicinal and health promoting properties [1-3]. Anecdotal evidence often points to these beneficial properties however in this report we will concentrate on one family of molecules, the glucosinolates, a family of Sulphur containing glucosides with a very extensive scientific and nutritional literature [1] which documents their properties as a nutritive supplement and as medicinal compounds which are of potential application in a number of therapeutic areas in biomedicine [3-12].

Table 1. Examples of Glucosinolate rich Edible Cruciferous plants of the *Brassicacea* family order Capparales

Brocolli
Brocolli Sprouts
Cabbage
Brussell Sprouts
Cauliflower
Daikon (Japanese radish)
Daikon sprouts
Garden Cress (*Lepidum sativum*)
Kale
Rapeseed (*Brassica napus*)
Wasabi (*Wasabia japonica*)
White Mustard (*Sinapis alba*)
Yellow Mustard (*Brassica juncea*)
Bok Choi
Arugula, Rocket (*Eruca sativa*)
Collard Greens
Horseradish
Kohlrabi
Radish
Rutabaga/turnip
Watercress

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Cruciferous plants such as those listed in Table 1. represent an important nutritious component of the healthy diet and have characteristic spicy flavor profiles which are appealing to many. However many of the compounds which impart these spicy or bitter flavours also have important effects in a number of physiological processes.

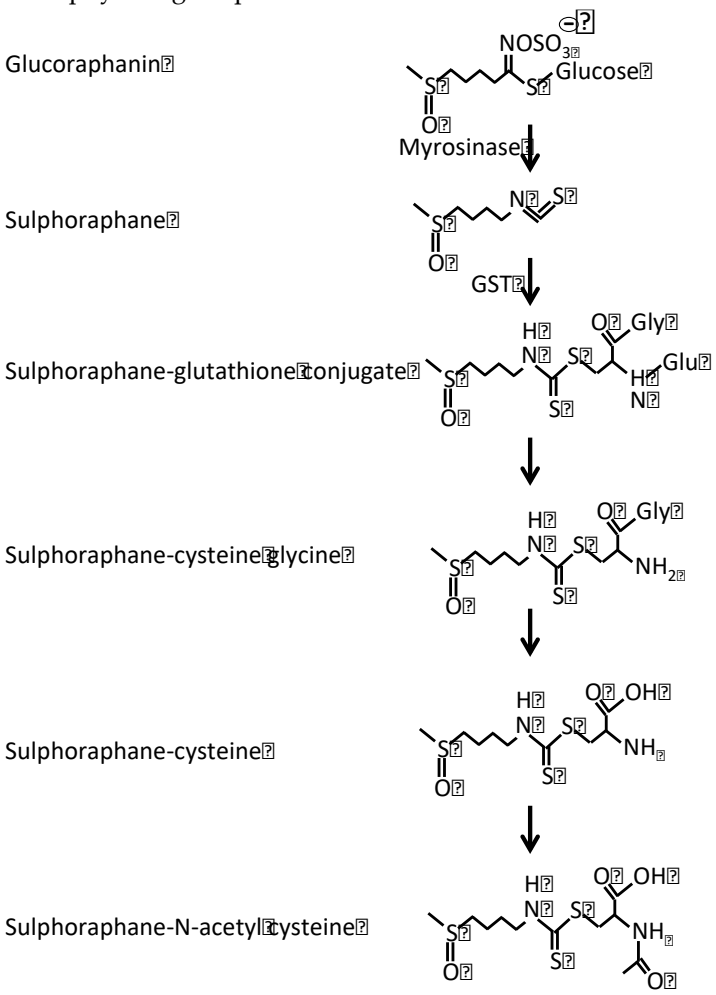

Figure 1. Interaction of Sulphoraphane with the glutathione S-transferase enzyme system as part of its elimination from the body via the mercapturic acid pathway.

Sulphoraphane-GSTs and specific roles in the brain

It is important to control the redox balance of oxidant and anti-oxidant species in the human brain since these control neuronal mitochondrial vitality and activity which under oxidant stress can diminish neuronal energetics and promote the development of neurodegenerative conditions such as Parkinson's and Alzheimers's disease [21]. Brain tissue is very rich in fatty acids and is especially sensitive to the action of excess oxidant activity which can occur focally when GST activity is insufficient [22]. The GSTs are scavengers of reactive oxygen species (ROS) and have essential roles to play in neuronal plasticity [21, 23, 24]. GSTs promote microglial activation and proinflammatory astrocyte-microglia communication [25]. GST polymorphisms can lead to neuronal dysfunction, with some GST isoforms associated with pathological processes in glioblastoma, Alzheimer's disease, Parkinson's disease, stroke, epilepsy [26, 27] and progression of multiple sclerosis [28] and may deleteriously impact on repair mechanisms following ischemic stroke [29-31]. Induction of the

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expression and activity of GSTs and other phase II detoxification enzymes by bioactive forms of the glucosinolates therefore is of importance in the maintenance of normal functional properties in the brain. Individuals deficient in GST activity may be more susceptible to the development of cancer.

The Brassicaceae family of plants

The Brassicaceae family of cruciferous plants, includes the model plant *Arabidopsis thaliana* which has proved useful for biochemical and phylogenetic studies which have defined the coordination of cell proliferation, expansion, and differentiative processes which underpin plant growth, and contains ~375 genera and over 3,000 species (Table 1). The Brassicaceae are a rich source of sulphur glucosides called the glucosinolates which impart a characteristic spicy bitter flavor profile to these vegetables.

Glucosinolates have been classified into three categories on the basis of their amino acid precursors (i) aliphatic (e.g., glucoraphanin; Ala, Leu, Ileu, Val, Met), (ii) indole (e.g., glucobrassicin; Trp), and (iii) aromatic (e.g., gluconasturtiin; Phe, Tyr). While ~140 glucosinolates have been identified to date, in a survey of 2,121 German participants in the European Prospective Investigation into Cancer and Nutrition (EPIC study), only five of these were commonly found in the human diet namely glucobrassicin, sinigrin, glucoraphasatin (dehydroerucin), glucoraphanin, and glucoiberin [32].

Glucosininolates have only been found in dicotyledonous plants and occur mainly in the Capparales order (Fig 1) including cruciferous vegetables and the mustards *Brassica juncea* (brown mustard) [33], *Brassica napus*. (rape seed) and the popular Japanese condiment horseradish Wasabi (*Eutrema japonicum* or *Wasabia japonica*) [34, 35](Fig 2). Several members of the mustard family of cruciferous plants produce abundant glucosinolate levels which are stored in the seed heads and can be recovered in cold pressed oils. Mustard seed oils have been harvested since Biblical times. Figure 2 illustrates the oil storage tissues of *Brassica napus* (Rape seed).

The glucosinolates and their roles in the preservation of normal tissue functions

When the bioactive glucosinolate derived isothiocyanates generated by exogenous myrosinase activity or from the microbiota of the gut are absorbed they are rapidly conjugated to glutathione by the phase II detoxification enzyme glutathione S-transferase (GST) and subsequently undergoes a series of chemical modifications leading to its elimination from the body via the Mercapturic pathway (Fig 1). The glucosinolates induce the production of phase II enzymes such as the GSTs through the KEAP1/Nrf2/ARE pathway, the phase II enzymes are critical in mutagen elimination [13]. Sulphoraphane has chemopreventive properties through its ability to inhibit phase I enzymes responsible for the activation of pro-carcinogens, and to induce phase II enzymes critical in mutagen elimination. Sulphoraphane also mediates a number of anticancer pathways, including the activation of apoptosis, induction of cell cycle arrest, disruption in tubulin assembly and tubular microdynamics and inhibition of NFκB [14]. A diet rich in cruciferous vegetables is associated with a lower risk of developing breast, lung, prostate, and colorectal cancer [15-19] with a diet containing three to five servings per week reported to be sufficient to decrease the risk of cancer development by ~30%–40% [20].

