University of Naples Federico II
Polytechnic and Basic Sciences School

Department of Chemical Sciences

Ph.D. in Chemical Sciences

New glycoconjugates with anti-oxidant activity for the development of supplements, useful in neurodegenerative diseases prevention

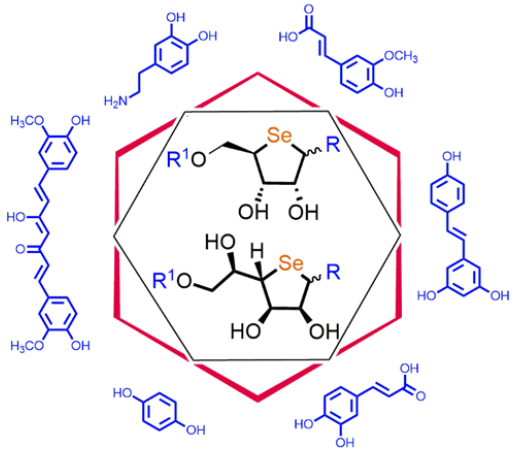

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Table of contents

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

ABBREVIATIONS

AA	ascorbic acid
AD	Alzheimer disease
ALB	Albumin
ApoER2	Apolipoprotein E-receptor type II
ATP	Adenosine triphosphate
Aβ	amyloid beta
AβPP	amyloid-β protein precursor
BDMC	bis-demethoxycurcumin
Br·	Atomic bromine
C	curcumin
CA	caffeic acid
CAT	catalase
CF	Cystic fibrosis
CFTR	CF transmembrane conductance regulator
Cl·	Atomic chlorine
CoQ10	Coenzyme Q10
CRP	C reactive protein
CVDs	Cardiovascular diseases
DCF	dichlorofluorescein

DCFH-DA	dichlorodihydrofluorescein diacetate
DMAP	dimethylaminopyridine
DMC	demethoxycurcumin
DNA	Deoxy ribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
ECM	extra cellular matrix
EHS	Engelbrecht–Holm–Swarm
EMT	Epithelial-mesenchymal Transition
ETC	Electron transport chain
FA	ferulic acid
FDA	Food and Drug Administration
FRAP	ferric reducing/antioxidant power
GPx	Glutathione peroxidase
Grx	Glutaredoxins
GSH	glutathion
GSR	Glutathione reductase
GSSG	Oxidized glutathione
H_2O_2	Hydrogen peroxide
HEMA	2-hydroxyethylmethacrylate
HIV	Human immunodeficiency virus

HO-1	Heme Oxygenase-1
HOCl	Hypochlorous acid
$^{l}O_2$	Singlet oxygen
LPO	Lipid peroxidation
LPS	lipopolysaccharide
MDs	Mitochondrial dysfunctions
MIC	minimum inhibitory concentration
MPO	Myeloperoxidase
MsCl	methanesulphonyl chloride
mtDNA	Mitochondrial DNA
NADPH	Nicotinamide adenine dinucleotide phosphate
NDs	Neurodegenerative diseases
NF-kB	nuclear factor kappa-light-chain-enhancer of activated B cells
NFT	neurofibrillary tangles
NO^{-}	Nitroxyl anion
NO_2^{-}	Nitrite
NOS	Nitric oxide synthase
NPC	Nutritional Prevention of Cancer Trial
Nrf 2	nuclear factor erythroid 2–related factor 2
$O_2^{\cdot -}$	Superoxide anion radical
O_2NOO-	Peroxynitrate

O_3	Ozone
OBr-	Hypobromite
OCl^-	Hypochlorite
OH·	Hydroxyl radical
ONOO-	Peroxynitrite
OS	Oxidative stress
OXPHOS	Oxidative phosphorylation
PAs	polyamines
PD	Parkinson disease
PEG	poly-etylene-glycole
PEGDA	poly(ethylene glycol) diacrylate
PUFA	Polyunsaturated fatty acids
RCO	Electronically excited carbonyls
RCS	Reactive carbonyl species
RNHCl	Chloramines
RNS	Reactive nitrogen species
RO	Alkoxyl radical
ROO	Peroxyl radical
ROOH	Organic hydroperoxide
ROS	Reactive oxygen species
RSeS	Reactive selenium species

RSS	Reactive sulphur species
SARS-COV2	Severe Acute Respiratory Syndrome Coronavirus-2
Se	Selenium
SELECT	Selenium and vitamin E Cancer Prevention Trial
SelP	Selenoprotein P
SeTal	1,4-Anhydro-4-seleno-D-talitol
SOD	Superoxide dismutase
T2DM	type 2 diabetes mellitus
TBAF	tetrabutylammonium fluoride
TBDPSCl	tert-butyl(chloro)diphenylsilane
TCA	Tricarboxylic acid
TEA	triethylamine
TF	Transferrin
TPP	Triphenylphosphine
TrxR	Thioredoxin Reductases
UV	Ultra violet
VEGF	Vascular endothelial growth factor
WHO	World Health Organization

ABSTRACT

ABSTRACT

Oxidative stress (OS) is a condition due to an imbalance between oxidants and antioxidants production. It occurs either when the former are prevalent, or the latter are reduced. If this alteration is prolonged in time, the physiological equilibrium is disrupted, leading to an oxidative damage in the involved tissues.[1]

It is now well established that OS is responsible for the initiation or development of many pathological processes, and may well become the salient feature to understand the pathogenesis of most of them.[2] Indeed, oxidative stress has been associated with age related disorders,[3,4] as well as cardiovascular diseases (CVDs),[5,6] metabolic disorders[7] like diabetes,[8,9] cancer,[10] neurodegenerative diseases[11] (NDs) and many others. Moreover, OS can be considered an important therapeutic target to prevent oxidative states.

In view of the foregoing, it is not surprising that the interest of scientific community has focused on preventive therapies for OS conditions, and inter alia, on those that involve the use of natural compounds as antioxidants. The latter - easily available in foods - can improve cellular defences helping the prevention of oxidative damage and have mostly no side effects for the body. Among natural antioxidants, polyphenols and selenium have been largely studied.

In particular, organoselenium compounds have been described as potent antioxidants [12,13] and – among them- selenosugars work well as oxidant scavengers, being even better tolerated by the human body.[14,15] Polyphenols, as well, own beneficial biological effects,[16] including those related to their antioxidant properties.[17]

Nowadays, the large data available show that selenium and polyphenols' dietary assumption are recommended both to guarantee a redox balancing, and to counteract eventual oxidative stress conditions. Hence, the idea of the project: to synthetize a new set of molecules consisting of selenium containing monosaccharides bound to a

phenolic moiety, which differ in the position of the bond. Furthermore, we investigated their potential antioxidant activity through chemical and cellular assays.

Finally, taking into account the already documented beneficial effects of antioxidant treatment in wound healing,[18,19] and the wide use of novel biocompatible hydrogels in wound dressing,[20] we approached to the design and the development of a new device for transdermal delivery of antioxidants. In details, it consists of a high biocompatible thin polymeric hydrogel membrane containing the aforementioned synthesised organoselenium compounds.

Publications

Avicenna Natural Institute, S. Dello Iacono, M. De Nisco, L. De Stefano, S. Pedatella, L. Serpico. "GLICOCONIUGATO SINTETICO E MEMBRANA POLIMERICA COMPRENDENTE TALE GLICOCONIUGATO". Patent application n.102021000012806. 18/05/21.

L. Serpico, M. De Nisco, F. Cermola, M. Manfra, S. Pedatella. 2021. "Stereoselective Synthesis of Selenium-Containing Glycoconjugates via the Mitsunobu Reaction" *Molecules* 26, no. 9: 2541. **https://doi.org/10.3390/molecules26092541**

P. Dardano, M. Battisti, I. Rea, L. Serpico, M. Terracciano, A. Cammarano, L. Nicolais, L. De Stefano, (2019). "Polymeric Microneedle Arrays: Versatile Tools for an Innovative Approach to Drug Administration" Adv. Therap., 1900036 (2019). **https://doi.org/10.1002/adtp.201900036**

Communications

L. Serpico, M. De Nisco, F. Cermola, M. Manfra, Claus Jacob, S. Pedatella "Antioxidant activity of new glycoconjugates containing Selenium" Poster presentation at 6th International Conference on New Trends in Chemistry OCTOBER 16-18, 2020 (Kyrenia, North Cyprus) ICNTC-2020.

L. Serpico, M. De Nisco, F. Cermola, M. Manfra, Claus Jacob, S. Pedatella "Selenium as Antioxidant: New Glycoconjugates to Prevent Oxidative Stress" Poster ICCST-14 2019.

M. De Nisco, L. Serpico, F. Cermola, I. Vespoli, M. Manfra, S. Pedatella "Supplements for Neurodegenerative Disease Prevention: Antioxidant Glycoconjugates" Poster IASOC 2018;

M. De Nisco, I. Vespoli, F. Cermola, L. Serpico, R. Gaglione, S. Pacifico, D. Mastroianni, M. Manfra, S. Pedatella "Towards New β-Sheet Breakers: Design, Synthesis and Evaluation of Short Peptides" Poster IASOC 2018

Schools

IASOC Ischia Advanced Organic School 2018, 22-25 September, Naples

Meetings

ICNTC-2020 6th International Conference on New Trends in Chemistry OCTOBER 16-18, 2020 (Kyrenia, North Cyprus)

"Workshop on NanoBioMedicine in Naples: the Next Future of Theranostics", 22nd March 2019, Naples.

ICCST-14 14th International Conference on the Chemistry of Selenium and Tellurium ICCST-14, 2019 3rd-7th June, Santa Margherita di Pula (CA), Sardinia, Italy

INTRODUCTION

1. INTRODUCTION

1.1. Oxidative stress

Oxidative stress (OS) is a condition due to an imbalance between oxidants and antioxidants production. It occurs either when the former are prevalent, or the latter are reduced. If this alteration is prolonged in time, the physiological equilibrium between oxidation and reduction is disrupted, leading to an oxidative damage in the involved tissues (Figure 1).[1]

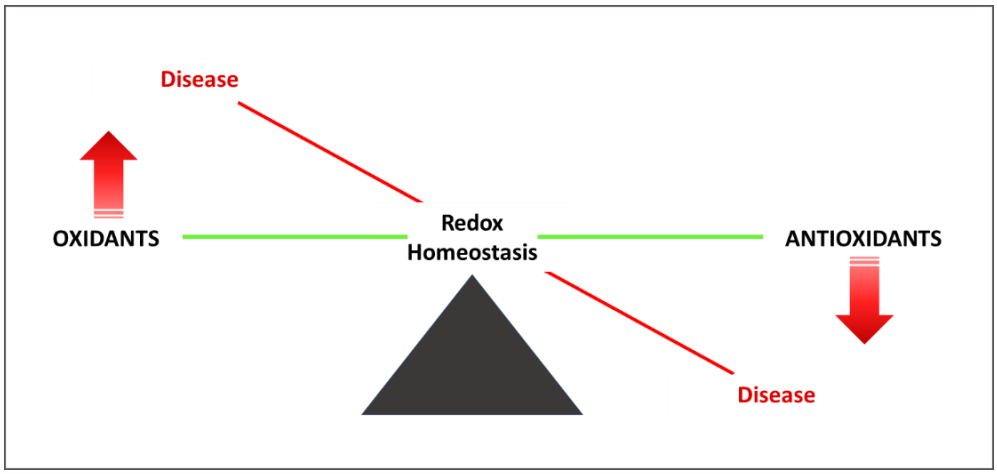


Figure 1. Redox equilibrium

The concept of OS was introduced for the first time in 1985 by Sies, who defined OS as "a disturbance in the prooxidant-antioxidant balance in favour of the former".[21] Since then, the study of reactive oxygen species has attracted attention in several fields of research, including chemistry, medicine and biology. The raising interest of researchers towards this topic also gave impulse to the foundation of two new journals in that same year: *Free Radical in Biology and Medicine* and *Free Radical Research*. Over the years the concept has been revised and expanded. Indeed, in 2007, Sies himself updated the definition of OS, describing it as "an imbalance between oxidants

and antioxidants in favour of the oxidants, leading to a disruption of redox signalling and control and/or molecular damage".[22]

The elucidation of several cellular and molecular mechanisms have contributed to the current understanding of this phenomenon. Among them, the comprehension of the basic chemical process of oxidation-reduction, (involving electron transfer, free radicals' formation, and oxygen metabolites) as well as the biological concept of stress, has been crucial. The latter was first presented by Selye in 1936,[23] and was later applied by himself to the study of complex organisms, leading to the introducing, in 1975, of the concepts of ***oxidative eustress***, and ***oxidative distress***.

Oxidative eustress represents a state in which an adaptive response to transitory stressful insults is carried out. The stimulus eliciting this kind of response does not necessarily come from dangerous sources, like a pathogen. Indeed, it can also be ascribed to an "intense stress" deriving from normal activities such as physical exercise or hormone synthesis stimulation (e.g. cortisol production). Physical activity as a stressor has been studied in mice by Wisløff.[24] Based on these studies, he concluded that mice that do not perform exercise develop mitochondrial dysfunction and cardio vascular diseases (CVDs); conversely, mice alternating periods of exercise and rest improve their mitochondrial activity and maintain a state of oxidative eustress.[25,26]

In eukaryote organisms, stressors induce an adaptive, defensive response mainly modulating gene expression (through epigenetic modifications[a]). Indeed, several studies demonstrated that low levels of oxidative stress correspond to an increased expression of antioxidants, proteins, and enzymes, leading to beneficial health effects.[27,28,29] This epigenetic modulation leads to the activation of defence systems

[a] Epigenetics has been defined and today is generally accepted as ''the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence".[323]

represented by transcriptional factors like nuclear factor kappa-light-chain-enhancer of activated B cells NF-kB/I-kB,[30] that plays a key role in cell's response to environmental changes and stress; or nuclear factor erythroid 2–related factor 2 (Nrf-2), that mediates the Heme Oxygenase-1 (HO-1) upregulation in low stress conditions[27], just to cite a few.

The main function of these systems is to maintain a dynamic equilibrium, referred to as "redox homeostasis", in which the level of oxidants is balanced by the level of reductants. In these conditions, oxidants are only able to transform specific targets, that are particularly prone to oxidation and are physically close to the oxidant.[31]

Oxidative eustress is considered a "positive stress", so much so, that, in 2013, Aschbacher defined it as "manageable levels of life stress that may enhance psychobiological resilience to oxidative damage".[32] Aschbacher and her group came to this conclusion by studying the adaptive response to an increased cortisol secretion induced by stressors. They defined the U-shaped relationship between the hormone and stress response in properly stimulated mice, demonstrating that a moderate stress can enhance resilience, helping the organism to improve response to repeatedly faced stressors (Figure 2).[33]

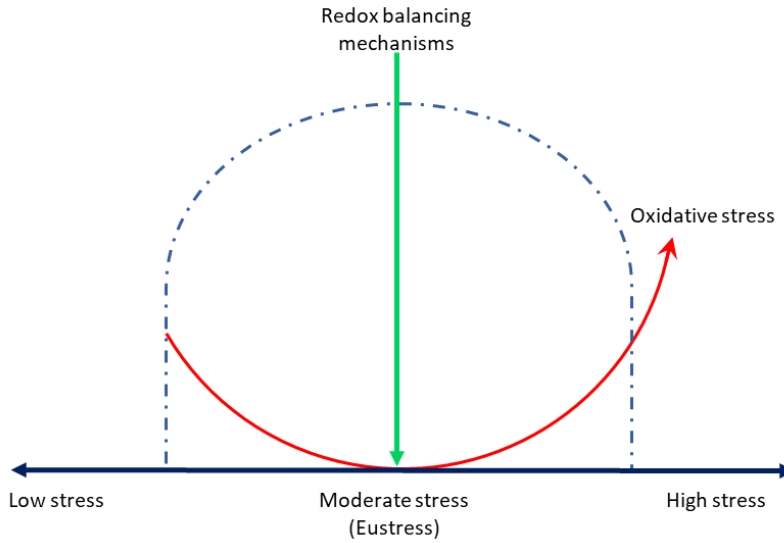


Figure 2. Moderate stress may enhance resilience.
Adapted from Aschbacher[33]

As clear from the above, in non-pathological conditions there is a tightly regulated equilibrium: when oxidant compounds are produced as result of fundamental oxidative processes, reducing systems are activated to synthetize antioxidants that neutralize the former, assuring a dynamic equilibrium called *redox homeostasis*.

Thanks to this complex balancing system, the necessary cellular activities which require oxidant species - drugs metabolism, 5'-adenosine triphosphate (ATP) production, oxidative phosphorylation, inflammatory response - are carried out without injury for cells.[34]

Conversely, *oxidative distress* is a toxic situation, characterized by the accumulation of reactive species (e.g. reactive oxygen species, ROS), due to either an overproduction of oxidants or to a low level of antioxidants.[35] In fact, a high exposure to dangerous stimuli exerts in a non-specific targeting by oxidant species and could

result in a widespread tissue damage; in this process several cellular death programs such as autophagy[b] and apoptosis[c] are activated (Figure 3).

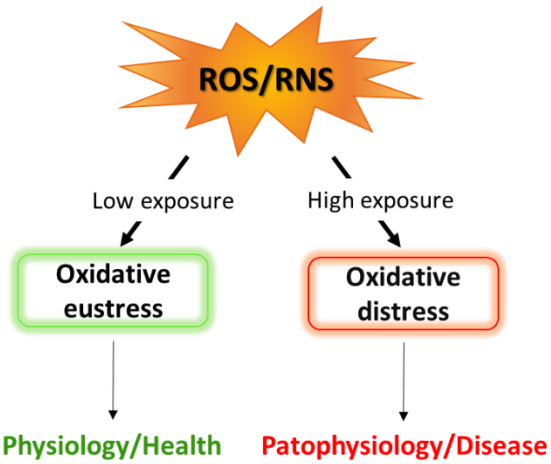


Figure 3. Oxidative eustress and distress.
Adapted from Sies et al. 2020[22]

In the last decades, intense research has been carried out in order to set a dividing line between good and negative stress. The transition of a eustress condition into a distress one is strictly correlated to the concept of hormesis. Hormesis is defined as 'a process in which exposure to a low dose of a chemical agent or environmental factor, that is damaging at higher doses, induces an adaptive beneficial effect on the cell or organism', so it is characterized by a biphasic response to increasing amounts of a stressor.[36] Generally, at low doses of any stressor corresponds a beneficial biological response (hormetic zone), although at higher levels cell damage occurs (Figure 4).

[b]Autophagy is a catabolic process that lead to the degradation of intracellular damaged organelles and molecules, by using lysosome; it is activated under several stress stimuli.[324]
[c] Apoptosis is a mechanism for programed cell death, it is used by cells to remove any unnecessary or unwanted cells and is a highly regulated process; it is involved in pathological phenomena like chronic inflammation and cancer.[325]

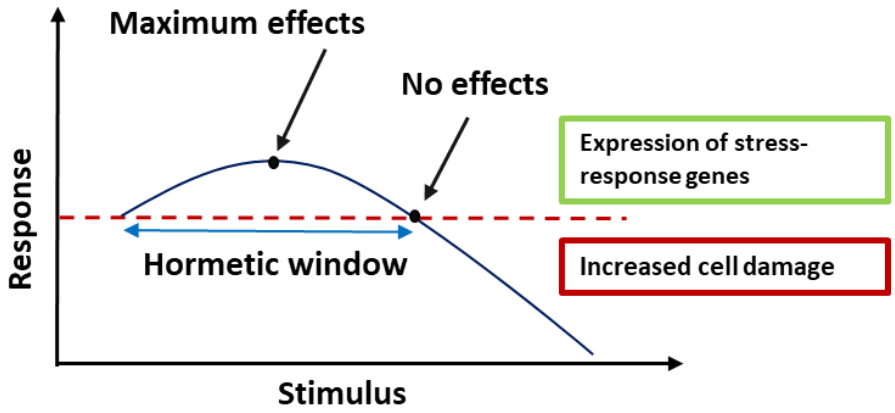


Figure 4. The biphasic hormetic response to stressors.
Adapted from Leri et al 2020[37]

In conclusion, a physiological redox homeostasis is associated with transitory processes and is related to a basal level of stress response, while an over activation of defence systems is linked to pathophysiological consequences such as inflammation and ultimately cell death.

The transition from a homeostatic response to an altered state can be also analysed as evolution from molecular to clinical manifestations: at the beginning molecular events start because of an oxidative stimulus; these molecular events increase radical species formation. This leads to inflammation and immune responses, and to an up-regulation of pro-inflammatory cytokines synthesis. Over time these events lead to an extensive damage that results first in an acute phase, then in a chronic condition, leading to the development of several diseases (Figure 5).[38]

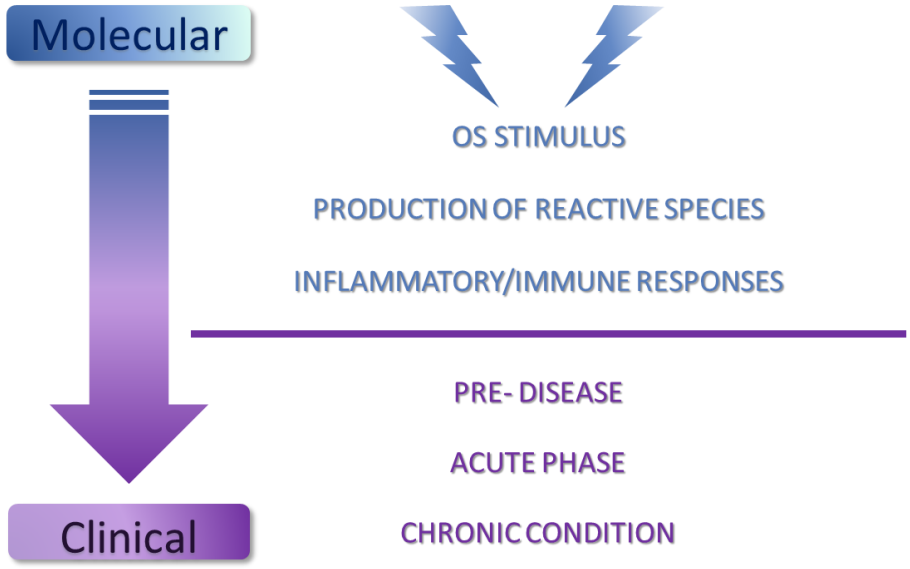


Figure 5. Oxidative stress evolution.
Adapted from Armstrong et al., 2016[38]

The molecular events leading to the formation of reactive species, both in normal and altered conditions, mainly take place in a specific cellular compartment: the mitochondria, multifunctional organelles surrounded by a double-membrane.[39]

Mitochondria have an important role in a series of biological pathways like amino acid synthesis, lipid metabolism, energy production, free radical generation, apoptosis, signalling processes and thermogenesis.[39] They own a specific genome, the mitochondrial DNA (mtDNA), which consist of a circular molecule of DNA. mtDNA encodes 13 proteins that are part of the respiratory chain, as well as the rRNAs and tRNAs necessary for local protein synthesis.[40] The main function of these organelles is to produce energy for the cell, in form of high amounts of 5'-adenosine triphosphate; this occurs through the electron transport chain (ETC). Moreover, they are the sites where the tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS) take place, and, because of these activities mitochondria produce reactive oxygen species (ROS).[41,42] Mitochondrial dysfunctions (MDs) are associated with

several and different disturbs[43] such as aging,[44,45] neurologic,[46] dermatologic,[47] metabolic disorders like diabetes, CVDs etc.[24] Recently, it has been demonstrated that mitochondria-derived ROS directly promotes activation of innate immune responses,[48] leading to an inflammatory condition and to an up-regulation of pro-inflammatory cytokines synthesis.[49]

Despite their biological relevance, ROS are not the only reactive species involved in the metabolic reactions taking place in our body. The main reactive species present in biological systems are discussed in the next section.

1.1.1. Oxidants

In biological systems, several metabolic processes entail redox reactions and thus, involve the participation of oxidant molecules. These molecules are also known as reactive species and, depending on the reactive atom, they are mainly classified in:

- reactive oxygen species (**ROS**) like H_2O_2, HOCl;
- reactive nitrogen species (**RNS**)[50], such as nitric oxide, nitrogen dioxide peroxynitrite, and nitrite/nitrate;
- reactive sulphur species (**RSS**)[51,52] like cysteine, methionine, and small compounds like glutathione[53].
- reactive carbonyl species (**RCS**)[54] which include metabolically generated aldehydes and electronically excited (triplet) carbonyls;
- reactive selenium species (**RSeS**)[55] including low-molecular-mass compounds as well as selenocysteine and selenomethionine residues in proteins.

Reactive species can be also classified according to their reactivity in: Free Radical and Non-Radicals (Table 1).

Table 1. Classification of reactive species.

Free radicals	Non-radicals
ROS	
Superoxide anion radical ($O_2^{\cdot -}$)	Hydrogen peroxide (H_2O_2)
Hydroxyl radical (OH·)	Organic hydroperoxide (ROOH)
Peroxyl radical (ROO·)	Singlet molecular oxygen ($^1O_2$)
Alkoxyl radical (RO·)	Electronically excited carbonyls (RCO)
	Ozone (O_3)
RNS	
Nitrogen monoxide	Nitrite (NO_2^-)
Nitrogen dioxide	Nitroxyl anion (NO^-)
	Peroxynitrite ($ONOO^-$)
	Peroxynitrate (O_2NOO^-)
RSeS	
	Selenite
	Selenate
	Selenocysteine
	Selenomethionine
Reactive chlorine/bromine species	
Atomic chlorine (Cl·)	Hypochlorite (OCl^-)
Atomic bromine (Br·)	Chloramines (RNHCl)
	Hypobromite (OBr^-)

In human body, reactive species that are mainly involved into cellular metabolism and oxidative processes are the ROS, the oxygen-centred oxidants.

Oxygen is a non-metallic element, present in the atmosphere as $^3O_2$, its ground state, and it is reduced to water through a multistep process.[56] Molecular oxygen owns two unpaired electrons with parallel spins in its two separate anti-bonding orbitals, so it can accept a pair of electrons from an electron donor.[57] Molecular oxygen is, indeed, the ultimate electron acceptor in the electron flow system.[57,58]Oxygen is therefore a highly reactive element that can form oxides with both other elements and other compounds. In particular, molecular oxygen undergoes four-electron reduction to water (reaction (1)).

$$O_2 + e^- \rightarrow O_2^{\bullet -} + e^-(+2H^+) \rightarrow H_2O_2 + e^- \rightarrow \cdot OH + (HO^-) + e^-(+2H^+) \rightarrow 2H_2O \quad \textbf{(1)}$$

In biological systems, oxygen participates in redox reactions that are essential for aerobic metabolism wherein cells mainly use oxygen to provide energy in the form of ATP (adenosine triphosphate).[59] All these processes generate intermediates that are very reactive and are known as ROS.[56]

1.1.1.1. ROS

It is noteworthy that the word ROS refers to a class of molecules that have different chemical, biological and functional characteristics, but have in common the oxygen as reactive atom.[60,61] ROS are produced in various reactions involved in the normal cell metabolism. Part of them derives from the multistep reduction of molecular oxygen (reaction (1)).[62,63]

This reaction normally occurs in the mitochondrial respiratory chain, and it causes the production of both H_2O and a relatively small amount of ROS.

Besides being produced during cellular metabolism in mitochondria,[42] ROS can also originate from drugs metabolism, physical exercise or during immune and inflammatory responses.[49] Moreover, their synthesis can be induced by external factors such as tobacco smoke, certain pollutants, organic solvents, UV and ionizing radiations.[1]

In addition, ROS can also be produced in several other oxidative reactions occurring in the cell, that are catalysed by different enzyme families such as the NADPH oxidases, NOS (nitric oxide synthase) and the cytochrome P450.

Recent studies have shown that ROS, when kept at physiological concentrations, are essential for governing life processes. Indeed, ROS are key molecules in cell signalling,[64] gene expression, maintenance of redox homeostasis and signal transduction pathways involved in all cell functions, in its growth, differentiation and death.[65–67]

The human body is thus constantly exposed to ROS and it also needs these molecules in several processes, but, as for all reactive species, the excessive accumulation of ROS is harmful and can ultimately cause cell death.[68] Indeed, ROS can damage several biomolecules including lipids, carbohydrates, nucleic acids, and proteins.

As mentioned above, ROS can be classified according to their reactivity in Free Radical ROS and Non-Radical ROS (Table 1). For example, hydroxyl (HO·) and

alkoxyl (RO·) free radicals are very reactive, while $O_2^{\cdot -}$ and lipid hydroperoxides are less reactive.[69] Among non-radical ROS, hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl) are the most reactive. The entity of damage induced by ROS may be influenced by their reactivity.

1.1.1.2. Free radical ROS

Free radicals are atoms, molecules or ions with unpaired electrons in the outermost orbital, characteristic that makes them extremely unstable and chemically active. The reactions in which free radicals take part are generally characterised by different steps and follow a chain-reacting behaviour: an initiation step, a propagation step, and a termination step. Free radicals can react with other molecules through different mechanisms like electron donation, electron acceptance, reduction or oxidation of radicals, hydrogen abstraction, self-annihilation reactions, addition reactions and disproportionation.[70] The main free radical ROS present in the human body are illustrated below.

Superoxide Anion ($O_2^{\cdot -}$) is generated by the addition of one electron to the molecular oxygen in its ground state (1); it also derives from dissociation at pH 7 of hydroperoxyl radical (HO_2·). Superoxide anion, usually the "primary" ROS, can further interact with organic substrates to generate "secondary" ROS, both directly or via enzyme- or metal-catalysed reactions. Indeed, in biological systems, its toxicity is mostly indirect, and occurs through the generation of highly reactive secondary species. For instance, in the Haber-Weiss reaction (catalysed by iron), it reacts with H_2O_2 to afford ·OH radicals (shown below).[71] Furthermore, $O_2^{\bullet -}$ can also react with NO· to generate the highly-reactive peroxynitrite ONOO-, a potent compound that targets DNA, proteins and lipids.[72]

Hydroxyl radical (·OH) is the neutral form of the hydroxide anion (OH^-). This radical is very reactive and it is easily transformed in hydroxyl groups. It has indeed

a short half-life, which has been estimated at about 10^{-9} s.[73] It reacts very quickly, in the site where it is produced, with almost every type of molecule present within the cellular environment: sugars, amino acids, phospholipids, DNA, and organic acids.

In the biological environment ·OH can be generated by different reactions including:

- Fenton reaction: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {\cdot}OH + HO^-$
- Haber-Weiss reaction: $O_2^{\bullet-} + H_2O_2 \rightarrow {\cdot}OH + O_2 + HO^-$

Hydroxyl radical can be also generated by an activation of the immune response: microglia cells and macrophages generate ·OH when are exposed to specific pathogens and certain bacteria. In addition, it might be formed in vivo on high-energy irradiation by haemolytic cleavage of body water.

·OH mainly reacts in three different ways: electron abstraction, hydrogen abstraction and double bond addition; it is an electrophilic species, thus, it has a strong affinity for electron-rich sites of molecules, especially for aromatic or sulphur-containing molecules. For instance, ·OH is able to oxidise different biomolecules through addition; the conversion of guanine into 8-oxoguanine is an example of one such reaction.[74]

Peroxyl (ROO·) Radicals. ROO· are a group of radicals with an important role in biological systems. Among them, the simplest is HOO·, known as hydroperoxyl radical or perhydroxyl radical. It is the protonated form of anion superoxide ($O_2{\cdot}^-$) and it is known for its role as initiator of lipid peroxidation (LPO) which starts with the abstraction of an H atom from polyunsaturated fatty acids (PUFA).[75] Additional molecules formed in lipid peroxidation are alkoxyl radicals (RO·) and organic hydroperoxides (ROOH).