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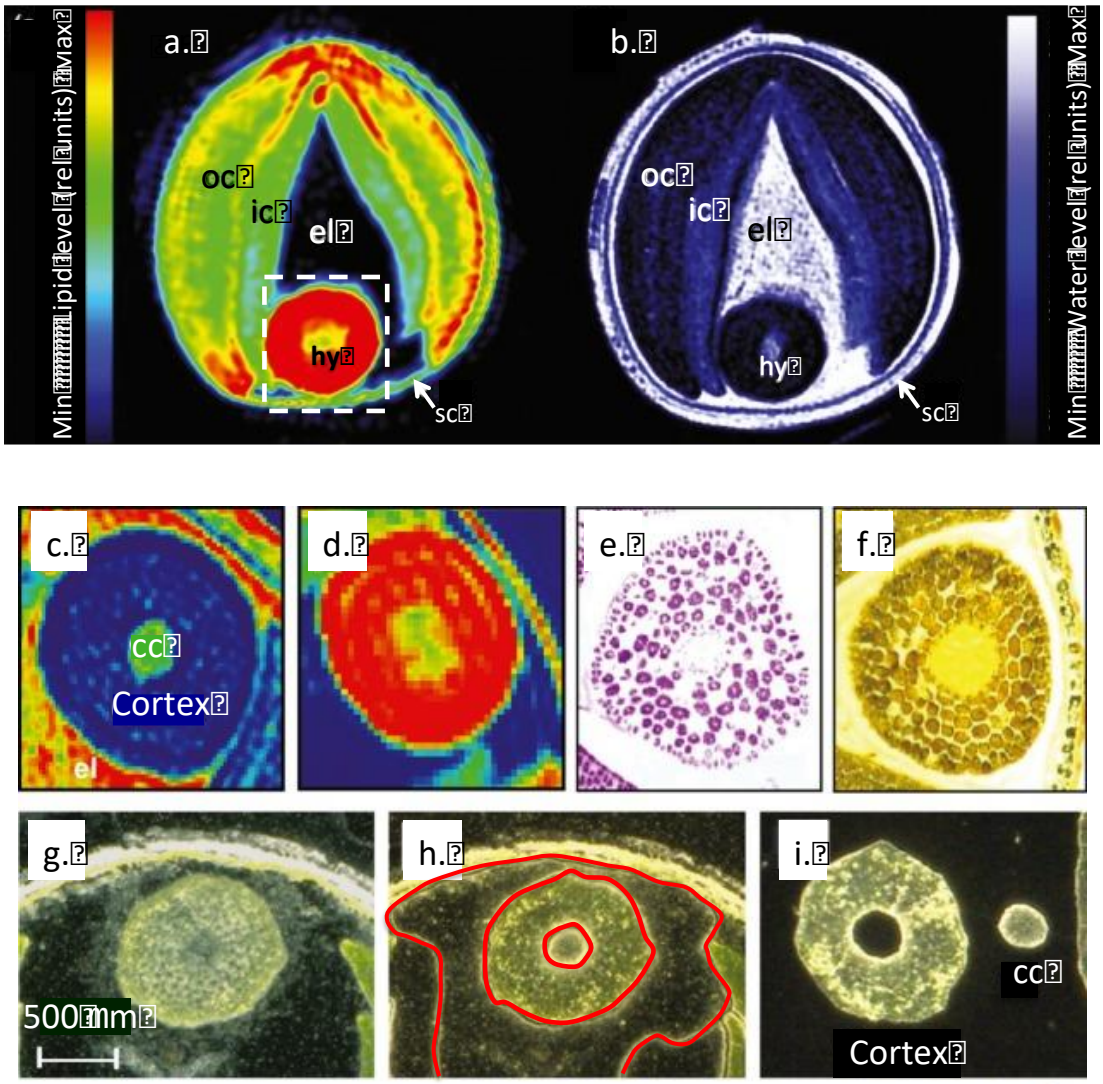

Figure 2. Lipid oil storage and moisture profiles in a cross-section of a *Brassica napus* seed visualized by non-invasive MRI (a, b) and hypocotyl (c, d). The boxed area is and can be recovered in cold pressed oil products depicted at higher magnification in (c-i). The concentration of water and oil are colour coded red high; blue, low and given in relative units. Crucifern immunolocalisation (e) and starch visualised by iodine staining (f). Laser dissection of hypocotyl into the cortex and central cylinder of the cotyledon. Image modified from [36] in accordance with the Creative Commons Deed, Attribution licence 2.5.

The mustard plant, Rape seed, yellow, white and brown mustard is widely distributed and has a characteristic yellow flower head. Figure 3 illustrates mustard growing in the wild and under crop cultivation. *Brassica napus* is an ancient plant crop and is mentioned in the texts of the Bible.

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Figure 3. Characteristic four petal flower head of mustard, inflorescence and seed pod arrangements (a, b). Wild mustard growing in the Vosges Mountains, France (c). Cultivated field of *Brassica napus (d).* Images from Colza oléagineux d'hiver SYNERGY (Coobtention INRA-SERASEM)-3-cliche Jean Weber. Figure reproduced under CC BY 2.0 licence from https://commons.wikimedia.org/w/inde

Under Australian climatic conditions yields of 11.7-24 ◎mol glucosinolate/g of seed dry weight are typical for these mustard species and can be extracted as a cold pressed oil from the seed head. Rapeseed (*Brassica napus*), also known as rape, oilseed rape [37] is a bright yellow flowering member of the *Brassicacea*, mustard or cabbage family. The term "rape" is derived from the Latin word for turnip, *rapum* [38]. This is an ancient plant known of since Biblical times and has even been identified

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in the fossil record as early as the Mesozoic era and mid-Devonian period as a component of the diet of some herbivorous dinosaurs. More recently *Brassica napus* was described and published in *Species Plantarum* by Carl Linnaeus who introduced the binomial name *Brassica napus* for the first time [37] (Fig 4).

Figure 4. Anatomical description of a mustard (*Brassica napus*) plant showing its characteristic four petal flower head, stamen, seed pods, leaf arrangements and mustard seeds. Image from Franz Eugen Koehler archive, Kohlers Medicinal Plants, Germany 1887. Image reproduced from Wikimedia Commons Repository through Open Access. [File:Brassica napus - Köhler–s Medizinal-Pflanzen-169.jpg|Brassica napus - Köhler–s Medizinal-Pflanzen-169].

Rapeseed oil is one of the oldest known vegetable oils, but historically has been used in limited quantities as a food item due to its high levels of erucic acid and glucosinolate. Natural rapeseed oil can contain up to 54% w/v erucic acid [39] while mustard oil typically contains 42% w/v erucic acid. Rapeseed cultivated for food production typically contains levels ~0.5-5% erucic acid. Erucic acid is a very long C22 chain mono-unsaturated omega-9-fatty acid, formula $C_{22}H_{42}O_2$. A strain of mustard plant was subsequently developed in Canada with low erucic acid and glucosinolate levels, this was termed Canola, a contraction of the terms "*Canada*" and "*ola*", meaning oil, a "double low" (low erucic acid and low glucosinolate) rapeseed product [40]. Canola oil is limited by government regulation to a maximum of 2% erucic acid by weight in the USA and 5% in the EU. In 1992, the health promoting properties of Rapeseed oil gained publicity with the release of the George Miller feature film

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"Lorenzo's Oil" starring Nick Nolte and Susan Sarandon which documented the work of a British chemist , Don Suddaby, and Augusto Odone in 1985 who developed a blend of rapeseed and olive oils which halted the progression of Adrenoleukodystrophy, a genetic disorder characterized by an enzyme abnormality which results in the build up of toxic fatty acid levels in the brain that damages the myelin sheaths and impairs neuronal function resulting in convulsions, seizures and hyperactivity.

Besides the harvesting of the rapeseed oil component, a high protein animal feed is also produced from the pressed Canola rapeseed residue which is of a similar nutritional profile to that of soybean based animal food products [41]. Besides the use of rapeseed oil as a human food product it is also used as biodiesel, in heated fuel systems, or in a blend with petroleum products to power motor vehicles and is suitable for use in pure form in newer engines without engine damage but is also combined with fossil-fuel diesel in ratios varying from 2% to 20% in a form known as biodiesel [42]. Canola oil is an edible oil with a flavor profile acceptable to Western palates, however mustard oil from *Brassica juncea* has a characteristic spicy flavor enjoyed in the Asian sub-continent and represents ~30% of the total edible oil market in this region.