1.1.1.3. Non-radical ROS

Non-radical ROS are molecules that do not contain unpaired electrons and are included in two-electron oxidants; they generally result more stable and with a longer half-life than free radicals.

Singlet oxygen ($^1O_2$) is the common name used for the diamagnetic form of molecular oxygen (O_2), which is less stable than the normal triplet oxygen. It is a highly reactive molecule, and its generation by photoexcitation is responsible for the skin damage induced by ultraviolet irradiation, as well as for the cytotoxic anti-cancer effect of photodynamic therapy. Despite its prominent roles, the biological effects of $^1O_2$ are not fully understood.[76] $^1O_2$ can react with multiple cellular components; its preference for reacting with conjugated double bonds is very high, and hence it preferentially targets polyunsaturated fatty acids (PUFA) or guanine in cells' DNA .[77]

Hydrogen peroxide (H_2O_2) is the simplest peroxide containing an oxygen–oxygen single bond and it is formed by the two-electron reduction of O_2. It is unstable and slowly decomposes in the presence of light.[78] It is the reactive species most frequently involved in biological processes.[79]

In physiological conditions, H_2O_2 acts as a signalling messenger, and it is maintained in specific intracellular compartments, at a concentration in the order of the nanomolar, thanks to dedicated control systems.[80] Its production is stimulated by stressors such as growth factors and chemokines, while it is removed by antioxidant systems like superoxide dismutase (SOD). In physiological concentration, H_2O_2 exerts its functions by oxidizing –SH groups in specific target proteins. However, when its concentration exceed the critic value of 100 nM, H_2O_2 starts oxidising different biological targets such as DNA, thus triggering a series of collateral pathways that lead to the development of an oxidative distress condition.[67]

Moreover, H_2O_2 can act like a primary ROS generating secondary oxidants as ·OH via either the Fenton or the Haber–Weiss reaction.[81] The damage is thus amplified by the oxidized molecules, that act as secondary oxidant signals.

Given the participation of H_2O_2 in a wide variety of metabolic reactions, a shift towards an over production of this species leads to unspecific oxidation of molecular targets and altered response patterns that, in turn, cause oxidative damage and ultimately cell death, with associated pathological states.[82]

Hypochlorous acid (HOCl) Hypochlorous acid is a nonspecific chlorinating and oxidizing compound, it owns a two-electron reduction potentials of 1.28 V. It is able to target several molecules, comprising amino acids, proteins, carbohydrates, lipids, and DNA.[83] In human body HOCl is produced by the reaction of hydrogen peroxide with chloride anion (Figure 6).

Figure 6. MPO reaction

This reaction is catalysed by myeloperoxidase (MPO), an enzyme involved in the immune response and primarily localized in the azurophilic granules of leukocytes, including neutrophils, macrophages/monocytes,[84] and microglia cells in brain.[85] It has been estimated that the local concentration of HOCl in the inflammation loci can reach even several millimoles per liter.[86] HOCl has a pK_a of 7.5, thus, at physiological pH (7.4), it is in equilibrium with its conjugate base and it is present as both forms, HOCl and OCl^- (with an approximate ratio of 1:1).[87]

HOCl plays an important role as an antibacterial agent during the immune response,[88] and as a highly and rapidly effective antimicrobial agent, has been used as a universal disinfectant in clinical practice for more than 100 years.[89] Nevertheless, its overproduction leads to the oxidation/chlorination of several targets[90] in involved tissues, and the consequent oxidative damage concurs to the pathogenesis of various

diseases, including Alzheimer disease (AD), cardiovascular diseases (CVDs), atherosclerosis,[91] ischemia-reperfusion injury in stroke,[92] inflammatory diseases,[83] myocardial infarction, and even cancer.[93] HOCl can react with primary amines and other N-compounds to afford chloramines and N-Cl derivatives.[94] The rates of the reaction of HOCl with some important biomolecules have been studied. The highest rate constants (of the order of 10^7–10^8 $M^{-1}s^{-1}$ at pH 7.4) have been determined for methionine, glutathione, and cysteine.[95]

1.1.1.4. RNS

Nitric oxide (NO·) is a bioactive, potentially toxic, relatively unstable free radical gas. Due to its highly lipophilic nature, NO· can diffuse through cell membranes without the aid of specific membrane transporters. It is an important cellular messenger molecule involved in many physiological and pathological processes.

NO is formed by NO synthase (NOS) enzymes, starting from arginine and has a relaxing effect on the smooth muscles in blood-vessel walls. It is also produced by activated macrophages, contributing to the primary immune defence. An excess of NO· is cytotoxic and is found in a series of disorders comprising cancer and neurodegenerative diseases.[96] It might react directly with biomolecules or combine with $O_2^{\cdot -}$ to form peroxynitrite ($ONOO^-$).[97]

Peroxynitrite (ONOO–) Peroxynitrite is an oxidant and nitrating agent. It can induce lipid peroxidation and also interfere with cellular signalling by nitrating tyrosine residues in proteins. Because of its oxidizing properties, $ONOO^-$ can damage a wide array of molecules in cells, including proteins and DNA; it can also oxidise electron-rich groups like sulfhydryls, zinc-thiolates, iron–sulphur centres and the active site sulfhydryl in tyrosine phosphatases.[98]

1.1.2. Antioxidants

According to Halliwell, "antioxidant is any substance that delay, prevents, or remove oxidative damage to a target molecule".[99]

As stated before, oxidants are by-products of several and fundamental cellular processes. They are physiologically required, but they become harmful for cells when they are over accumulated.[82] The human body owns different balancing/defence systems - that consist of antioxidant species- to preserve a redox equilibrium both in physiological conditions and in oxidative eustress situations.[100]

In physiological conditions, basal levels of antioxidants are produced to guarantee redox homeostasis.

When an OS stimulus occurs, the synthesis of antioxidants is increased; if ROS levels exceed the scavenging capacity of these systems, a series of repair pathways are initiated to counteract the oxidative damage. [101,102]

Since oxidants are produced in a wide array of aerobic cellular pathways, redox homeostasis is ensured by complex antioxidant defence systems, of both enzymatic and non-enzymatic nature.[2]

1.1.2.1. Enzymatic antioxidant

Enzymatic antioxidants include superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and glutathione reductase (GSR).

Superoxide dismutases (SODs) SODs are a group of metalloenzymes that catalyze the dismutation of superoxide anion into molecular oxygen and hydrogen peroxide decreasing $O_2^{\cdot -}$ levels (Figure 7).[103]

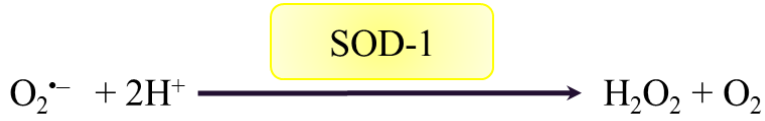


Figure 7. SOD's reaction.

This reaction is coupled with oxidation-reduction cycles of metal ions present in the active site of SODs. Furthermore, depending on the metal cofactors existent in the active sites, SODs can be classified into four distinct groups: Copper-Zinc-SOD (Cu, Zn-SOD), Iron SOD (Fe-SOD), Manganese SOD (Mn-SOD), and Nickel SOD.[104]

SODs are a very important constituent of human antioxidant system, different forms are placed in different subcellular compartments, their levels change during the lifetime and depending health conditions; for instance, in the old age their levels decrease, indeed, in the elderly the oxidative stress is much more frequent, and, with it, the related pathologies. Furthermore, some studies have found a strong association between the deficiency of SOD-1 and Alzheimer's disease, one of the most prevalent neurological diseases affecting the elderly in west countries.[105]

Glutathione peroxidases (GPxs) are a family of enzymes mainly represented in human body by GPx-1. Identified in 1957 by Mills, crystallographic studies demonstrated that GPx is a tetramer, with two asymmetric units containing two dimers. Each dimer owns two selenocysteine residues in its active sites.[106] The selenocysteine residue in the active site is crucial for its action, indeed, it has been demonstrated that selenium activity is fundamental for the antioxidant mechanism of GPxs.[13]

In humans, eight GPx isotypes have been identified with different cellular and tissue localizations, but the most abundant one is GPx-1, the cytosolic form, whose main action is to reduce H_2O_2 oxidising GSH at the same time.

that principally reduces coupling the reaction with oxidation. GPx-1 is thus crucial in preventing the harmful accumulation of intracellular hydrogen peroxide (Figure 8).

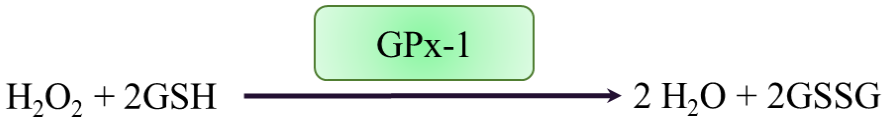


Figure 8. GPx reaction.

Special attention has been paid to understand and mimic the activity of the GPx; nowadays an entire class of synthetic GPx-like selenium containing compounds exists. The first compound of this class is the ebselen,[107] a small molecule approved by the FDA for its use as antioxidant drug.[108]

Glutathione reductase (GR) is another GSH related enzyme, which reduces oxidized glutathione, namely glutathione disulphide (GSSG) to its reduced form GSH. GR uses NADPH as reducing equivalent to carry out the reduction of GSSG (Figure 9). In addition, GR can reduce peroxide radicals to alcohols and oxygen.[109]

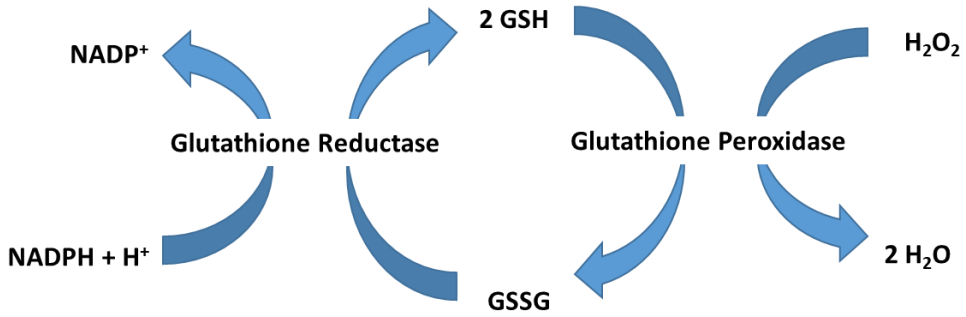


Figure 9. GR's mechanism of action

Catalase (Cat) is a highly effective enzyme that scavenges hydrogen peroxide. It is primarily localized in peroxisomes; it is a tetrameric enzyme containing four polypeptide chains, each with one porphyrin heme group which allow the enzyme to react with the hydrogen.[110] CAT uses either iron or manganese as a cofactor and splits H_2O_2 into H_2O and O_2 ensuring the redox homeostasis (Figure 10).

Figure 10. CAT reaction

1.1.2.2. Non enzymatic antioxidants

Non-enzymatic antioxidants comprise both synthetic antioxidants and dietary supplements; these may include vitamin C, vitamin E, β-carotene, selenium, zinc, taurine, glutathione and so on. In addition, there are also endogenous non-enzymatic antioxidants like albumin, ferritin, lactoferrin, myoglobin, etc.[111]

The non-enzymatic antioxidants include endogenous and exogenous (dietary) molecules that, with the other antioxidant species (enzymatic ones), guarantee the redox homeostasis in the human body. Non enzymatic compounds include proteins - like ceruloplasmin, ferritin, transferrin and albumin - and low molecular mass molecules, such as glutathione (GSH) and polyamines (PAs). The endogenous antioxidants can be further divided, according with the mechanism, into true scavengers and metal buffering proteins - chelators for redox- active metals (Fe, Mn, Cu) and chelators of redox-stable metals (Zn, Cd). Dietary antioxidants such as vitamin E, vitamin C, carotenoids, some minerals (e.g. ZnMn, Cu, Se) and polyphenols constitute the exogenous type of non-enzymatic of antioxidants; they are contained in a wide array of foods and significantly contribute to the function of human antioxidant defence system. [112]

In the following paragraphs we will address endogenous antioxidant systems only; exogenous systems will be treated separately.

Transferrin (TF) is a blood plasma protein that transports iron from the intestine to the bone marrow, where iron is required to red blood cells production. Binding ferrous ions, TF contributes to reduce the Fenton reaction, in which Fe^{2+} transforms H_2O_2 in

the more toxic ·OH, thus TF is considered an important antioxidant.[113] Moreover, free irons ions represent a risk factor in neurodegenerative disorders, including Parkinson's and Alzhiemr's diseases.[114] TF is synthetized in several tissues, including brain, where it contributes to correct iron's metabolism, limiting oxidative stress damage.

Albumin (ALB) is considered the principal extracellular antioxidant thanks to its capacity to bind ferrous and cupric ions.[115] ALB contains sulphur amino acids, methionine and cysteine, in particular it is the free sulfhydryl group of cysteine that allows ALB to scavenge hydroxyl radicals.[116]

Glutathione (GSH) is a small molecule, constituted only by three amino acids: glycine, cysteine and glutamic acid. It can be found in several redox forms, whose most abundant are reduced GSH and oxidized glutathione (GSSG). In physiological conditions GSH is present with a ratio of 100:1 respect GSSG.[117] Despite its multiple biological functions, GSH is also a potent antioxidant because reduces ROS during the enzymatic and non-enzymatic reactions and it is able to regenerate other oxidized antioxidants, vitamin C and vitamin E for example.[112,118]

Coenzyme (CoQ10) is a 1,4-benzoquinone derivative owning several biochemical functions. It is involved in the transport of electrons in the mitochondrial respiratory chain and in the electron transport outside mitochondria.[119] Furthermore, CoQ10 participates in the redox reactions of dehydrogenases, cytochromes or other non-heme proteins.[120] In particular, reduced forms of CoQ10 - ubiquinol ($CoQ_{10}H_2$), and ubisemiquinone radical ($CoQ_{10}H$) - exert these functions. Ubiquinol binds hydrogen to free radicals, transforming ubiquinol in $CoQ_{10}H$. This radical, in turn, can react with molecular oxygen and other free radicals, exerting an antioxidant action too.[121] Ubiquinol may also reduce oxidized α-tocopherol. The reduced form of tocopherol shows strong antioxidant properties.

Dietary antioxidants are mostly assumed with diet, indeed they are contained in fruits, vegetables, beverages (juices, tea, coffee), nuts and cereal products.[122] In the last

decades, exogenous antioxidants attracted the scientific interest thanks to their ability to affect the antioxidant defence system contributing to increase the antioxidant response: they positively contribute in the counteract of aging[123] and play a role in the contrast of diabetes'[124] and cardiovascular diseases' complications.[125]

Selenium and polyphenols will be treated in dedicated sections in this thesis.

1.1.3. OS in human diseases

It is now well established that OS is responsible for the initiation or development of many pathological processes, and may well become the salient feature to understand the pathogenesis of most of them[2]. Indeed, oxidative stress has been associated with age related disorders,[3,4] as well as CVDs,[5,6] reproductive disorders, metabolic disorders[7] like diabetes,[8,9] chronic kidney disease,[126] chronic obstructive pulmonary diseases,[127] cancer,[10] NDs[11] and many others, including immune-mediated inflammatory disorders.