The bioactivity of glucosinolates

The glucosinolate family contains 130 members, this report will focus on two specific glucosinolate molecules, glucoraphanin and sinigrin (Fig 5). The glucosinolates constitute part of an innovative defence mechanism which the Brassicaceae have developed over several hundred million years of evolution to protect them from attack by parasites (aphids, ticks, nematodes) and herbivores. Fortuitously, these compounds also display beneficial biological activities in a number of mammalian physiological processes and herein lies the interest in these compounds in a number of biotherapeutic applications. In the plant, the glucosinolates, are stored concentrated in particular phloem cells however when plant tissue is damaged by a parasite, an enzyme, myrosinase, a ⍵-thioglucosidase, is released from an adjacent cell type and this converts the glucosinolate into a bioactive molecule which has fungicidal, bactericidal and nematocidal properties (Fig 6). When activated by myrosinase, glucoraphanin is converted to bioactive sulphoraphane (SFN) (Fig 3d), sinigrin is also activated into a bioactive form, allyl isothiocyanate (AITC) by the action of myrosinase. Myrosinase is the only known enzyme which can cleave thio-gluose. The glucosinolates themselves are benign molecules thus if cruciferous plants are consumed as part of the diet the glucosinolates will have undergone partial conversion to their active forms when they are chopped up during food preparation when the myrosinase containing cells are also mechanically disrupted (Fig 7-9) prior to the cooking stage. However cooking inactivates the myrosinase but there is some evidence that the gut microbiota in humans may provide myrosinase activity which converts the glucosinolates in the diet to the bioactive glucosinolate forms which can be absorbed in the intestine, myrosinase is not a component of the human genome. The activated glucosinolate products are potent inducers of mammalian phase II detoxication enzymes, which aid in the deactivation and excretion of many carcinogens from the body. Many observational and case studies have demonstrated a beneficial effect on a number of human cancers. This has led to a number of glucosinolate compounds being evaluated in dietary strategies to combat cancer in worldwide clinical trials. The glucosinolates also have potent anti-oxidant, anti-inflammatory and anti-microbial properties relevant to their application in several areas of biomedicine.

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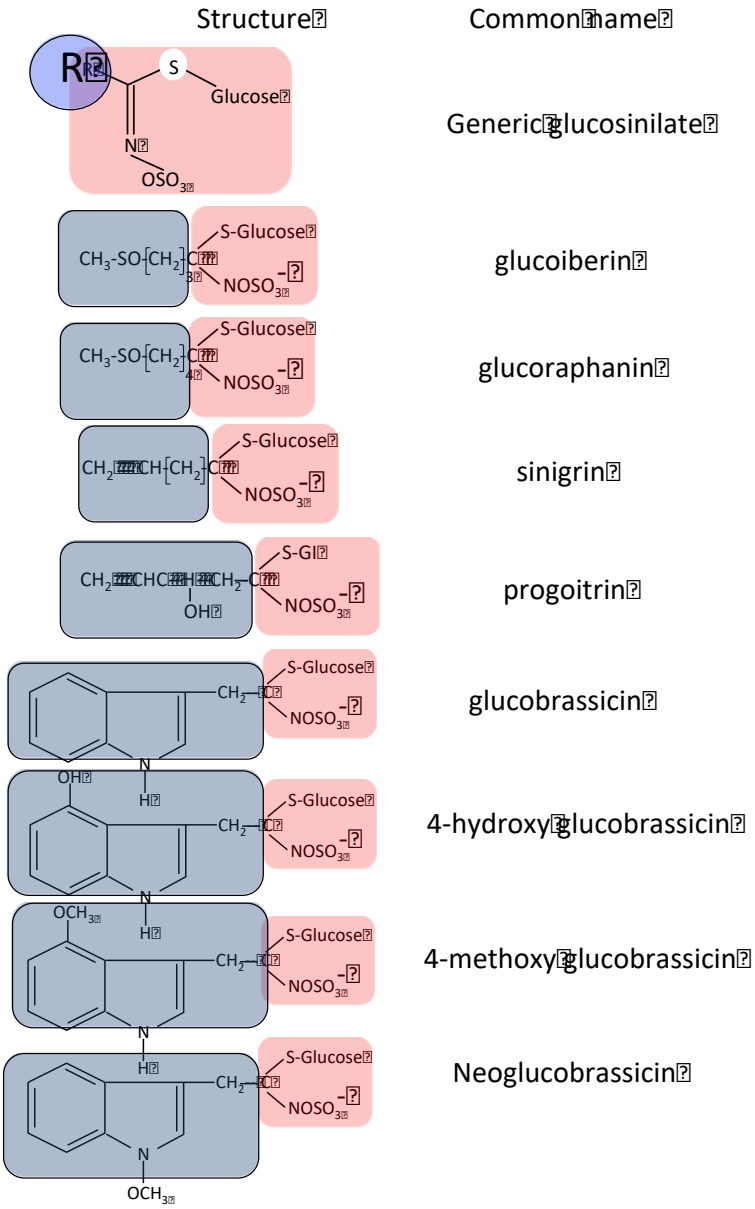

Figure 5. The structure of some members of the glucosinolate family

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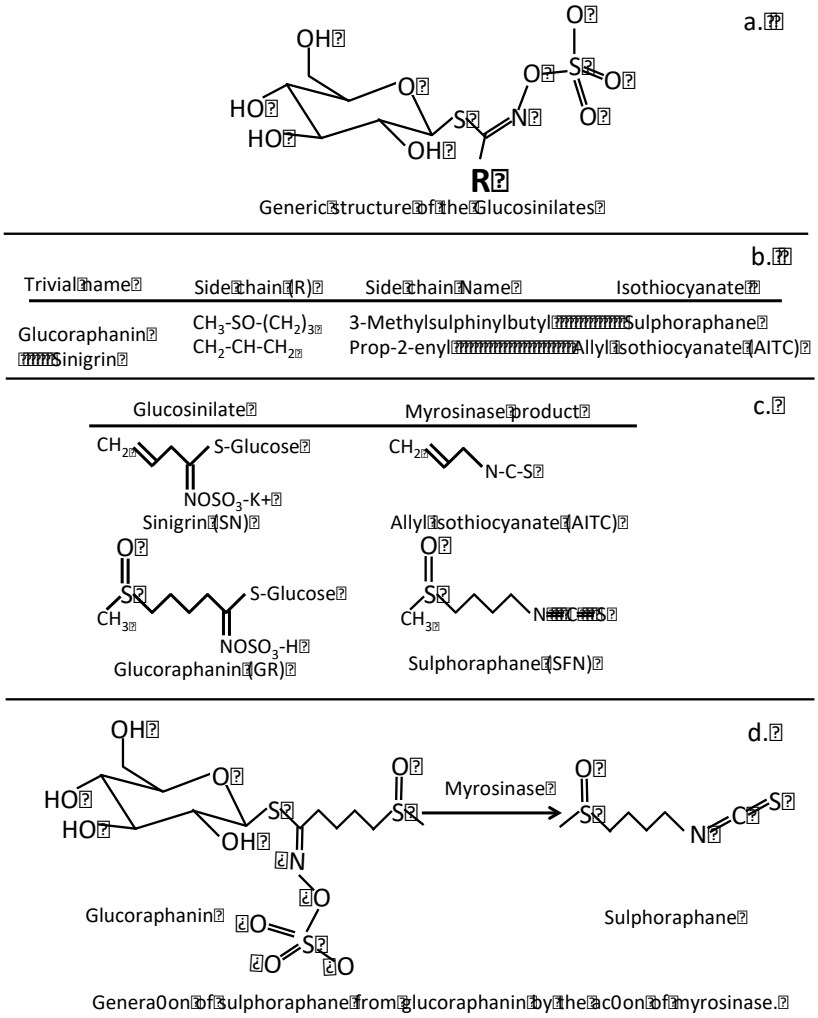

Figure 6. General structural information (a) of selected glucosinolate members and their bioactive forms (b, c) generated by myrosinase (d).

Mechanical disruption or trauma to the myrosinase and glucosinolate containing cells sets in motion a series of events which generate the bioactive SFN and AITC which combat the perceived threat. The distribution of the cell types which contain these compounds is shown in *Brassica juncea* (yellow mustard, Figure 7a-c). The distribution of the myrosinase and glucosinolate containing cells in a flower stalk of *Arabidopsis thaliana* which is also a member of the *Brassicaceae family* is also shown (Fig 7d*).* Epidermal cells of the *A. thaliana* flower stalk also express epithiospecifier protein which may have regulatory roles in the myrosinase-glucosinolate conversion reaction (Fig 7d).

The activation of the glucosinolates by myrosinase in the Brassicaceae while at first appearing as a simple inter-conversion may actually be more complicated in-vivo. Myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1.) in Brassicaceae species such as *Brassica napus* and *Sinapis alba* is encoded by two differentially expressed gene families, MA and MB, consisting of about 4 and 10 genes, respectively. Two of these genes (TGG1 and TGG2) have been sequenced and shown to share 75% nucleotide sequence homology. Phylogenetic analyses however shows that these two gene families arose after *Arabidopsis* had diverged from the other Brassicaceae species [43, 44]. The TGG1 and TGG2 genes are differentially expressed in the leaf, sepal, petal, and gynoecium in

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developing seeds. Moreover, In mature seeds of *Brassica napus* three major and three minor myrosinase isoenzymes have been identified which are differentially expressed in the embryos, seedlings, and mature tissues with up to 6 isoenzymes of 75, 73, 70, 68, 66, and 65 kDa detected in any one tissue. Differential expression of the MA and MB genes was also evident, MA genes were expressed only in developing seeds, whereas MB genes were most highly expressed in seeds, seedling cotyledons, young leaves, and to a lesser extent tissues of the mature plant. The mature 75kDa myrosinase isoform was encoded by the MA gene family [45].