1.1.3.1. OS in cardiovascular diseases

CVDs are the first cause of death in the western world. Common independent risk factors for these diseases include genetic susceptibility, previous pathological conditions - such as diabetes, dyslipidemia,[128] hypertension - incorrect lifestyle, unbalanced diet, stress, air pollution, and sedentary life.[129] Of note, several studies have shown that subjects with CVDs display alterations in cell metabolism, immune and inflammatory response, and point to a role for OS in the development of CVDs. Indeed, ROS are involved in atherosclerotic plaques formation, and the role of oxidative stress has been demonstrated in atherosclerosis, ischemia, hypertension, cardiomyopathy, cardiac hypertrophy, and congestive heart failure.[130] OS is thus considered an independent risk factor for *cardiovascular diseases.*

1.1.3.2. OS in reproduction

OS is associated with the development of pathological conditions affecting both female and male reproductive apparatus.[131] Indeed, although ROS, at low concentration, are necessary for the correct development of female sexual organs and feto-placental growth, in higher concentrations, they can significantly affect the reproductive process, in all its steps.[132] In addition, it has been demonstrated that OS has a crucial role in endometriosis and ovarian cancer.[133] Moreover, in more than one study it has been assumed that ROS can increase the synthesis of such miRNA – actively involved in these pathologies - by ROS responsive transcription factors.[134]

Male germ cells are also particularly susceptible to ROS damage. This is mainly due to their unique architecture and the high content of oxidizable substrates versus limited intracellular antioxidant defences.[135] Thus, OS can lead to germ cell dysfunction and apoptosis, thus potentially causing male infertility. OS has also been causally linked to lesions in the motility profile of mature spermatozoa.[136]

1.1.3.3. OS in cystic fibrosis

Cystic fibrosis (CF) is the most common autosomal recessive disease among Caucasians. It is a lethal disorder and it is caused by mutations of a single gene, the gene encoding a channel-protein, called CF transmembrane conductance regulator (CFTR).[137] The defective function of CFTR protein causes an accumulation of thick mucus in the small airways with a consequent compromised elimination of bacteria that, in turn, leads to recurrent pulmonary infections and excessive neutrophilic activation.[138] Significant evidences indicate that systemic oxidative stress is a feature of Cystic fibrosis.[139,140] CF-related defects of the pulmonary epithelium cause a persistent activation of inflammatory pathways that in turn provokes an over accumulation of ROS in the lung, causing chronic inflammation syndrome.[141] In this environment, the activated neutrophils - recruited in the site of inflammation - synthesise high amounts of hypochlorous acid, which is toxic for lung epithelial

cells,[142] and contributes to damage to CF airways. Consequently, targeting oxidative stress and in particular HOCl in CF has therapeutic potential.

1.1.3.4. OS in cancer

Presently, cancer is the second most-frequent cause of death worldwide and its incidence is expected to increase in the coming years.[143]

The word cancer indicates a group of more than one hundred neoplastic disorders with specific characteristics depending on involved tissue, grade of malignancy etc. These features are named hallmarks of cancer and they are studied as collective characteristics of cancer cells and include sustaining proliferative signalling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis.[144] All these acquisitions have in common the genomic instability which leads to genetic diversity[145] both between different kind of tumours, and within the same tumour. Understanding the mechanisms of tumour development and progression is crucial for decoding the complex biology of cancer; their molecular investigation has led to the conclusion that oxidative stress plays a crucial role in almost all phases of neoplastic disorders. ROS are involved in the initiation, progression, angiogenesis as well as metastasis and the suppression of apoptotic cell death in different ways.[146] Neoplastic cells show an altered redox homeostasis, so much so that increased ROS levels and increased antioxidant ability constitute one of the many hallmarks of a cancer cell.[147] In particular, ROS contribute to generate an inflammatory condition in tumour microenvironment activating key molecules, such as NF-kB,[148] nuclear factor erythroid 2–related factor 2 (Nrf 2), and p53;[149] furthermore, ROS have a role in the key biological cancer processes, as illustrated below.

Tumour recurrence. Several studies have shown that high levels of ROS in neoplastic cells and corresponding stem cells are associated with this phenomenon. As mentioned above, ROS can promote the expression of Nrf 2, which is clearly

involved in tumour recurrence.[150] Thus, the modulation of oxidative stress response might represent a potential approach to eradicate cancer in combination with FDA-approved chemotherapies, radiotherapies as well as immunotherapies. Indeed, most therapeutic agents, already approved, exert their activity by increasing intracellular/extracellular ROS levels.

Epithelial-mesenchymal Transition (EMT). Different researchers have proved ROS to be a major cause of EMT; they act by different mechanisms based on epigenetic modifications, for instance activating the transcription of NF-kB.[148] EMT is a process normally occurring in embryological development, it is important during the embryogenesis for gastrulation;[d] in the adulthood it physiologically occurs in wound healing, while it is pathologically involved in cancer progression, generating mesenchymal cells with the ability to migrate.[151,152] The crucial event of this process is the loss of E-cadherin, a protein involved in the cell-cell adhesion. When cancer cells start this evolution, they loss an epithelial-like aspect, to acquire a mesenchymal feature and properties, and evolve towards a less differentiated state. This favours cancer cells migration and formation of metastasis (Figure 11).

[d] Gastrulation is a crucial process of animal embryogenesis. It is characterized by cellular rearrangements and migration which lead to the formation of the three germ layers: ectoderm, mesoderm and endoderm.[326]

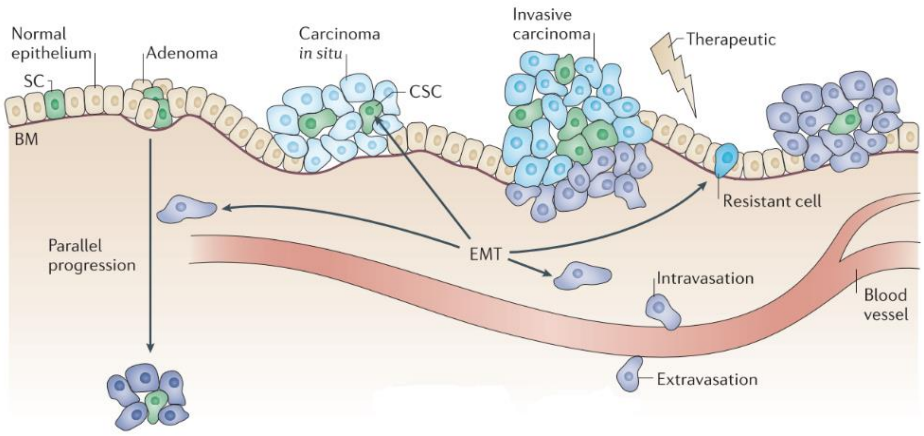


Figure 11. Epithelial mesenchymal transition
Adapted from De Craene and Berx[152]

Angiogenesis. ROS play a role in angiogenesis which consists in the formation of new blood vessels and it is necessary for tumour growth and survival in the first steps of its evolution.[153,154] OS - deriving from an increased cell metabolism - leads to an increase in ROS levels, causing the secretion of angiogenetic modulators, like growth factors, such as HiF-1α.[155] The latter, in turn, increases the production of vascular endothelial growth factor (VEGF), favouring tumour's migration and proliferation.

Considering the above, it is clear that ROS have a crucial role in every step of cancer development. However, ROS can paradoxically induce apoptotic cell death, a mechanism activated by several anti-cancer treatments to detain tumour growth.[156] In particular, ROS start the apoptotic cascade via different pathways.[157–159] Various studies have illustrated that numerous anticancer agents induce ROS-mediated neoplastic cells' apoptosis and autophagy.[b] For instance, it has been demonstrated that resveratrol, a polyphenol naturally present in red wine, can cause mitochondrial accumulation of H_2O_2, which in turn, induce apoptosis in several human cell lines.[160]

Taking into account all these evidences, it is clear that ROS concentration in tumour microenvironment is critical. Although it is true that high ROS levels induce cytotoxicity, it is also proved that they are responsible for chemotherapeutic resistance

in neoplastic cells. Indeed, several investigations suggest that the increase of ROS levels is a key occurrence for the efficacy of conventional chemotherapies inducing cell death.[161]

The evidence discussed so far highlights the importance of a balanced redox homeostasis. Hence, all the approaches aimed at tackling oxidative stress may contribute to the prevention of cancer development. In particular, agents able to modulate ROS levels may serve as adjuvant therapy to induce cell death, as well as, to limit the chemotherapy induced damages in non-neoplastic tissues.

1.1.3.5. OS in age-related diseases

Age-related diseases are one of the most relevant and significant health problems of our time. They are mostly linked to the increased life expectancy registered in the last decades. During the past years, indeed, the average life expectancy has increased steadily, reaching a global average of 73 years in 2019 (World Health Organization, 2020) (Figure 12).

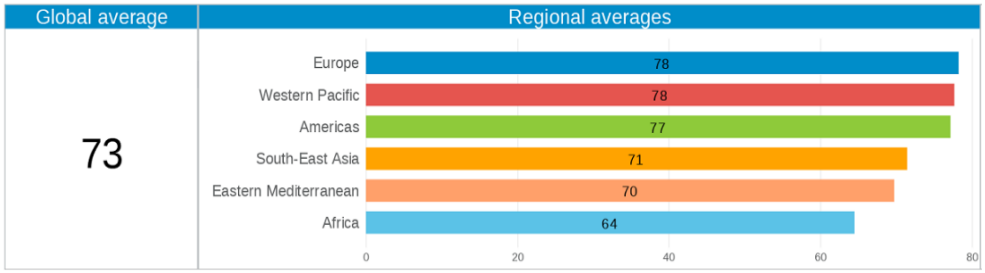


Figure 12. Global average life expectancy in years (from birth)
Source: WHO website

Although this data is a remarkable achievement, attained thanks to medical, social and human development, it also means a growth of the elderly population, and an increased prevalence of a series of chronic health problems such as physical frailty, psychological impairment, and cognitive decline.[162] All these conditions are consequent to a physiological and gradual process called aging.

Aging is defined as the progressive loss of tissue and organ function over time and increased vulnerability to death;[163] it is a multifactorial, universal and intrinsic phenomenon characterized as degenerative in nature; it can be identified by a series of hallmarks like genomic instability, nuclear and mitochondrial DNA damage, epigenetic modifications, stem cell exhaustion, cellular senescence (Figure 13).[164]

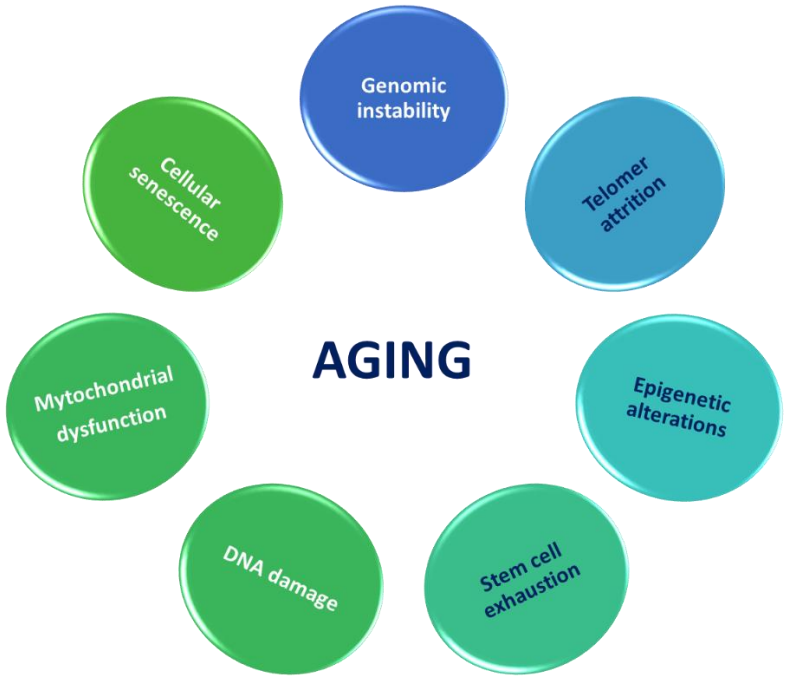


Figure 13. Most actors of aging

The theory that better explain this process is the oxidative stress theory of aging: it assumes that age-associated impairments are attributable to an accumulation of oxidised macromolecules (lipids, DNA, and proteins), this accumulation, in turn, is a consequence of the failure of several antioxidant defensive mechanisms.[165] At a cellular level this situation reflects in both structural and functional changes in mitochondria, leading to a redox disequilibrium and enhance vulnerability to oxidative stress.[44,45,166]

Thus, over time, the efficiency of antioxidant systems declines, making the elderly more susceptible to oxidative stress. Given the high demand of oxygen and relative limited respiration capacity of organs like the brain and the heart, it is not surprising that the prevalence of CVDs and NDs is substantially higher in the elderly.[167]

However, oxidative stress has been clearly associated with the pathogenesis of many other age-related diseases[168] including diabetes, vascular diseases, obesity, osteoporosis and metabolic syndromes (Figure 14).[167]

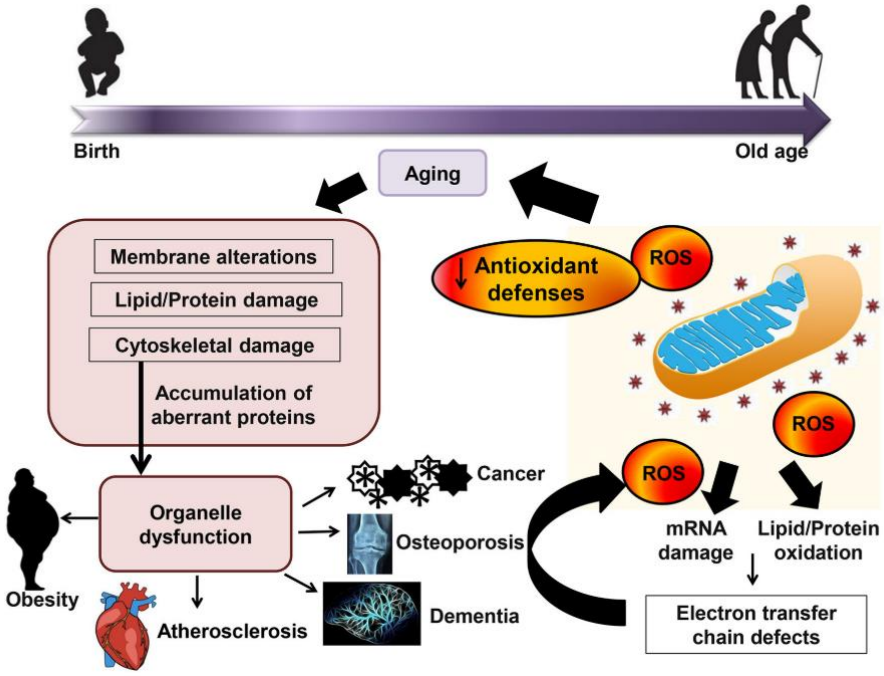


Figure 14. Oxidative stress in aging and age-related diseases
Tan et al., 2018[167]

1.1.3.6. OS in neurodegenerative diseases

Neurodegenerative Diseases, likely Parkinson's[169] and Alzheimer diseases, are tightly linked with the concept of aging. Neuroinflammation and OS are hallmark of neurodegeneration and clearly linked to this kind of disorders (Figure 15).[170] Among them, we will focus on the AD.

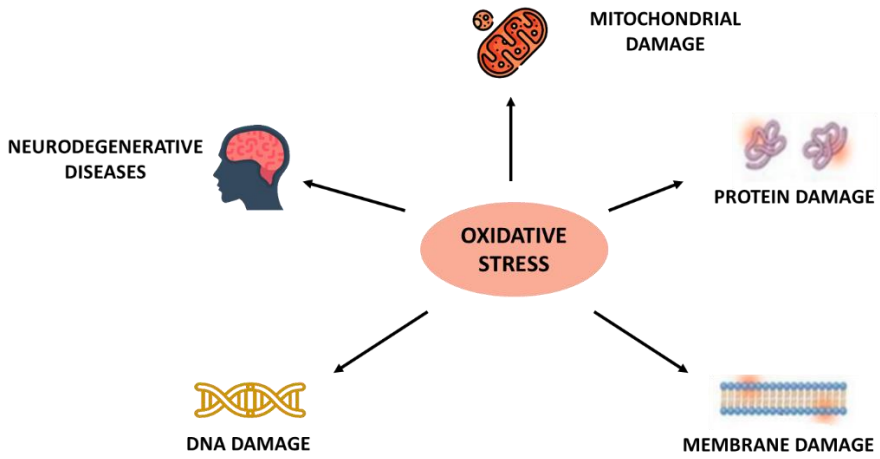


Figure 15. Interconnection between OS and NDs[171]
This picture has been prepared based on the image from Scuderi et al.[171] *and with resources from Flaticon.com*

AD is the most prevalent neurodegenerative disorder and is the first cause of dementia in the elderly; nowadays, there is no cure for AD: the treatments currently available are not able to reverse the progression of the disease; they can only temporarily attenuate the symptoms.

AD is characterized by the progressive and irreversible loss of neuronal populations that leads to loss of memory and cognitive capacities. The pathogenesis of AD is mostly related to an extracellular accumulation of amyloid beta (Aβ) plaques and the amassing of intracellular tau neurofibrillary tangles (NFT).[172,173] This disorder is mostly sporadic and both modifiable and non-modifiable risk factors (environmental exposure, genetic risk factors, mitochondrial haplotypes, age, and sex) play a crucial role in its onset.

Only about 1% of AD cases is *familial* and these patients carry mutations in the genes that encode amyloid-β protein precursor (AβPP), or proteins presenilin 1 (PS1) and presenilin 2 (PS2), which are directly involved in the AβPP processing. Regarding *sporadic* cases, it has been well documented that there is a link between mitochondrial dysfunction, which cause an increased production of ROS, and the development of AD. In neurodegenerative disorders, indeed, markers of OS have been found to be present already in asymptomatic stages.[174]

OS is involved in the development of AD through different mechanisms;[173] for instance, it has been demonstrated that the increased oxidative damage to lipids and proteins and the decline of antioxidant systems at the synapses level, correlates with the severity of the disease, suggesting that oxidative stress induce AD-related synaptic loss.[175] Taking all these considerations into account, it is clear that OS can be considered as a hallmark of AD disease, as well as a potential therapeutic target.

1.2. Selenium

Selenium (Se) is an element positioned in P Block, Period IV and Group 16 of the Mendeleev's periodic table. It was discovered in 1817 by Jacob Berzelius who gave it this name in honour of Selene, the Greek goddess of the moon. This attribution is probably due the characteristic silver colour that this element assumes when it cools after fusion.

Selenium is classified as a non-metal, but it possesses properties of both metal and non-metal. Due to its properties, it is also been classified as metalloid.[176] This bivalent nature of Se is due to its existence in different allotropic forms; indeed, it can be found as amorphous Se (brick-red dust or black vitreous shape), crystalline Se (grey hexagonal form) - the most stable form - and monoclinic selenium, the less stable one. Thanks to these characteristics, Se is utilized in several fields, from chemical and electrical industries to medicinal chemistry. Se is a commonly occurring element in

nature; it is present in the atmosphere as dimethyl selenide; it can be found in trace in water, and, in different amounts, in the soil.

The soil is the main source of Se, however the distribution of this element is variable and depends on geographic areas: some areas have selenium rich soils, while others are lacking it. Soils with a high selenium content are present in Canada, United States, Australia, Russia, and numerous regions of China,[177] while selenium-deficient areas include New Zealand and an extensive part of Europe.

Selenium can be found in the environment in four different oxidation states: elemental state (Se^0), selenides (Se^{2-}), selenates (SeO_4^{2-}), or selenites (SeO_3^{2-}).[178] The oxidation state of selenium can easily change, as it is influenced by several factors such as humidity, pH, concentration of free oxygen and redox potential. For instance, in anaerobic conditions and acidic environment Se is present in lower oxidation states, while, in aerobic circumstances and at alkaline pH, Se is found in higher oxidation states.[177]

Until the first decades of 20th century, Se did not attract the interest of the scientific community, maybe because of its bad odour, the instability of some of its derivatives and its similarity with sulphur (S). Nevertheless, since 1970 a growing attention on Se has been recorded and the number of related publications has shown a rapidly increasing trend. This change is associated to the discovery of several properties of this element over time. Indeed, although Se has been traditionally known for its toxicity, nowadays it is well established that it is involved in different and fundamental biological processes, and its deficiency is associated with several pathologies.

1.2.1. Selenium in human health

Se is a trace element, critical for the correct functioning of all organism and especially for human health. It is recognised as an essential micronutrient and it is typically assumed with diet. The main food sources are Brazilian nuts cereals, meat, dairy

products and fishes.[179] It can be also found in sea salt, eggs (only in case of feed supplemented with Se-yeast), yeast (yeasts containing selenium), bread, mushrooms, garlic, asparagus.[180] It is important to consider that the same food can contain a different amount of selenium depending on the geographic area it originates from. Moreover, different forms of selenium come from different sources: organic Se (MetSe, CysSeMet) is present in legumes, rice, garlic, onions, broccoli, Se-yeast, grains and legumes, vegetables and fish. Inorganic forms, selenite and selenate, are also observed in some grains, legumes and fish and are usually used in selenium supplementation (Figure 16).

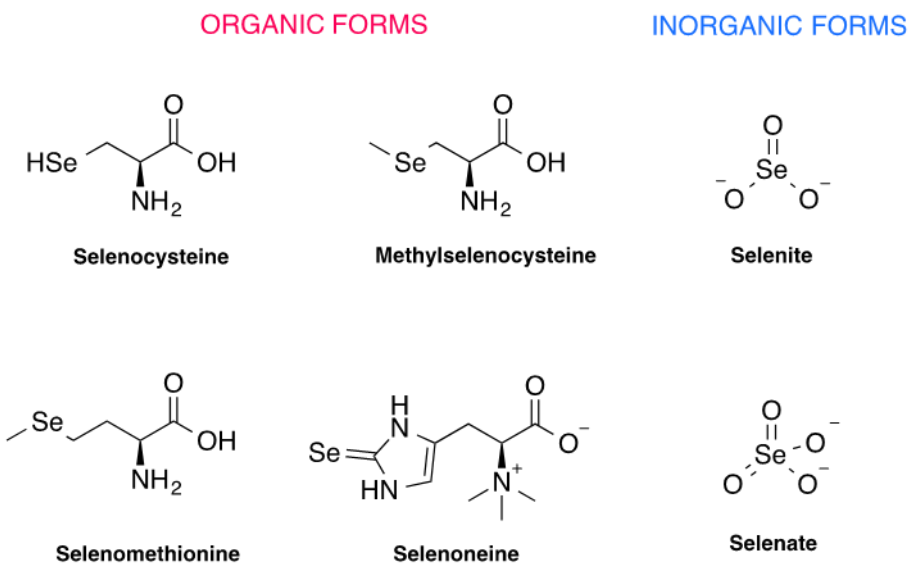


Figure 16. Forms of selenium

In 2004, the World Health Organization (WHO) established the recommended daily allowance (RDA) of Se in 50 µg for women and 70 µg for men. In the human body Se is present in trace, indeed, the total amount is approximatively between 3 and 20 mg/L, while serum levels are estimated at 60–120 ng/mL.[181] Maintaining the correct concentration of selenium is crucial for human wellbeing because this element possesses a series of biological functions (Figure 17).

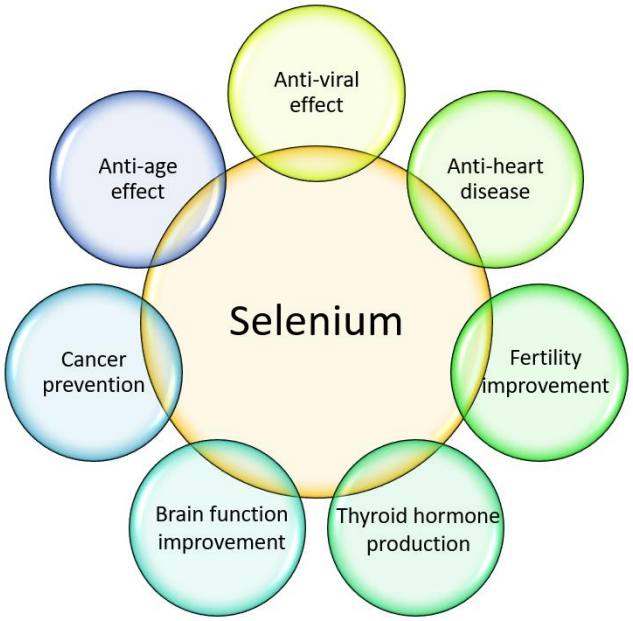


Figure 17. Main biological functions of selenium

The concentration is variable across different populations because of several factors like diet, geographic area, age: the maximum levels are recorded in adulthood, to decrease in people with more than 60 years. Both high and low levels of this element are harmful and are associated with pathological conditions; moreover, the difference between a beneficial dose and an unsafe dose is small,[182] this is why most selenium compounds have a narrow therapeutic window.

Nevertheless, Se-deficiency pathologies are far more common, and a prolonged deficiency of selenium leads to serious diseases, for instance, Kashin-Beck and Keshan Diseases in humans. The latter is a severe and lethal cardiomyopathy that broke out in 1935 in the Keshan region of China, where it became an endemic disease. Several years later, it was discovered that this disorder is linked to decreased levels of Glutathione Peroxidase, that in turn is due to a low level of selenium (Figure 18). This explained why the disease was endemic in those areas where the soil was poor in selenium.[183] In addition, selenium supplementation with sodium selenite was effective

in preventing Keshan disease and in mitigating the clinical manifestations in affected patients.[184]

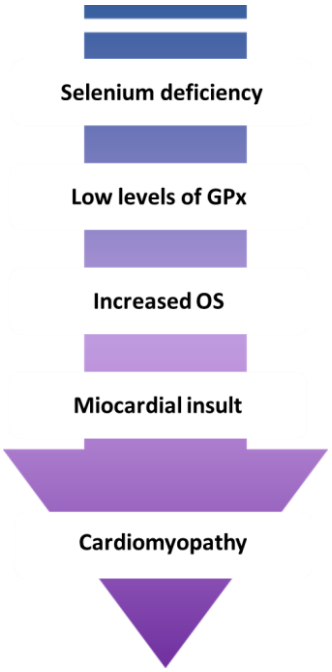


Figure 18. Selenium deficiency in heart diseases
Adapted from Loscalzo 2014

The discovery of Keshan Disease and mammalian Se-proteins established the role of selenium as a trace essential element.[185]

It has been shown that Selenium deficiency can also affect the physiological functioning of the nervous system,[186,187] leading to harmful neurological consequences[186] and can become lethal in extreme cases.[188,188] Se participates in most molecular mechanisms (redox pathways, myelopathies, signal transduction), associated with neurodegenerative diseases and particularly in AD.[189] Subjects with lack of selenium may also develop depression and anxiety.

Se appears to also be involved in the antiviral immune response. Indeed, it has been shown to reduce the virulence of human immunodeficiency virus (HIV),[190] and recent

studies show its possible involvement in the immune response against Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-COV2).[191–193]

However, it should be noted that also an excess of selenium is toxic for the organism. The toxic effects can be caused by both inorganic and organic forms.[182,194] When over-accumulated, Se can compete and replace sulphur in amino acidic residues during protein synthesis, altering both structure and function of proteins. Selenium excess is associated to various symptoms, like skin and nail lesions, hypotension, anaemia, and fever.[195] Also neurological symptoms as tremor and muscle contractions have been described.

As clear from the above, Se owns different and opposite biological and chemical proprieties, depending on the dose (hormesis) and because of this it is considered a *Janus element*.[196] At low concentration, Se acts as a potent antioxidant and cytoprotective and chemoprotective agent,[12] furthermore, it is involved in a series of biological process as physiological required element.

By contrast, at high concentrations, selenium acts as a pro-oxidant inducing thiol groups oxidation, and oxidative cellular damage. Indeed, this specific activity is used to induce cell death in certain kind of tumours.[197,198]

1.2.2. Se as antioxidant

Several studies have shown that Se acts like a potent antioxidant.[186,199–201] It is found in specific aminoacidic residues, usually a selenocysteine, in which an atom of sulfur is replaced by a Se one. Selenium containing proteins are known as selenoproteins (Sel). Until now, 25 Sel have been described in the human body;[202] among them, there are oxidoreductases - mainly represented by GPx - Thioredoxin Reductases (TrxR) and Glutaredoxins (Grx) families.[200]

In addition to their antioxidant role, Sel play others and different biological functions: they are involved in signalling modulation of proteins and growth factors, in thyroid

hormone and calcium metabolism, highlighting that Se is crucial for some physiological cellular processes.

For these antioxidant properties, Se has been proposed for treating or preventing a series of pathologies related to OS conditions, like AD.[203] It has been showed, indeed, that selenium is able to interfere with both the formation of senile plaques – responsible of the increasing of oxidative stress - and NFTs.[152]

Particularly, a selenoprotein, the Selenoprotein P (SelP) plays an important role in the transport of Se to the brain for the synthesis of essential selenoproteins[187] such as GPX and thioredoxin reductase (TrxR). In addition, SelP contains a redox motif and owns antioxidant properties contributing to redox regulation.[204,205] It is the only selenoprotein containing more than ten Sec residues.[206] SelP exerts its transporting action by binding a specific receptor: the apolipoprotein E-receptor type II (ApoER2). The depletion of SelP or its receptor leads to a decrease in brain Se levels, causing neurological damage and dysfunctions related to oxidative stress.[207] Furthermore, ApoE polymorphisms and mutations are a strong genetic risk factor for AD.[208] Finally, several post-mortem investigations on human brains of subjects with AD, showed the association between AD and Se levels.[209]

The recognised importance of Se pushed the researchers to create synthetic or semi-synthetic organic selenium compounds, that have found their use in medicinal chemistry during the last decades. One of the first Se-compounds to be approved as a drug by the FDA, is the ebselen (Figure 19), a synthetic GPx mimetic with a Se-N bound, with antioxidant properties. Starting from this compound, a series of GPx mimetic ebselen-like molecules, have been obtained.

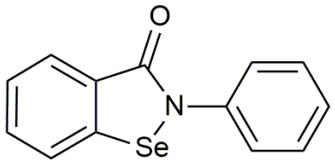


Figure 19. Ebselen's structure.

Se antioxidant compounds also comprise a different class of molecules: the selenosugars. Selenougars are a group of Se-containing compounds with peculiar physicochemical properties.[15] Indeed, they combine the antioxidant properties of selenium and the good solubility in water of sugars. This increases their bioavailability and their use for human applications. They own an important antioxidant activity as HOCl scavengers[14] and have recently found a use in wound healing.[18] The most known compound in this category is 1,4-Anhydro-4-seleno-D-talitol,[14] alternatively known as SeTal (Figure 20). This compound is patent-protected by a [210,211] and it is under technological development out by an Australian company, to formulate a pomade for skin repair.

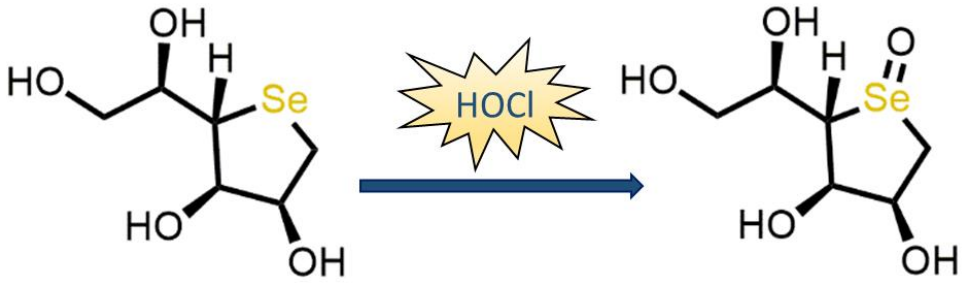


Figure 20. HOCl scavenging mechanism.
Adapted from Storkey et al.[14]

1.2.3. Se supplementation

The data discussed above show that a proper intake of Se can help maintaining wellbeing in humans. Diet is the principal source of selenium; however, it is not always possible to follow an appropriately selected and balanced diet.[182].Therefore, selenium supplementation can be useful to cover the daily demand for this element; furthermore, it might be helpful to prevent a series of human disorders, including those related to oxidative stress conditions, such as AD[212] and CVDs.[213]

Selenium supplementation can be direct or indirect. The latter is realized adding this element to soil, to fertilizers or fodder used to feed animals.[214] Conversely, the direct

one involves the administration of supplements. In particular, the selenium contained in supplements can be in its inorganic or organic forms. The latter is mainly represented by selenium yeast supplementation which mostly provides selenomethionine. This is a more bioavailable form of selenium (as compared to selenium salts), it is safe and exerts a series of beneficial effects in human body. Furthermore, selenium yeast preparations entails low production costs.[215]

To confirm the possibility of guaranteeing an appropriate Se balancing, by modulating its intake, a series of Se supplementation studies have been conducted in the past decades. Nevertheless, the data currently available are not conclusive.