A number of myrosinase-binding proteins (MBPs) and myrosinase-associated proteins (MyAPs/ESM) have also been identified[46] which may have accessory roles to play in the regulation of the myrosinase-glucosinolate interconversion reaction to nitriles, isothiocyanates, epithionitriles and thiocyanates [47].

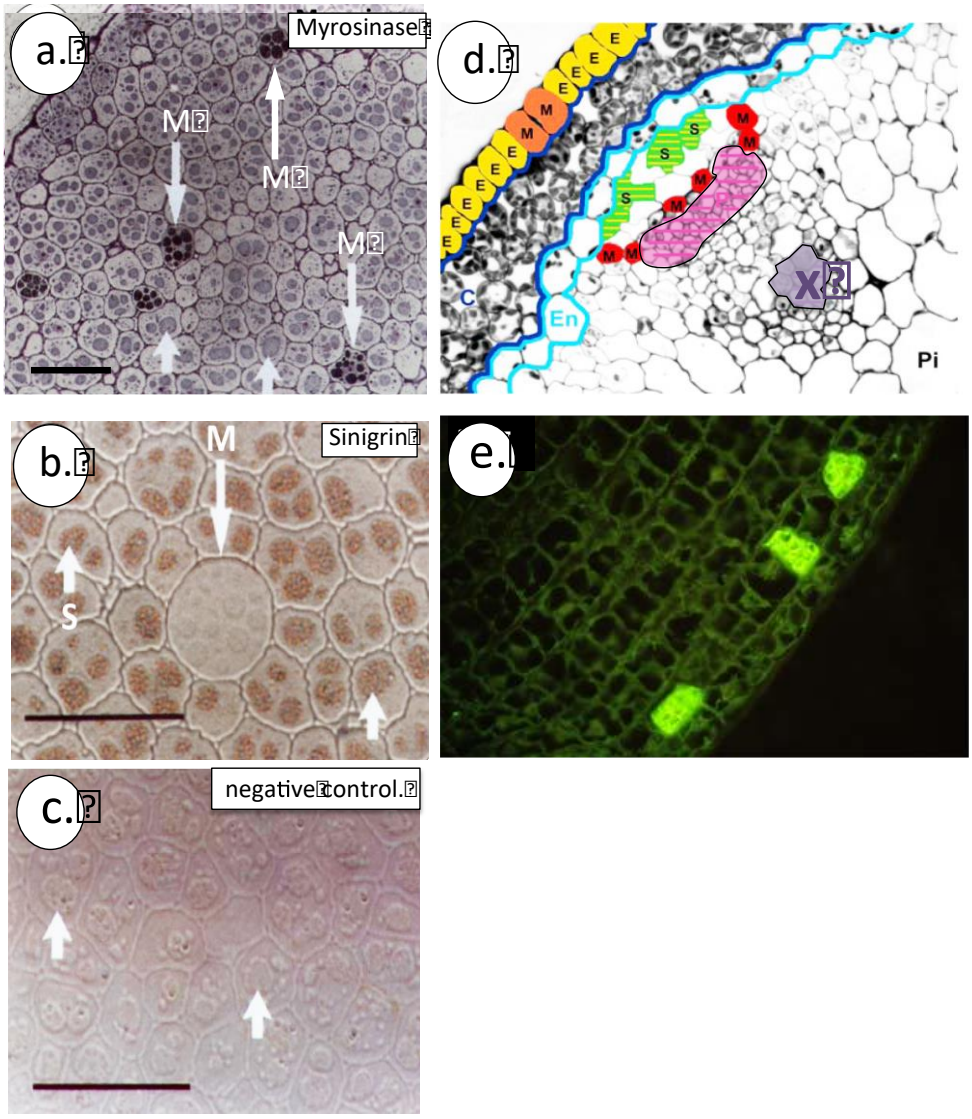

Figure 7. Cellular distribution of myrosinase and glucosinolate in plants of the Capparales order. Interference contrast immunogold-silver light micrographs of *Brassica juncea* cotyledons showing the cellular immunolocalization of myrosinase (M) and sinigrin (S) localized using anti-myrosinase antibody (a) and anti-sinigrin-BSA conjugate (b) or pre-immune sinigrin anti-serum (c). Schematic representation of the spatial distribution of known components of the glucosinolate–myrosinase system in a portion of an *Arabidopsis thaliana* flower stalk transverse section (d). Cell types labelled green represent S-cells containing glucosinolates;

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myrosinase (M)-expressing phloem cells and guard cells are labeled red and orange respectively; epidermal cells (E) expressing ESP [47], epithiospecifier protein are labeled yellow; stripes in S-cells indicate cellular colocalization of glucosinolates and ESP. For orientation purposes some tissues are marked by coloured lines: P: Phloem (pink); C: cortex (dark blue); En: endodermis (light blue); X: xylem (purple); Pi: pith. FITC labeled myrosinase cells using polyclonal Ab K059 in the outer cell layer of White mustard *Sinapis alba* hypocotyl (e). Segments a-c and d, e reproduced from [48] and [46] with permission.

In *A. thaliana* epidermal cells also express epithiospecifier protein (ESP) and the thiocyanate forming protein (TFP) that divert the glucosinolate hydrolysis from isothiocyanate production to nitrile/epithionitrile or thiocyanate production [47] (Fig 7d). The high glucosinolate content of S-cells in *A thaliana* can also be visualized by toluidine blue and periodic acid Schiff staining (Fig 8) and by histological elemental analysis (Fig 9a, b) showing the localization of the Sulphur component of the glucosinolate and by laser dissection of cellular components and elemental analysis of the sap contents (Fig 9c, d).

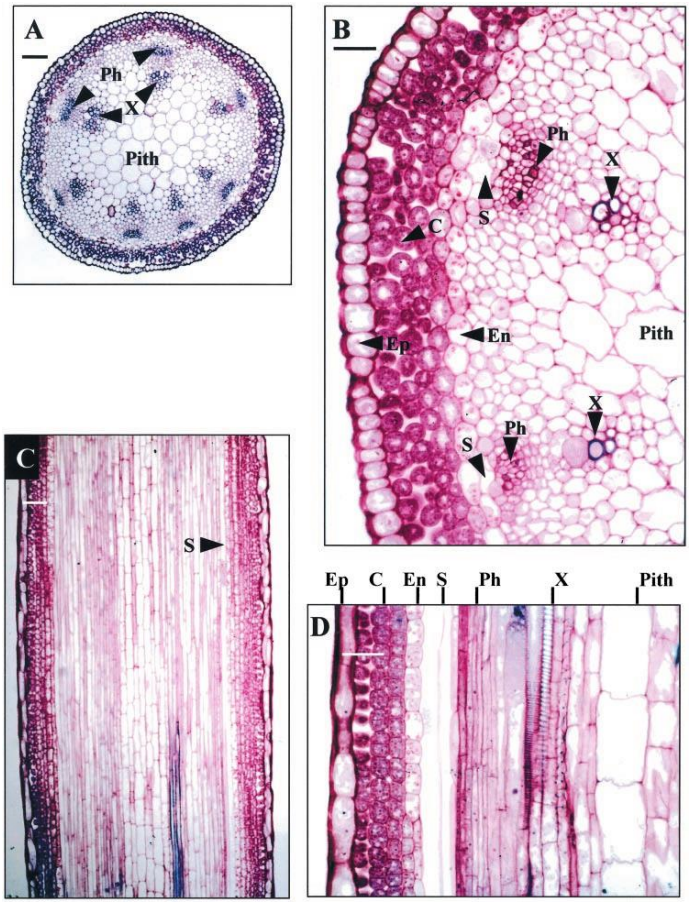

Figure 8. Histology of a flowering stalk of arabidopsis stained with toluidine blue (a), and periodic acid-Schiff (b, c) and identification of S cells which have extremely high levels of glucosinolate. Transverse (a, b) and longitudinal section (c) showing characteristic distributions of cells in different regions of the epidermis, endoderm, parenchyma, phloem, xylem and central pith. Images modified from [49] with permission.

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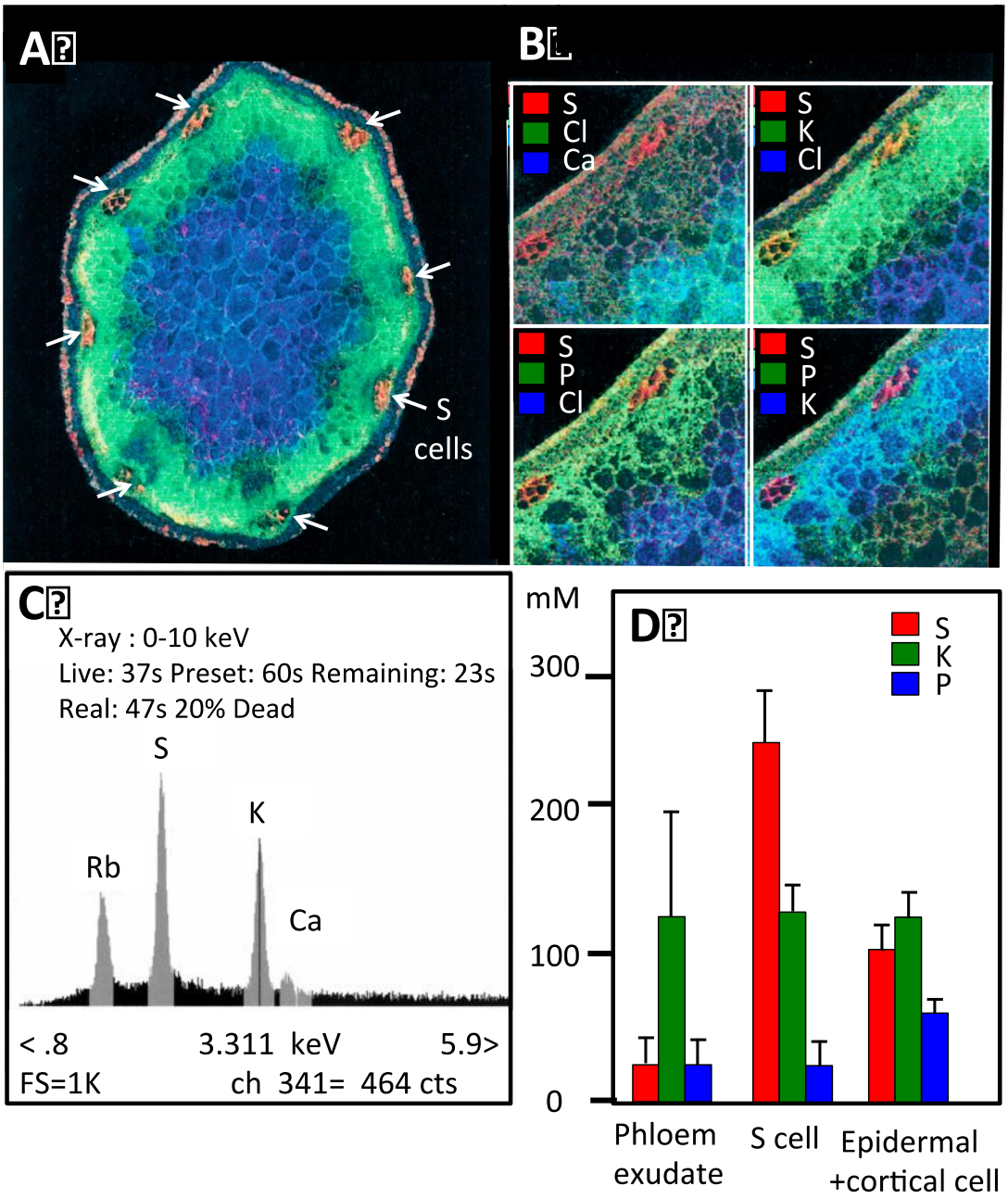

Figure 9. Elemental energy dispersive X-ray image of a cross-sectioned freeze dried Arabidopsis flower stalk, pseudo coloured Sulphur (red), potassium (green) and calcium (blue) (A, B). Elemental energy dispersive X-ray spectrum of sap collected from a secretory cell rich in glucosinolate. Rb -$RbNO_3$ internal standard (200 mM). (C) Quantitive measure of S, K, P elemental composition of phloem exudate, S-cells and a mixture of epidermal and corticol cells. Images modified from [49] with permission (D).

Treatment of antibiotic resistant bacterial infections

Antibiotics and antimicrobial agents, have been used for the last 70 years to treat human infectious diseases. Since the 1940s, these drugs have greatly reduced illness and death from infectious diseases. However, these drugs have been used so widely and for so long that the infectious organisms the antibiotics are designed to kill have adapted to them, making these drugs far less effective. Each year in the USA, at least 2 million people become infected with bacteria that are resistant to antibiotics with at least 23,000 deaths recorded as a direct result of these infections. Multi drug resistant bacterial infections were also responsible for an estimated 25,000 deaths per year in the

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EEC in 2015-2017 and these cost Eur 1.5 billion per year in healthcare treatment costs and lost productivity. If these current infection rates are not reversed then 10 million deaths globally per year are predicted by 2050, (317,000 in USA; 392,000 in S.America; 392,000 in EEC; 4.1 million in Africa; 4.7 million in Asia and 22,000 in Australia). Moreover it is estimated that additional hospital costs per patient will be in the order of 10-40K USD in OECD countries. Furthermore, the associated impact of lost economic output due to increased mortality, prolonged sickness and reduced labour efficiency may double this figure [50-54].

Despite the fact that bacterial infections are already one of the leading causes of death globally and that mortality rates are escelating at alarming rates, no new antibiotics have been produced by the pharmaceutical industry in more than a decade. The WHO has warned of the possibility that we may be entering a "post-antibiotic era" within this century. Bacteria resistant against all known antibiotics are becoming increasingly common and already producing untreatable infections.

The repurposing of anticancer drugs for the treatment of bacterial infections has been suggested since some of these have proven to be effective in vitro for eliminating recalcitrant, multidrug tolerant bacteria, other antibiotics have proved useful as anti-cancer compounds [55-58]. Among the most harmful human pathogenic bacteria, *Staphylococcus aureus (Golden Staph)* stands out as one of the most virulent and troublesome due to its ability to cause life-threatening infections and to adapt to changing environmental conditions [59, 60]. The ability of *S aureus* to establish itself in various community home and hospital environments, and its resistance to antibiotic treatment make this an important healthcare threat [61]. The emergence of methicillin resistant *S. a*ureus (MRSA) almost 5 decades ago further compounds the serious nature of such infections. Furthermore, hospital environments are conducive to *S.aureus* colonisation and its virulence is a major threat particularly to patients with reduced immune function [62]. Particularly virulent strains of *Enterococcus*, resistant to conventional antibiotic treatment, have also emerged in hospitalized patients [63]. Of particular concern are the vancomycin-resistant enterococci (VRE), that lead to infections of the urinary tract associated with catheter use or to catheter mediated bloodstream infections [64]. There is therefore an increasing global interest in the identification of bioactive compounds from plant sources, which display antibacterial and antifungal properties that are pharmacologically effective but which display limited or no side effects.

The glucosinolates produced by the Brassicacea family, order Capparales contain compounds with potent anti-bacterial, anti-fungal, anti-nematodicidal, anti-viral and insecticidal properties making them obvious candidates in the search for compounds to counter bacterial infections [4, 10, 11, 65-71]. Morever many of the glucosinolates have been shown to act synergistically with existing antibiotic regimens improving their effectiveness [68, 72].

The World Health Organization (WHO) has implemented a number of initiatives to collect data on the incidence of antimicrobial resistance and to co-ordinate methods to combat this problem. These include the Global Antimicrobial Resistance Surveillance System (GLASS), Global Antibiotic Research and Development Partnership (GARDP), a joint initiative of WHO and Drugs for Neglected Diseases initiative (DNDI), Interagency Coordination Group on Antimicrobial Resistance (IACG), an initiative of the United Nations Secretary-General established to improve coordination between international organizations ensuring effective global action against this threat to health security [73].

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WHO published a list of antibiotic-resistant "priority pathogens" in 2017 covering 12 families of bacteria posing the greatest threat to human health [74] highlighting the threat of gram-negative bacteria that are resistant to multiple antibiotics and pose particular threats to global public health. http://www.who.int/news-room/detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed

Further studies have appeared describing these antibiotic resistant bacterial strains which have been referred to as Super-bugs [75-77].