Indeed, although the results of the Nutritional Prevention of Cancer Trial (NPC), indicated that a daily supplementation with 200 µg Se-yeast was associated with a decrease in lung, colon or prostate cancer risk, further studies have shown no risk decrease. In particular, the Selenium and vitamin E Cancer Prevention Trial (SELECT), showed no beneficial effects of SeMet supplementation, and was hence prematurely discontinued.[216] In addition, an increase in type-2 diabetes risk was found in subjects with adequate Se status, that had received Se supplementation.

These controversial results may be explained by the strict dependency of Se activity on multiple factors. Indeed, further analyses of these studies have revealed that both the baseline levels of Se in the plasma of the studied subject, and the form of supplemented Se may influence the effects of Se supplementation.[217]

Despite these divisive data, recent studies have proved the advantages of this practice. Asbaghi at al. have conducted a systematic review and meta-analysis showing that selenium supplementation, at a dose of 200 µg/day for 8 weeks, is able to reduce C reactive protein (CRP) in the serum of treated people. CRP increases during initial stages of inflammation and stress responses; it is also a strong predictive factor of cardiovascular events. Thus, selenium administration could be useful to prevent this kind of events.[218]

In a recent review Zakery at al. have analysed the effect of selenium supplementation in oxidative stress conditions, proving that it can positively affect glutathione levels in treated population, decreasing the levels of oxygen and nitrogen free radicals.[219]

AD is a neuropathology due to several risk factors and several pathogenic mechanisms. Selenium is involved in the protection of cognitive decline, as reported by a series of studies, summarized by Aaseth et al in a review published in 2016. [212]

More recent studies have hypothesized that selenium supplementation could be useful in the prevention of viral infections, including SARS-COV2.[220] It also seems to relieve the clinical complications associated to COVID-19.[221]

In conclusion, even though selenium supplementation benefits are still discussed and this field requires further research, the data accumulated so far, require a close evaluation of this practice.

1.3. Polyphenols

Polyphenols are a wide class of natural compounds including different substances. They have one or more aromatic rings with one or more hydroxyl groups attached to them.[222] This family of compounds is markedly heterogeneous, with simple structured molecules, such as phenolic acids and stilbenes, and more complex compounds, such as tannins (Figure 21).[16]

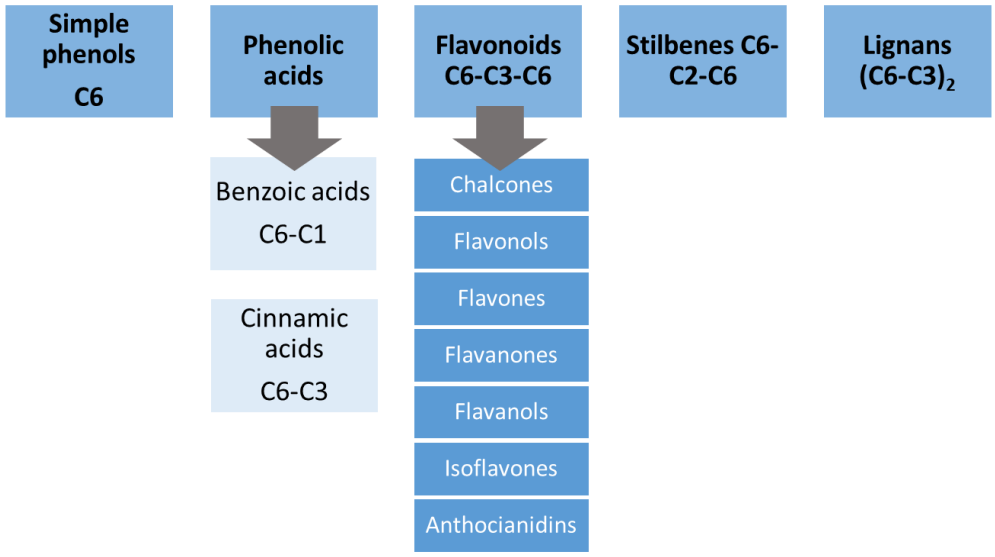


Figure 21. Phenolic compounds classification.

They are mostly assumed with diet as they are contained in fruits, vegetables, grains, bark, stems, tea and wine;[223] they are usually present in conjugated form where they are linked to sugar residues.

Polyphenols have raised great interest in the scientific community since the 1990s, due to their beneficial effects on human health. Indeed, if these compounds show strong antioxidant properties in vitro, several studies have furthermore proved that their dietary consumption contribute to the prevention of degenerative diseases, like

CVDs,[125] metabolic disease such as type 2 diabetes mellitus (T2DM),[124,224] cancer[225–227] and NDs, particularly AD[228] and Parkinson disease (PD).[229–231] The incidence of these pathologies increases with age and it has followed a constant increasing trend in the last decades, mainly due to the increase in life expectancy, especially in developed countries. Thus, reducing the incidence of these pathologies, by preventing their development, is crucial goal for health authorities worldwide, in order to ensure a better health and quality of life to the population. Therefore, it is not surprising that research has gradually shifted its efforts towards the achievement of an effective preventive approach, in addition to the "drugs", and to promoting "lifestyle" concept, including "diet", and hence "food".

Taking these considerations into account, a large part of research focused on the understanding of the effects of plant polyphenols on cellular metabolic and redox equilibria, as well as on inflammatory response. As a result, an increasingly solid molecular basis for the health effects of these molecules has been established. The data now available are paving the way for the possible use of natural polyphenols, or their molecular scaffolds, as nutraceuticals to counteract aging and to prevent associated pathologies.

1.3.1. Classification and chemistry of polyphenols

Phenolic compounds are a wide group of compounds synthetized by plants. These compounds are characterized by the so-called phenolic structure (C6H5OH), which consists of at least one hydroxyl group (–OH) bound to one or more benzene aromatic rings (C6H5 or C6), forming the phenol structure. If the compound exhibit a single aromatic ring, they are termed phenolics (or monophenolics). Polyphenols, instead, have two or more aromatic rings with at least one hydroxyl group as substituents.[232,17] Since this description can refer to a large series of compounds, polyphenols can be further subdivided into two main groups: flavonoids, non-flavonoids, which, in turn, include numerous subclasses depending on diverse structural and chemical characteristics.

1.3.1.1. Flavonoids

Flavonoids are plant secondary metabolites, principally found in fruits and vegetables, with variable phenolic structures. It has been shown that they have a series of beneficial effects on human health,[233] linked to their antioxidant, anti-inflammatory and anti-mutagenic properties. Due to their properties they are used for nutraceutical, pharmaceutical, medicinal and cosmetic applications. They have also proved beneficial in pathological conditions such as CVDs,[234] cancer,[235,236] and Alzheimer's disease.[237]

Flavonoids have the C6–C3–C6 general structural backbone in which the two C6 units (Ring A and Ring B) are of phenolic nature. They are linked by a heterocyclic ring (Ring C) that usually is a closed pyran (Figure 22Figure 22).

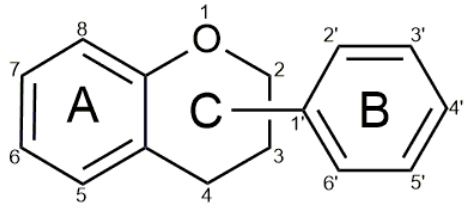


Figure 22. Flavonoid general structure.

According to the heterocyclic ring (Ring C) variation, the different number of hydroxyl groups, the degree of unsaturation and oxidation state of central carbon, flavonoids can be classified into the following subgroups: anthocyanins, flavanols, isoflavones, flavones, flavanones and chalcones. If in most flavonoids the Ring B is attached to the C2 position of Ring C, in isoflavones the Ring B is connected to the C3 position of Ring C (Figure 1Figure 23); conversely, chalcones are characterized by the lack of the heterocyclic Ring C (Figure 23Figure 23).

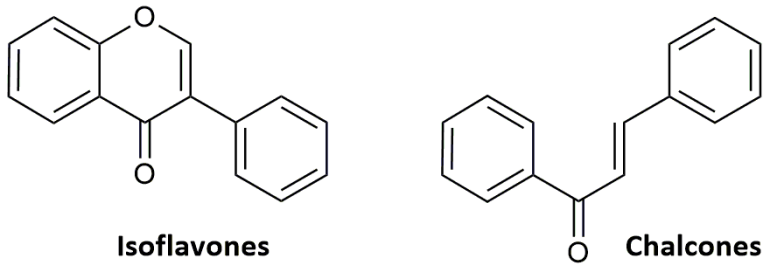


Figure 23. General structures of isoflavones and chalcones

Flavones, together with their 3-hydroxy derivatives flavonols, their glycosides, methoxides and other acylated products, represent the largest polyphenols subgroup (Figure 24). For instance, quercetin and kaempferol, which are the most common flavonol aglycones, have approximately 300 different glycosidic combinations, each.[238]

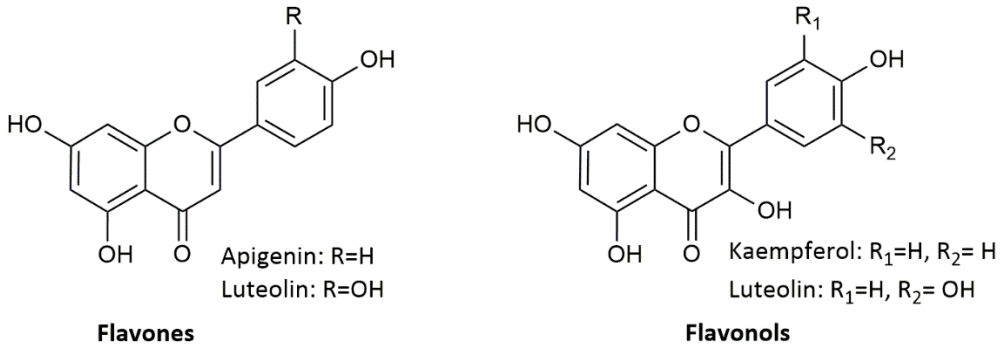


Figure 24. Flavones and Flavonols

1.3.1.2. Nonflavonoids

Nonflavonoids are the other principal group of phenolic compounds with dietary importance, and generally have a simpler chemical structure. Nonflavonoid compounds include phenolic acids, the hydrolysable tannins (ellagitannins and gallotannins), stilbenes, and lignans.[239] Less abundant in food, although of a high importance, is also the curcuminoids class.[240]

1.3.1.2.1. Phenolic acids

Phenolic acids are a group of natural compounds which, in turn, are represented by two main sub-groups: hydroxybenzoic and hydroxycinnamic acids. They are based on C1–C6 and C3–C6 backbones, respectively (Figure 25Figure 25). Phenolic acids can be found in a free form in fruits and vegetables, while they are usually in bound form (as their amides, esters, or glycosides) in grains and seeds.[241] In fact, in foods, hydroxycinnamic acids are more present than hydroxybenzoic ones. The main hydroxybenzoic acids are gallic, ellagic, protocatechuic and 4-hydrobenzoic acids (Figure 25),[242] while hydroxycinnamic acids are mainly represented by caffeic, ferulic, sinapic and *p*-coumaric acids.[243]

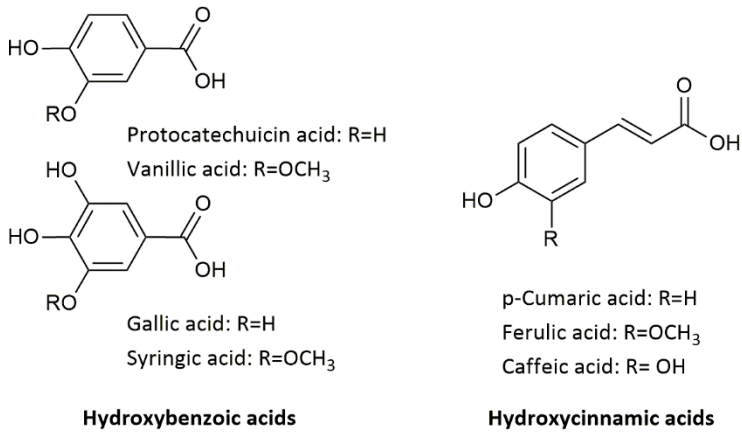


Figure 25. Most common phenolic acids.

As natural compounds, phenolic acids have attracted the interest of the scientific community for their antioxidant behaviour and promising health benefits.[244]

The -OH group on the aromatic ring of phenolic acids is responsible for their antioxidant activity. The main mechanisms through which they exert this activity is hydrogen atom donation. However, the antioxidant properties of different phenolic acids change depending on the substituents on the aromatic ring –that affect the structure's stability - and on their bound or free forms.[245]

1.3.1.2.2. Stilbenes

The stilbene structure is based on the C6–C2–C6 backbone, defined by two aromatic rings linked by an ethylene bridge; thus they are structurally characterized by the presence of 1,2- diphenylethylene nucleus. More than 400 stilbene derivatives have been identified, hovewer, the most known and best characterized stilbene is resveratrol, 3,4′,5-trihydroxystilbene (Figure 26).

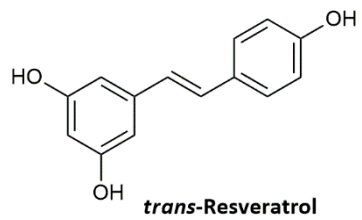


Figure 26. Resveratrol

Resveratrol is mainly contained in grape and grape products, but it is also contained, in lower amounts, in peanuts, pistachios, some berries, tomato skin and chocolate. It can be found in its free form, as glucoside (piceid) and as polymer (viniferins); both as cis and trans isomer. Resveratrol owns a series of biological properties: it can modulate cell proliferation, angiogenesis and redox intracellular equilibrium. Thanks to this ample spectrum of properties, resveratrol is widely used in a plethora of cosmetic and nutraceutical applications; indeed, a high number of resveratrol- based products are on the market.

1.3.1.2.3. Lignans

Lignans are phytoestrogens with a structure containing two phenylpropanoid units (C6-C3) linked at the central carbon (C8-C8) (Figure 27). Lignans are generally found in free forms and are metabolized by intestinal microbiota to be transformed in their corresponding bioactive derivatives.[246,247]

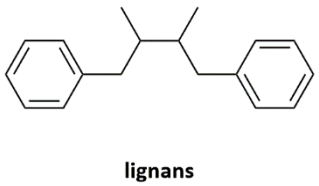
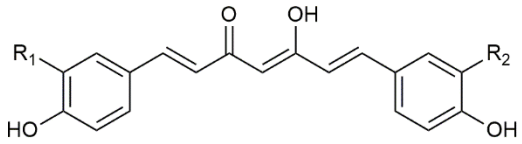


Figure 27. General structure of lignans

1.3.1.2.4. Curcuminoids

Curcuminoids are mainly represented by three chemically related components named curcumin (C), demethoxycurcumin (DMC) and bis-demethoxycurcumin (BDMC) (Figure 28).[248,249] Among the curcuminoids, C is the most abundant; it has been considered the most powerful for several years, but, new scientific results have demonstrated that DMC and BDMC also have comparable bioactive potential.[250]



Curcumin: $R_1=R_2=OCH_3$
demethoxycurcumin: $R_1=H$, $R_2=OCH_3$
demethoxycurcumin: $R_1=R_2=H$

curcuminoids

Figure 28. Most common Curcuminoids

Nowadays it is well established that curcuminoids possess different functional and nutraceutical functions, including antioxidant, anticarcinogenic, antidiabetic and hypocholesteremic activities.[251] Thus they are currently used in therapeutic foods, cosmetics, and pharmaceuticals.[249]

HYPOTESIS AND AIMS

2. HYPOTHESIS AND AIMS

Oxidative stress is implicated in several diseases including cancer, atherosclerosis, cystic fibrosis, rheumatoid arthritis and neurodegenerative diseases. Extensive data suggest that oxidative damage may play a major role both in in the causation of these disorders and in chronic associated processes. Several data demonstrated, for instance, that a redox disequilibrium is involved in neurodegenerative diseases like AD and PD, in which the alteration of key proteins induces OS leading to neurodegeneration.[252]

In view of the foregoing, it is not surprising that the interest of scientific community has focused on preventive therapies for oxidative stress conditions, and inter alia, on those that involve the use of natural compounds as antioxidants. The latter - easily available in foods - can improve cellular defences helping the prevention of oxidative damage and have mostly no side effects for the body. Among natural antioxidants, polyphenols and selenium and their antioxidant properties have been largely studied.