The effective antibiotics which are available for the treatment of bacterial infections are relatively small in number and in many cases have become largely ineffective. The last time a new antibiotic was released on to the world market was approximately 30 years ago so there is a strong need for one to be developed and a ready made world market eagerly awaiting this product. The WHO has established three treatment categories based on the urgent need for new antibiotics: these are critical, high and medium priority (Table 2). The most critical group of patients includes those infected with multidrug resistant bacteria that pose a particular threat in hospitals, nursing homes, and among patients whose care requires devices such as ventilators and blood catheters. These include *Acinetobacter*, *Pseudomonas* and various Enterobacteriaceae (including *Klebsiella*, *E. coli*, *Serratia*, and *Proteus*). These can cause severe and often deadly bloodstream infections and pneumonia. Such bacteria have become resistant to a large number of antibiotics, including carbapenems and third generation cephalosporins, now the best available antibiotics for treating multi-drug resistant bacteria.

The second and third tiers in this list, the high and medium priority categories contain other increasingly drug-resistant bacteria that result in gonorrhoea and food poisoning caused by *Salmonella*. Gonnorhoea is rapidly becoming a condition which will soon become untreatable.

Table 2. World Health Organization priority pathogen list*

Priority Category	Bacterium
Critical	1. *Acinetobacter baumannii*, carbapenem resistant
	2. *Pseudomonas aeruginosa*, carbapenem resistant
	3. *Enterobacteriaceae*, ESBL** producing carbapenem resistant
High	1. *Enterococcus faecium*, - Vancomycin resistant
	2. *Staphylococcus aureus*, - Methicillin/Vancomycin resistant
	3. *Helicobacter pylori*, - Clarithromycin resistant
	4. *Campylobacter spp.* - Fluoroquinolone resistant
	5. *Salmonellae* - Fluoroquinolone resistant
	6. *Neisseria gonorrhoeae* , Cephalosporin/Fluoroquinolone resistant
Medium	1. *Streptococcus pneumonia*, Penicillin resistant
	2. *Haemophilus influenzae*, Ampicillin resistant
	3. *Shigella sp*, Fluoroquinolone resistant

*http://www.who.int/news-room/detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed

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** Certain strains of bacteria are resistant to treatments with commonly used antibiotics such as penicillin and cephalosporins. These bacteria produce enzymes known as **E**xtended **S**pectrum **B**eta-**L**actamases (ESBL). ESBL producing bacteria are resistant to most types of third generation antibiotics and include strains of *Klebsiella pneumoniae, Klebsiella oxytoca and Escherichia coli, Enterobacter* spp., *Salmonella* spp., *Morganella morganii*, *Proteus mirabilis*, *Serratia marcescens* and *Pseudomonas aeruginosa* produce ESBLs relatively infrequently.

The deadliest drug resistant bacteria.

In the face of rising antibiotic resistance, the WHO published its first ever list of the deadliest superbugs that threaten human health. This so-called dirty dozen encompasses 12 families of dangerous bacteria that have developed resistance to the drugs used to treat common infections. Antibiotic-resistance costs some 700,000 lives each year, and if the phenomenon can't be halted, experts predict that this number could grow to 10 million deaths annually by 2050. *The publication of this list is a grave acknowledgement by the WHO that current pharmaceutical research efforts to curb antibiotic resistance is not doing enough to curb the risks posed by these superbugs*.

The myrosinase-glucosinolate system evolved in plants of the Capparales order as a protective mechanism aimed against, bacterial and fungal infection and attack by parasites or insects [4, 7, 11, 65, 78]. Significantly, the activated glucosinolates appear applicable to the treatment of human infections and may be used in combination therapy with existing antibiotics. Comparison of the antibiotic activities of SFN and AITCs with that of Vancomycin showed that all contained significant bactericidal activity [4, 7, 11, 65, 78]. Sulphoraphane was at least as effective an antibiotic as Vancomycin and was more potent in all bacteria tested. AITCs were also potent bactericidal agents under the conditions used [4, 65].

Application of Glucosinolates in cancer therapies.

The bioactivity of glucosinolates and their hydrolysis products have been well documented and an extensive literature exists on their nutritional benefits and potential in biomedical applications (Table 3, 4, 5). Over 300 scientific studies have documented the antioxidant properties of SFN and the roles it plays in the prevention of multiple diseases including several cancer types, high blood pressure, macular degeneration and stomach ulcers. Using 'sulphoraphane' as a search term, 1767 publications are currently listed in the PubMed data base. Glucosinolates are potent inducers of mammalian phase II detoxication enzyme systems in the human body, which aid in the deactivation and excretion of many carcinogens. A number of these compounds are currently being evaluated in dietary strategies for cancer prevention in worldwide clinical trials.

The myrosinase-glucosinolate system is a sophisticated system which evolved in plants as a protective mechanism against bacterial and fungal infections and parasitic attack from insects. The products of glucosinolate activation also display bioactivity in mammalian systems with epidemiological links to cancer chemoprevention in humans supported by in vitro, in vivo, and small clinical studies. The primary mechanism responsible for the observed chemoprevention afforded by activated glucosinolates lies in the induction of phase II antioxidant enzymes, such as NAD(P)H quinone reductase , heme oxygenase 1, glutamate-cysteine ligase catalytic subunit, and glutathione S transferases, through the Keap1-Nrf2-ARE cell signaling pathway [79-81]. The KEAP gene encodes

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Kelch-like ECH-associated protein 1. Under quiescent conditions, KEAP1 protein binds Nrf2 in the cytoplasm and represses its activation. Nrf2 is a nuclear receptor and transcription factor which is a master regulator of genes in many diseases and has a central regulatory role in the human diseaseome [80]. Nrf2 activation leads to a co-ordinated antioxidant and anti-inflammatory response in many disease states including many forms of cancer [6]. The Nrf2 gene encodes Nuclear factor (erythroid-derived 2)-like 2. Nrf2, is a transcription factor that is a master regulatory gene in humans. Significantly, sulphoraphane is a potent inducer of Nrf2 activity [82]. Nrf2 activation induces cytoprotective genes with key roles in cellular defence mechanisms including those that regulate redox status and detoxification processes [79]. Nrf2, is a transcription factor that regulates the expression of antioxidant proteins which have protective properties against the oxidative damage that may result from traumatic injury and inflammation in tissues. Cell signalling pathways triggered by Nrf2 prevent cancer initiation and progression in normal and premalignant tissues, however in fully malignant cells Nrf2 activity can actually enhance tumour cell growth. The Keap1-Nrf2 pathway is the major regulator of cytoprotective responses to endogenous and exogenous stresses caused by reactive oxygen species (ROS) and electrophiles. Nrf2 binds along with Maf proteins to the antioxidant response element (ARE) in the regulatory regions of target genes, and Keap1 (Kelch ECH associating protein 1), a repressor protein that binds to Nrf2 and promotes its degradation by the ubiquitin proteasome pathway. The Keap1-Nrf2 pathway regulates cytoprotective responses to oxidative stress and represents a promising therapeutic target to counteract oxidative damage in cancer and neurodegenerative disorders [79, 81, 83-86]. Numerous in vitro studies in human colon, leukemia, pancreatic, lung, and skin cancer cell lines have demonstrated SFN's inhibitory effects on cell cycle arrest [12, 87-89] and elevated apoptosis in human bladder[90] and prostate[91] cell lines. Sulphoraphane's ability to disrupt tubulin and actin polymerization, inhibits mitotic spindle formation and tumour cell growth in animal models of breast cancer[92, 93]. Sulphoraphane inhibits histone deacetylase increasing apoptosis in human colon, prostate, and kidney cell lines [94-97].

Table 3.

Combination Therapies of Sulphoraphane (SFN) used in Conventional Anti-Cancer and Anti-bacterial Treatments Often with Synergistic Effect

Compound used in Combination Therapy	Ref
SFN-Selenium nanoparticles	[98]
Paclitaxel	[9]
Cisplatin	[99]
Luteolin	[100]
Clofarabine	[101]
Doxorubicin	[102]
5-fluorouracil	[103]
HistoneH3	[104]
Withaferin A	[105]
Hispidulin	[106]
Carboplatin	[107]
Docetaxel	[108]
Lapatinib	[109]
PR-104A	[110]

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Histone acetylation plays a crucial role in chromatin remodeling and regulates its packing density around chromosomes and their constituent genes. Dense packing can deny transcription factors access to genes thus histone acetylation-deacetylation has a librarian type organizational role which controls chromatin structure and gene accessibility indirectly regulating gene expression [14, 111-113]. The first direct observation of SFN's inhibitory effect on cancer in a human population was observed in 200 healthy adults (ages 25-65) from the Jiangsu Province of China, a region with a high rate of hepatocellular carcinoma due to excessive dietary aflatoxin consumption and endemic chronic hepatitis B infection rates[114].

Use of Glucosinolates in the treatment of skin lesions.

Glucosinolates promote skin wound healing [115-117]. Cress Oil has been used to treat thermal and acid burns in rabbits [118]. Epidermolysis bullosa simplex, a rare inherited condition in which the epidermis loses its integrity after mechanical trauma, has been treated with SFN, which prevented blistering in this chronic painful condition. SFN activates Nrf2 expression in basal epidermal keratinocytes resulting in up regulation in Keratin 14 to alleviate blister production and restore skin integrity [119]. The annual incidence of melanoma reported by the WHO and National Cancer Institute, NIH, USA Surveillance, Epidemiology and End Result program (SEER) lists approximately 160,000 cases with an associated 48,000 deaths worldwide each year [120-123]. Sulphoraphane induces cell growth arrest dose dependently and cell death through apoptosis in ME-18 melanoma cells [124]. AITCs and SFN inhibit psoriatic skin lesion development and related pro-inflammatory factors in skin by prevention of inflammation development and they also reduce ongoing inflammation by down regulating interleukin-1 (IL-1) and tumour necrosis factor-alpha (TNF◎) production by skin ◎◎◎◎◎◎◎◎◎◎◎ [125]. Glucosinolates have also been shown to provide photo protection to skin to UVB irradiation [8].

Table 4.

The Diverse Areas of Application of Sulphoraphane (SFN) in Biomedicine

Miscellaneous medical conditions treated with Sulphoraphane	Ref
Spatial learning and memory dysfunction	[126]
Chemotherapy-induced neuropathic pain	[127]
SFN-decorated gold nanoparticle for anti-cancer treatment	[128]
Protection of granulosa cells against oxidative stress	[129]
Epigenetic Nrf2 signaling pathway	[130]
Cadmium-mediated carcinogenesis	[131]
Oxidative stress in cultured adult cardiomyocytes	[132]
Protective effects of glucosinolate hydrolysis products in neurodegenerative diseases	[133]
Clearance of Amyloid-β and Tau protein in a mouse model of AD	[134]
Experimental diabetic peripheral neuropathy	[135]
Joint inflammation in a murine adjuvant-induced mono-arthritis	[136]
Protection against cognitive impairment in AD-like lesions in diabetes	[137]
Anti-inflammatory effect of SFN on human THP-1 macrophages in a murine AD model	[138]
Improved cardiac function by inhibiting oxidative stress and inflammation in a Rabbit Model of Chronic Heart Failure	[139]

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Inhibition of class IIa histone deacetylase activity	[140]
Apoptosis via microtubule disruption in cancer	[141]
Inhibition of LPS-Induced Inflammation/cytotoxicity/oxidative microglial stress	[142]
Down-regulation of MAPK/NF-κB signaling in LPS-activated BV-2 microglia	[143]
Epigenetic modification of Nrf2 signalling in a model of AD	[144]
Inhibition of oxidative stress in an In-vitro model of age-related macular degeneration	[145]
Prevention of angiotensin II-induced cardiomyopathy by activation of Nrf2 and Akt/GSK-3ß/Fyn pathway.	[146]
Suppression of NLRP3 inflammasome alleviating acute gouty inflammation	[147]
Modification of Histone H3, unpacking of chromatin, to prime defence	[104]
Nrf2-Inducers Counteract Neurodegeneration in Friedreich's Ataxia	[148]
Modulation of oxidative stress and inflammation in rats with toxic hepatitis	[149]
Modulation of oxidative damage in lead exposed rat hippocampus	[150]
Prevention of dexamethasone-induced myotube atrophy via Akt/Foxo1	[151]
Induction of p53 deficient SW480 cell apoptosis by ROS MAPK signalling	[152]
Role of microRNAs in the chemo preventive activity of SFN	[153]
Up regulation of Nrf2 protection in doxorubicin-induced chronic heart failure	[154]
Increased Nrf2 expression protects alveolar epithelial cells against oxidative injury	[155]
Novel phosphonate analogs of SFN with in vitro and in vivo anticancer activity	[156]
Inhibition of PDGF-induced vascular SMC proliferation by targeting mTOR/p70S6kinase signalling independently of Nrf2 activation	[157]
Gastrointestinal protection against *H. pylori* and NSAID-Induced Oxidative Stress	[158]
Protection from cerebral ischemic/reperfusion injury via inhibition of NLRP3 inflammasome activation in rats	[159]
Protection against sodium valproate-induced acute liver injury	[160]
Enhanced SFN cardioprotection against oxidative stress by 17β-Estradiol	[160]
Photoprotective Effects of SFN and Hispidulin	[106]
Differential modulation of mitochondrial biogenesis/dynamics in normal and tumour cells	[161]
Nrf2 targeting by SFN: A potential therapy for cancer treatment	[162, 163]
Improvement of neuronal mitochondrial function in brain tissue	[164]
Protection of pancreatic Acinar cell injury by modulating Nrf2-mediated oxidative stress and the NLRP3 inflammatory pathway	[165]
Improvement in chemotherapy efficacy targeting cancer stem cell-like properties	[166]
Protection against rotenone-induced neurotoxicity via mTOR, Nrf2, and autophagy	[167]
Chemoprevention of oxidative stress-associated with oral carcinogenesis	[168]
Amelioration of bladder dysfunction via activation of Nrf2-ARE Pathway	[169]
Broccoli sprout homogenate treatment for Sickle Cell Disease	[170]
Treatment of Autism Spectrum Disorder	[171, 172]
Protection against aortic complications in diabetes	[173]
Anti-inflammatory effect of SFN against amyloid-β peptide via STAT-1 dephosphorylation and activation of Nrf2/HO-1	[174]

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Table 5. Assessing the Efficacy of Sulphoraphane in Cancer Models

Cancer type	Ref
Leukemia	[87, 124, 175-179]
Prostate cancer	[91, 95, 180-182]
non-small cell lung cancer cells	[107, 183, 184]
Pancreatic cancer	[165, 185-187]
Breast cancer	[92, 93, 101-103, 105, 108, 109, 188-195]
Bladder cancer	[169, 196-201]
Ovarian cancer	[99]
HepG2 Carcinoma Cells	[202-206]
Gastric cancer	[207, 208]
Squamous cell carcinoma	[209, 210]
Nasopharangeal cancer	[211]
Melanoma	[212]
Glioma	[173, 213-215]
Colon cancer	[110, 216, 217]
Lung cancer	[218, 219]
Schwannoma	[220]
Colorectal cancer	[221]
Cervical cancer	[222]
Oral cancer	[223, 224]

Biofumigation and adverse effects on the ozone layer.

In the 1970s and 1980s concern in the international community on the adverse effects of compounds on atmospheric ozone depletion led to *The Vienna Convention for the Protection of the Ozone Layer* in 1985. This led to *The Montreal Protocol on Substances that Deplete the Ozone Layer* in 1987 where the international community agreed to co-operate on the diminished use of ozone depleting compounds with the eventual phasing out of their use. Methyl bromide was once considered an effective pre-plant soil fumigant that controlled soilborne diseases, nematodes, insects, and weeds in economically important crops. At the fourth meeting of the Montreal Protocol in Copenhagen in 1992 (*http://ozone.unep.org/en/treaties-and-decisions/montreal-protocol-substances-deplete-ozone-layer*), methyl bromide was listed as the primary source of stratospheric bromine, responsible for ozone depletion and 20-25% of the austral spring's Antarctic 'ozone hole' [225, 226]. Methyl bromide was subsequently banned as a soil fumigant in several nations, including the U.S. in accordance with the U.S. Clean Air Act. After the phase out of methyl bromide from use as a soil fumigant due to the 2005 Montreal Protocol, farmers sought an effective, sustainable soil fumigant.