Investigating Se's biological properties has allowed clarifying principal mechanisms through which it exerts this function:

- it is found in specific amino acidic residues, usually a selenocysteine, in which an atom of sulphur is replaced by a Se one. The selenoproteins which contain this amino acidic residue in their catalytic site are involved in redox reactions,
- it is able to coordinate metal ions like Cu(II) e Fe(II), preventing metal-oxidative DNA damage,[201,253]
- it acts as free radical scavenger, reducing the level of these toxic molecules.[254]

These properties gave impulse to the development of a new chemistry branch focused on the synthesis of active selenium containing compounds. Among them, selenosugars have attracted particular interest as they have shown antioxidant activity as oxidant scavengers and have demonstrated to be well tolerated by the human body.

Furthermore, as discussed above, polyphenols own a series of biological advantages, including those related to their antioxidant properties. In detail, dietary polyphenols act as free radical scavengers, metal binding chelators, or enzymatic activity modulators. Therefore, they have positive effects on a series of human pathologies linked to OS.[37]

Based on available data, selenium and polyphenols' dietary assumption is recommended, both to guarantee a redox balancing, and to counteract oxidative stress conditions. However, there are also drawbacks and challenges related to their intake:

- selenium is toxic in high amounts; indeed, the daily recommended amount is about 50-70 µg/day;
- the majority of dietary polyphenols loses the proper bioavailability during digestion.

Hence, the idea of the project: to synthetize new molecules – specifically glycoconjugates - with potential antioxidant activity, to use as supplements in the OS prevention. Thus, here we propose a mini-library of glyconconjugates containing both selenium and active functions of polyphenols (Figure 29).

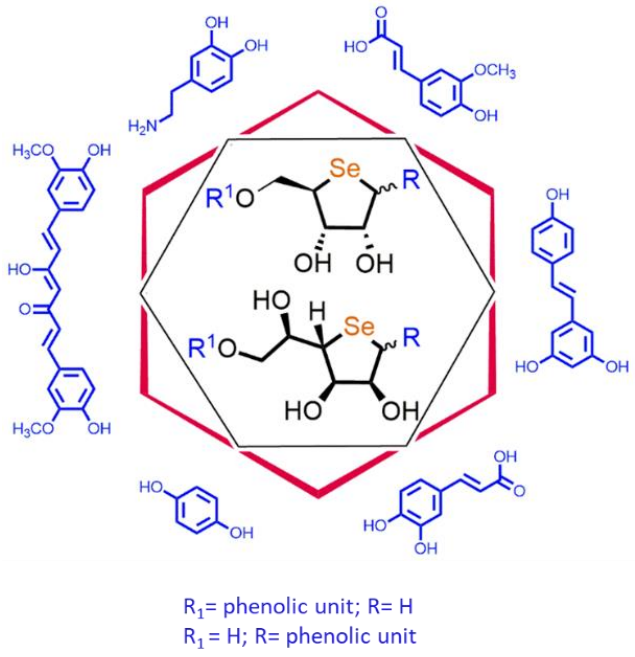


Figure 29. Aims of the project.

A series of polyphenolic molecules have been chosen to be linked to selenosugars. After an evaluation of structure and structure-related antioxidant properties, a selection has been made and a group of molecules has been chosen. Namely, caffeic acid (CA), ferulic acid (FA), curcumin (C) and dopamine.

In summary, the purpose of this work was to synthetize and to investigate the antioxidant properties of new glycoconjugates, consisting of selenium containing monosaccharides bound to a phenolic moiety, which differ in the position of the bond. The related aims are:

- to reduce the selenium amount to be administered, thanks to the presence of polyphenol moiety,
- to assess the impact, on both the antioxidant properties and bioavailability, of conjugating a selenosugar with a polyphenolic molecule.

The specific milestones of this thesis are outlined below:

- ✓ synthesis and purification of a series of new glycoconjugates containing both a selenium atom and a polyphenolic moiety.
- ✓ characterization of the novel compounds through ^{1}H and ^{13}C and ^{77}Se NMR spectroscopy,
- ✓ evaluation of the cytotoxic activity of the novel compounds in bacterial and yeast cell lines,
- ✓ assessment of the antioxidant profile of the new compounds by chemical assays,
- ✓ evaluation of the antioxidant activity of novel compounds in human skin cell lines.

All these goals, claiming to verify our initial hypothesis, are developed in the Results section. In the following sections, the synthetic strategies applied, and the related results will be discussed.

RESULTS AND DISCUSSION

3. RESULTS AND DISCUSSION

3.1. Synthesis

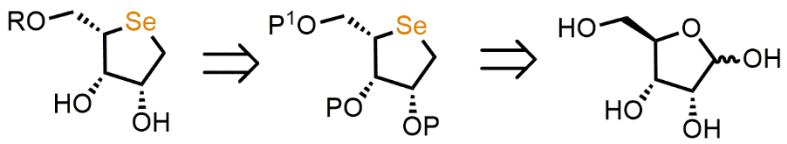
The introduction of Se into organic structures represents a synthetic challenge gaining an increasing importance in medicinal chemistry and natural sciences as well as in biology, for biologically active new compounds. Indeed, for more than a century, most researchers have ignored the chemistry and uses of selenium in organic compounds. This is probably due to its smell and toxicity, as well as the unpredictability of its biological effects as compared to the ones of sulphur, whose chemistry is well-known.

In the last three decades very important milestones in the selenium chemistry have been achieved via chemo- regio- and stereoselective methods, such as using nucleophile,[255] electrophile and/or radical procedures, overcoming the well-known instability and unusual behavior of early approaches.

More in deep, concerning the selenium ribose compounds, the results of Matsuda,[256] Pinto[257] and Jeong[258] represent the starting and successful pathways. Inspired by these studies and taking into account that, to the best of our knowledge, no examples of 5-conjugates have been described, our attention have been addressed to design these novel selenium containing structures. The related first synthesis is herein reported.

3.1.1. First approach

As outlined in the retrosynthetic analysis in the scheme 1, our first approach was based on the synthesis of the building block **1**. The latter is a L-deoxy-selenosugar prepared from the commercially available D-ribose.



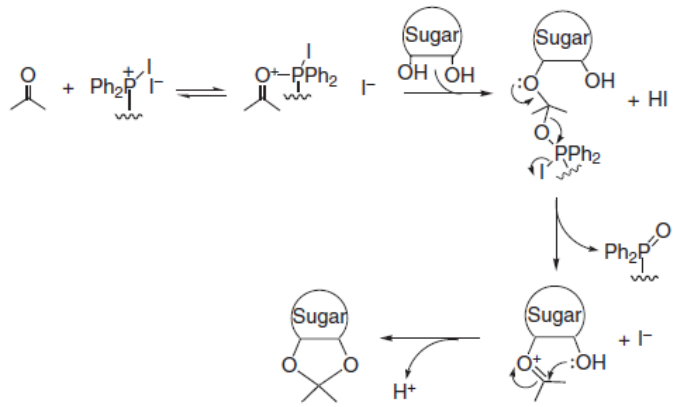
1 D-ribose

R= Phenolic moiety
P, P^1= orthogonal protecting groups

Scheme 1. Retrosynthesis of target.

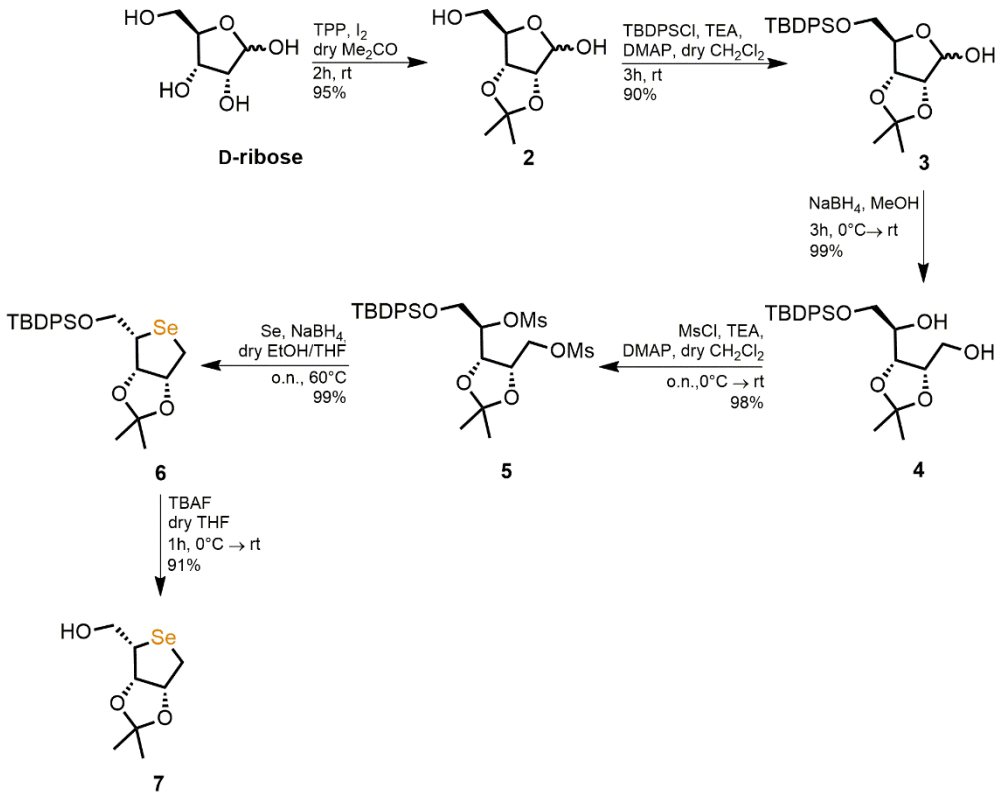
For the chemical conversion of D-ribose into an useful scaffold, we modified an already reported procedure, presented by Jeong at al.[259] The latter includes the choice of orthogonally protecting groups to allow their selective removing during the synthetic steps. For this purpose, isopropylidene was used to simultaneously protect the O-2 and O-3 positions. The used protocol was previously developed in the research group where this PhD was carried out. Specifically, the procedure (Scheme 2) is characterized by smooth reaction conditions with a low environmental impact.[260]

The sugar is added to a suspension of triphenyl phosphine polymer-bound/iodine complex (polystyryl diphenyl iodophosphonium iodide) in anhydrous acetone. An adduct is first formed, due to the presence of the positively charged phosphorous atom in the complex and the electron-rich carbonyl oxygen of acetone, which exposes the carbonyl carbon atom to undergo nucleophilic attack by a first hydroxyl group of the sugar molecule. The subsequent non-equilibrium step of the reaction is the loss of polymer-linked phosphine oxide and thereby formation of an oxygen-stabilized carbocation, known to be intermediate in the acetalization reaction.



Scheme 2. O-isopropylidene protection mechanism.

This carbocation can undergo the intramolecular attack by a second hydroxyl group present in the sugar molecule to afford the final *O*-isopropylidene derivative **2** in a 95% yield (Scheme 3). Then, compound **2**, without any further time consuming work up or purification procedure, was selectively protected by treatment with *tert*-butyl(chloro)diphenylsilane (TBDPSCl), in the presence of triethylamine (TEA) and dimethylaminopyridine (DMAP), to get the corresponding silyl ether **3.** The latter, in turn, provided the diol **4** with excellent yield (99%), under reductive conditions (sodium borohydride) Next, treatment of compound **4** with methanesulphonyl chloride (MsCl) under basic conditions led to compound **5**. This reaction allowed us to insert good leaving groups at C-1 and C-4, so that the subsequent treatment of **5** with selenium, in the presence of sodium borohydride in anhydrous ethanol and tetrahydrofuran at 60 °C, gave the L-deoxy-selenosugar derivative **6**. It is worth mentioning that this reaction takes place with an inversion of configuration of C-4,[259,261,262] leading to the corresponding L-sugar (Scheme 3).



Scheme 3. Synthesis of the scaffold 7.

Moreover, using dry conditions allowed the optimization of the reported procedure[259] affording **6** in a very significant improvement up to 99% yield. The final removal of the TBDPS group at C-5, obtained exploiting the great affinity of fluoride to silicon trough the treatment with tetrabutylammonium fluoride (TBAF), furnished the scaffold **7** with 75% overall yield.

3.1.1.1. Glycoconjugates synthesis

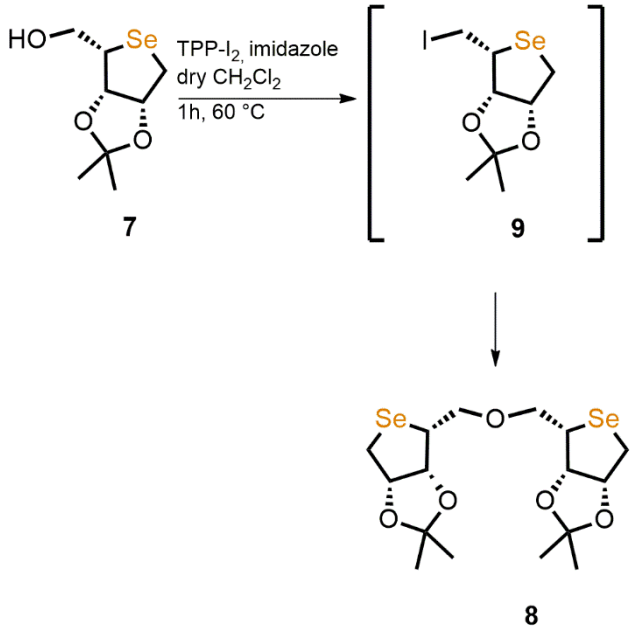
To covalently link the seleno scaffold **7** to the natural antioxidant molecules that we selected for our purpose, we attempted to enhance the reactivity of its electrophilic function at the C-5 position. Therefore, we firstly tried to convert **7** into the corresponding iododerivative using a TPP polymer-bound/iodine complex, already

reported on different substrates.[263] It is widely known that halogens react with trisubstituted phosphine promoting phosphorus(III)-phosphorus(V) conversion (Scheme 4).

$$RP_3 + XX \leftrightarrows [R_3P^+X]X^- \leftrightarrows R_3PX_2$$

Scheme 4. Polimer bound triarylphosphine halogen complex

The intermediate quaternary phosphonium salt, which is believed the active species, is a good Lewis' acid as well as a dehydrating agent. Such a one is capable to interact with nucleophilic oxygens inducing their substitution. In the fulfilled procedure phosphine iodide complex acts as a mild form of PX_5, whereas imidazole, that is reported to play a role of phosphine activating agent as well, behaves as a proton trap in the reaction mixture. The compound **7** represented the substrate for the iodination reaction (Scheme 5), which has been performed using the complex made of TPP and I_2 as reactive. Unfortunately, under the reported conditions, the only found product was the dimer **8** whose structure was investigated through ^1H and ^{13}C NMR and confirmed by mass spectrometry (Scheme 5), probably resulting from the quick formation of the corresponding iodide **9** that was attacked by the OH group at the C-5 position of the unreacted **7**.