Methyl bromide is an odorless, colorless biofumigant gas that was widely used to control a variety of pests in agriculture [227] and the prevention of their inadvertent on-shore release from shipping containers. Methyl bromide displays activity against fungi, bacteria, viruses, weeds, insects [228-230], nematodes [231] (or roundworms), and rodents. Methyl Bromide is a class I ozone depleting substance as defined by the *Montreal Protocol on Substances that Deplete the Ozone Layer*. Methyl bromide is a toxic substance, exposure to high concentrations of Methyl Bromide can cause CNS and respiratory failure damaging the lungs,

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eyes and skin [232]. The banning of the use of Methyl Bromide by the Environment Protection Agency (EPA) caught the farming community by surprise [233]. Anaerobic soil disinfestation and biofumigation using activated glucosinolate products (AITC, Sulphoraphane) is a potential non-chemical method for controlling soilborne plant pathogens [234]. Biofumigation via Brassica plantings was found to affect soil nematode and microbe populations [235-238]. Soils treated with mustard had higher microbial biomass carbon (average of 160mg/kg soil) than fallow treatment (130 mg/kg soil) [239, 240] thus the introduction of cruciferous plants as part of a crop rotation system made sense and helped to alleviate the dependence on chemical control of soil pests preventing toxic build up of chemical residues in the soil sub-structure. Cruciferous plants produce a number of members of the glucosinolate family which have natural anti-microbial, nematodicidal [231], mosquito larvicidal [P1], and insecticidal properties [228, 230, 241-245]. Thus the inclusion of cruciferous plants as part of a sensible sustainable crop-rotation program [33, 246-249] can have advantageous properties in long-term pest control in soils [230, 239, 249] and can also have favourable effects on organic matter content in the soil sub-structure.

Prevention of Biofilm Development

Biofilms have adverse effects on all types of instruments, sensors, and equipment used in industrial settings, in power plants, air filtration and air conditioning plants, food and beverage production plants, desalination facilities, and paper mills. Biofilms on pipelines, tanks, heat exchangers, filters and other equipment can cause reduction of heat transfer, increased pressure drop, corrosion of metallic surfaces, and can also be a source of bacterial contamination. Prevention of biofilm development represents an ongoing challenge for industrial engineers and instrument designers.

Biofilm formation on medical devices and implants such as catheters, mechanical heart valves, pacemakers, prosthetic joints, and contact lenses pose a critical medical problem. The most common biofilm-forming bacteria include *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus viridans*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* [250-255].

Among these biofilm-forming bacteria, *S.aureus* and *S.epidermidis* are most commonly found on cardiovascular devices [256-258]. It has been estimated that 40%–50% of prosthetic heart valve infections, and 50%–70% catheter biofilm infections are due to these bacteria [259, 260]. Despite the evaluation of a wide range of anti-fouling compounds [255, 261, 262] improvements are still required in this area, glucosinolates could fill this void. Glucosinolates, have antifungal, antibacterial, bioherbicidal, antioxidant, antimutagenic, anticancer, and anti-inflammatory properties and combat the development of bio-films of *Pseudomonas aeruginosa* [5, 78, 115, 263].

Cancer and dietary sulphoraphane and AITC levels

Numerous meta-analyses of observational and case studies show an association between consumption of cruciferous vegetables and a reduction in the risk of development of a number of human cancers (Table 7). In many cases however the power of these analyses have been hindered by low sample sizes and in some cases no associations were established which limit the conclusions

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that can be made from such findings. More studies, especially high quality cohort studies with larger sample sizes, and well controlled confounding factors will be required to confirm the benefit of cruciferous vegetable consumption and reduced chance of developing various cancers. These initial studies have nevertheless delivered sufficient evidence to warrant such studies. The bioavailability of glucosinolates following different food processing methods has been evaluated in order to improve on the bioavailable dietary content of the bioactive forms of glucosinolate [264] Supplementation of the diet with broccoli sprouts or myrosinase containing uustard products have also been examined as a means of increasing the sulphoraphane and AITC content in the diet [265] and high yielding broccoli strains have also been developed in an attempt to improve dietary glucosinolate levels.

The beneficial bioactivities of sinigrin and applications in biomedicine.

Although the scientific literature on sinigrin is less extensive as sulphoraphane they share similar bioactivities and areas of application in biomedicine [115].

Table 6 The Varied Applications of Sinigrin in Biomedicine

Application	Ref
Reduction of liver fibrosis	[266]
Suppression of NF-κB/MAPK and NLRP3 inflammasome activation in macrophages	[267]
Promotion of wound healing	[115, 116]
Anti-cancer properties in methyl glyoxal modification	[268]
.Anti-proliferative activitiy on carcinogen-induced hepatotoxicity	[269]
Biofumigation of potato cyst nematode	[33]
Inhibition of Listeria monocytogenes on bologna sausages	[263]
Suppression of metastasis via inhibition of invasion, migration, and MMP-2/-9 activities in SK-Hep 1 human hepatoma cells	[270]
Brussel sprout juice mediatedeffects on cell cycle and adhesion of human colorectal carcinoma cells (HT29) in vitro	[271]
AITC mediated mitotic block, loss of cell adhesion/disrupted cytoskeleton in HT29 cells	[272]

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Cytotoxicity and genotoxicity of allyl and phenethyl isothiocyanates, glucosinolates, sinigrin and gluconasturtiin	[273]
Inhibition of microbial growth	[70, 72, 274]
Effects of dietary sinigrin or indole-3-carbinol on O6-methylguanine-DNA-transmethylase activity and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced DNA methylation and tumourigenicity in F344 rats	[275]

Table 7.*

Meta-analyses on observational and case studies demonstrating an association between cruciferous vegetable consumption and the prevention of human cancers.

Cancer type	Category of Study	Relative Risk (RR) or Odds ratio (OR) (95% confidence interval)	Reference
Bladder cancer	cohort (n=5), case control (n=5)	RR: 0.80 (0.69-0.92)	[276]
	Cohort & case studies (n=12)	RR: 0.84 (0.77-0.91)	[277]
	Cohort & case studies (n=7)	RR: 0.85 (0.69-1.06)	[278]
	Cohort studies (n=8)	RR:0.97 (0.93-1.01)	[279]
Breast Cancer	Case control (n=12)	RR: 0.85 (0.77-0.94)	[280]
Colorectal cancer	Cohort (n=11) Case control (n=24)	RR: 0.82 (0.75-0.90)	[281]
	Cohort (n=11) Case control (n=18)	OR: 0.92 (0.83-1.01)	[282]
Endometrial cancer	Cohort (n=1) Case control (n=16)	OR: 0.79 (0.69-0.90)	[283]
Gastric Cancer	Cohort (n=6) Case control (n=16)	RR:0.81 (0.75-0.88)	[284]
Lung cancer	Cohort (n=5) Case control (n=6)	RR: 0.75 (0.63-0.89)	[285]
Ovarian cancer	Cohort (n=5) Case control (n=6)	RR: 0.90 (0.82-0.98)	[286]
	Cohort (n=4) Case control (n=4)	RR: 0.89 (0.81-0.99)	[287]
Pancreatic cancer	Cohort (n=4) Case control (n=5)	RR: 0.79 (0.64-0.91)	[288]
Prostate cancer	Cohort (n=7) Case control (n=6)	RR: 0.90 (0.85-0.96)	[289]
Renal cell carcinoma	Cohort (n=6) Case control (n=6)	RR: 0.81 (0.72-0.91)	[290]
	Cohort (n=3) Case control (n=7)	RR: 0.73 (0.63-0.83)	[291]

*Modified from Cruciferous Vegetables and Cancer Risk: Meta-analyses of Observational Studies, Micronutrient information centre, Linus Pauling Institute, Oregon State University based on data in [292].

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Gaseous phase delivery of AITCs and Advanced Packaging as a prospective means of preventing spoilage of food products to increase their shelf-life properties.

The antifungal properties of AITCs have been employed as a gaseous phase product in an active packaging format to prolong shelf storage of bread products [293, 294]. The characteristic spicy flavour profile of mustard products is appealing to the Eastern palate and this has found acceptance in bread production in Japan however some adjustment in the aroma of these volatile compounds may be required for the Western palate [295, 296]. Restrictions exist in Europe in the additives which are permissible in bread production, however this approach has proved popular in Japan [295]. Glucosinolates have also been applied in food packaging formats to prolong the shelf-life storage of premium quality fruit and protein rich products [297].

Glucosinolates have also been examined to combat bacterial contamination of meat products during food processing. *AITCs have been used in food production steps to prevent bacterial spoilage* [298-303].

Concluding remarks

The myrosinase-glucosinolate system is a sophisticated protective mechanism in plants developed through several hundred million years of evolution. With a greater understanding of its component parts it has now been possible to apply some of these components to physiological processes in man and these show much potential benefit in biomedicine. Some of these compounds may be useful in biofumigation and prevention of fouling of plant equipment, and sterilisation of medical implants and in biomedicine in wound healing and the prevention of cancer. A very extensive literature documents the biodiversity of areas of application for glucosinolate products in Biomedicine indicating considerable promise in future areas of investigation in the following areas.

1. Antibiotics, anti-fungal and anti-viral agents
2. Biofilm prevention in medical implants, catheters and Industrial plant equipment
3. Nutritive additives with anti-cancer properties
4. Advanced food packaging formats to improve shelf-life of products.

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