Scheme 5. Iodination reaction

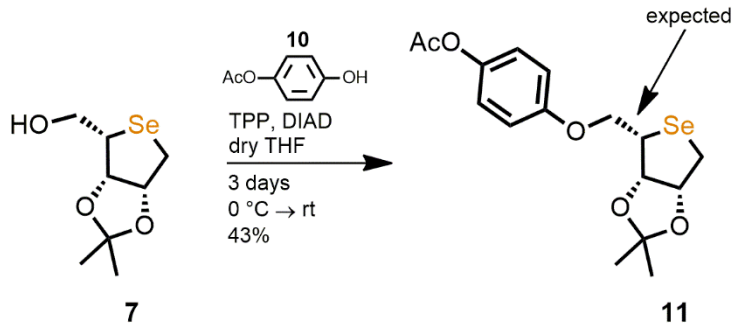
Besides, it was not possible to evaluate the efficiency of mesyl or tosyl derivatives since both were obtained in very low yields.

3.1.1.2. Mitsunobu reaction

These findings prompted us to design a different coupling method based on the Mitsunobu reaction. The latter is generally carried out with an activated alcohol, prepared *in situ* using a complex between TPP and an azodicarboxylate as promoter. With the aim to verify the feasibility of this strategy, monoacetylated hydroquinone **10** was selected as the acceptor model. It was prepared by treating hydroquinone with acetic anhydride (1 eq) in dry pyridine, in the presence of DMAP at room temperature. The reaction afforded the acceptor derivative with 70% yield, in addition to small amount of diacetylated corresponding compound.

Coupling reaction between monoacetylated hydroquinone **10** and the sugar donor **7** under Mitsunobu conditions afforded the glycoconjugate **11** which was isolated by

silica gel chromatography in 43% yield (Scheme 6).[264] However, the interpretation of NMR spectra gave an ambiguous result concerning the configuration and the conformation of the sugar moiety. In particular, we found that there could be some uncertainty in the assignment of C-4 configuration, which - in our knowledge- is not involved in the coupling reaction mechanism. This anomalous stereochemical trend pushed us to a further investigation.



Scheme 6. Mitsunobu reaction; synthesis of **11**.

Unfortunately, it was not possible to perform the X-ray crystallography analysis, thus we decide to carry out deeper NMR studies. It is well-known that the stereostructure of the furanose moiety is often difficult to analyze due to the low energy barriers between various puckering states.[265] Furanose rings in carbohydrate and nucleoside exist, in fact, as various puckering states that are separated from each other by relatively low energy barriers.[266] Consequently, the dihedral angles of each pair of hydrogen atoms in a furanose ring, which needs to be known for NMR stereochemical characterization, are not defined with certainty. In most cases, conformational analysis of furanose rings using NMR is performed based on a model that considers only two states: the north (C3′-endo) and south (C2′-endo) puckers (Figure 30).[267]

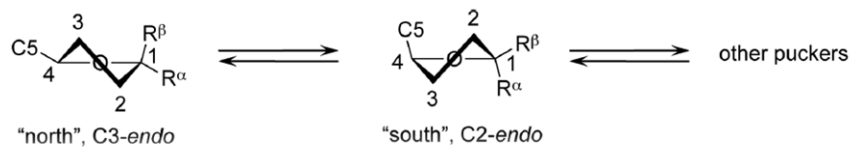
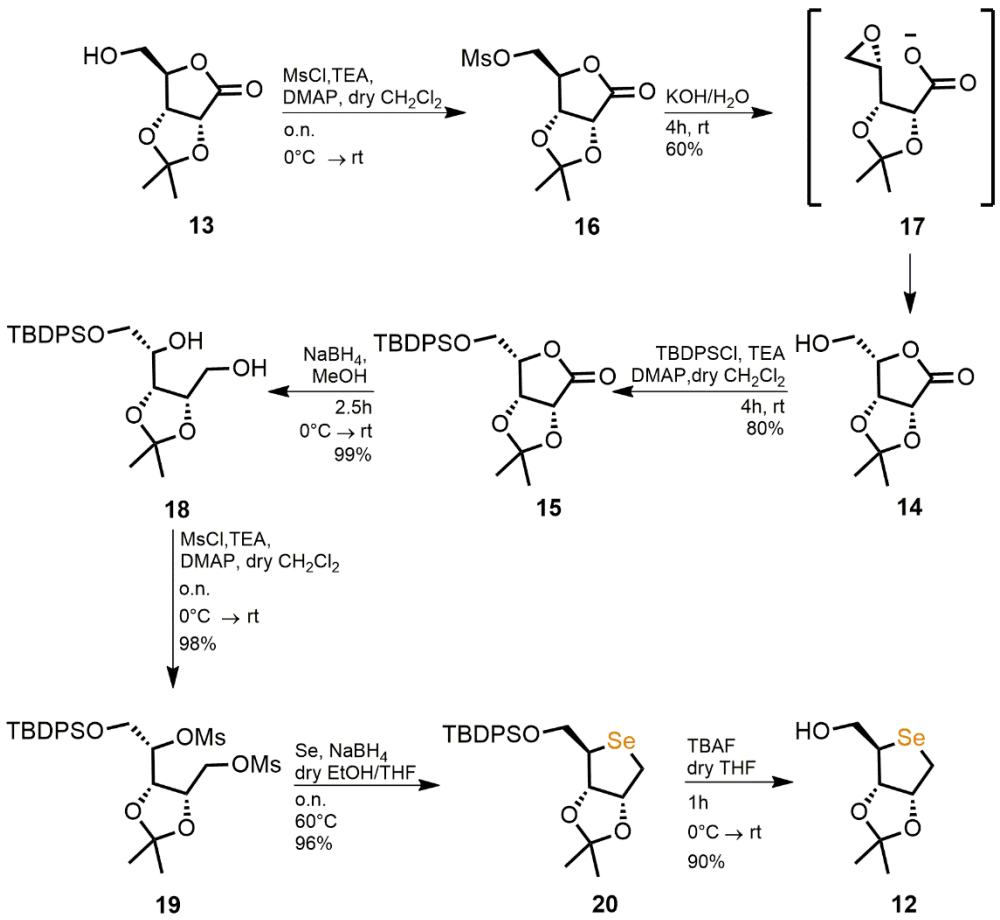


Figure 30. Puckered states of furanose rings.

It has already been reported in the literature that the bulky selenium atom in the 4'-selenoribonuclesosides leads to a south (C2′-endo) pucker state.[258] However, since significant NOE effects were not detected for the hydrogens at C-2 and C-4 and the coupling constants of **11** were not supportive for the stereochemistry assessment with certainty, we approached to a comparative evaluation of the outcomes between the L- and D-deoxy-selenosugars **7** and **12**, respectively.

3.1.1.3. Synthesis of the D-epimer

Starting from the commercially available D-ribonolactone-2,3-*O*-isopropylidene (**13**), we applied a synthetic strategy where, exploiting the procedure reported by Batra et al.,[268] a first inversion of configuration was accomplished, leading then to the intermediate **14**, which, in turn, was converted into the D-target **12** (Scheme 7). Starting from compound **15**, we followed the same route previously described for the isomer **7**.



Scheme 7. Synthesis of the D-donor **12**.

In details, the first step of this synthetic pathway is represented by the activation of C-5 position of D-ribonolactone-2,3-*O*-isopropylidene (**13**) which was treated with MsCl in the presence of TEA and DMAP in anhydrous CH_2Cl_2; this reaction gave the compound **16**. Without any purification, the crude mesylate **16** was subjected to aqueous potassium hydroxide (KOH) treatment. After workup and crystallization of the crude from 2-propanol, the product **14** was obtained in 60% yield. This reaction includes the aforementioned inversion of the configuration at the C-4 chiral center, a process in which an intermediate open-chained epoxide **17** of the retained configuration at C-4 undergoes subsequent intramolecular ring opening of the epoxide

by the carboxylate nucleophile in a favourable 5-endo-*tet* process with an inversion of configuration to yield the L-lyxonolactone derivative **14**.

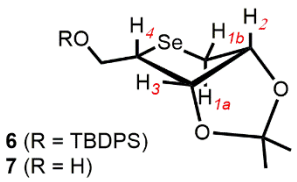
Then, position C-5 was protected with TBDPS group in presence of DMAP, under basic conditions (TEA), to give the fully-protected compound **15**. The latter was reduced with $NaBH_4$ to 1,4-diol **18**, that, in turn, after a treatment with an excess of MsCl, in presence of TEA and DMAP, afforded the dimesylate **19**, ready to the selenium attack and subsequent ring closing with the expected inversion of configuration at C-4, that, in this case, led to the D-derivative **20**. The final removal of TBDPS protection on the primary hydroxyl group provided the derivative **12** achieved in very high yields (47%, overall yield).

At this point, a comparative analysis of ^{1}H NMR coupling constants in compounds **6** and **20** as well as **7** and **12** (

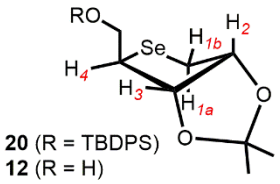
Table 2) was carried out to investigate the range of the vicinal coupling constants. The $^{3}J_{3,2}$ medium value was 5.6 Hz, the $^{3}J_{2,1b}$ was 4.8 Hz, and the $^{3}J_{2,1a}$ was 2.0 Hz. More interestingly, however, was that for **6** and **7,** the $^{3}J_{3,4}$ medium value was 4.3 Hz, while for **20** and **12** the same coupling value was 1.6 Hz.[264] Taking this into account, in the first glycoconjugate, we obtained the $^{3}J_{3,4}$ medium value of 1.6 Hz is consistent with the 3,4-*anti*-configuration.[269]

This unexpected result prompted us to repeat the Mitsunobu reaction using **12** as electrophile and the monoacetylated hydroquinone **10** as nucleophile. The spectral data of the only obtained product showed that we afforded the glycoconjugate **21** with the 3,4-*anti*-configuration also in this case.

Table 2. Vicinal coupling constants. Comparative analysis.



6 (R = TBDPS)
7 (R = H)

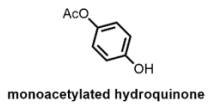
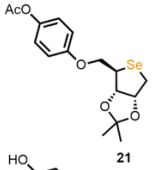
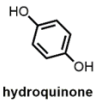
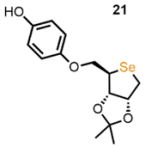
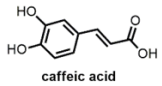
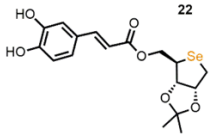
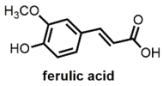
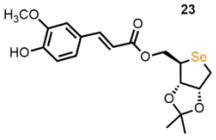
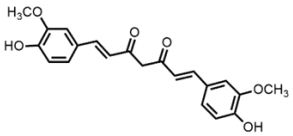
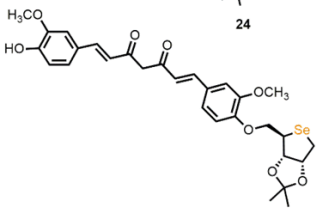


20 (R = TBDPS)
12 (R = H)

Compound	$^3J_{2,1a}$	$^3J_{2,1b}$	$^3J_{3,2}$	$^3J_{3,4}$
6	1.5	4.7	5.3	4.5
7	2.0	4.1	6.1	4.2
20	2.3	5.1	5.3	1.8
12	2.1	5.2	5.5	1.4

The selectivity seen in the model reaction, whose mechanism is under investigation, was also found out when the scope of our synthetic approach was explored by using other polyphenolic acceptors, whose phenolic or acidic function was exploited to our purpose. (Table 3).

Table 3 Glycoconjugates prepared starting from L- and D-donor **7** and **12**, respectively.[1]

Polyphenol	Glycoconjugate	Yield[2]
 monoacetylated hydroquinone	 **21**	43(44) [3]
 hydroquinone	 **22**	13(14) [3]
 caffeic acid	 **23**	70(72) [3]
 ferulic acid	 **24**	68(67) [3]
 curcumin	 **25**	30(28) [3]

[1]Reaction carried out on derivative **7** (1 eq), TPP (1.5 eq), diisopropyl azodicarboxyate (DIAD, 1.5 eq), and polyphenol (1.5 eq) in dry THF under N_2 atmosphere for 3 days. [2] Percentage yield; isolated yield by column chromatography; double reaction products of the polyphenol were never detected. [3] Percentage yield; reaction starting from derivative **12** under same condition.

As showed in the Table 3, all couplings exclusively led to the corresponding D-deoxy-seleno glycoconjugate (**21–25,** Table 3).[264] This happened when we used both the L- and the D-selenosugar as donor. These findings were clearly confirmed by spectral and analytical data. Moreover, they were obtained with related comparable yields. Indeed, the already reported unusual behaviour of selenium[270] is held responsible for these results. At this stage we can only take into account the significant nucleophilicity of selenium atom which is able to permit the formation of

unfavourable products never observed in the case of the corresponding 4-thiosugar derivative proposed in well-known report of Tanike et al.[270]

In spite of the unexpected stereochemical observed outcomes, the yields of couplings require some further considerations. In the preliminary attempts performed on hydroquinone, used as model compound, the desired conjugated **22** was obtained in a very low yield (13%), suggesting that the presence of two different hydroxyl groups could be a limit of reactivity for the methodology. In support of this hypothesis, the reaction on the monoacetylated hydroquinone **10** led to the product **21** in good yield. Taking into account the poor nucleophilicity of this phenolic function, the equivalent poor reactivity could have pushed us to a preliminary selective protection of hydroxyl group at C-4 position in curcumin, that instead was intentionally escaped because of the known handling problems of the molecule. Nevertheless, the yield was reasonable with the challenging purification procedure currently under improvement.

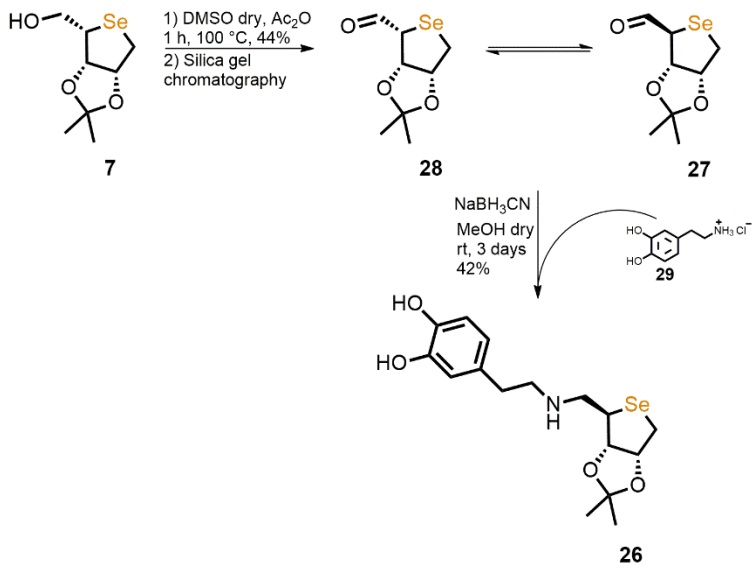
Finally, the lower pKa of the carboxylic acid in comparison to the phenolic one is responsible for the expected highest yields achieved for caffeic and ferulic acids (67–72%), that are a good example of a protecting-free approach, which, in turn, is very convenient in multistep programs.[271]

3.1.1.4. Synthesis of the dopamine glycoconjugate

Dopamine is a molecule of particular interest because of both its phenolic nature and biological properties. Thus, if on one side it is a good antioxidant thanks to its catechol function, on the other it should be considered that it is also a neurotransmitter, whose loss is involved in the early stages of AD.[272] Dopamine, in fact, is a natural substrate for neuronal cells, furthermore – in dopamine containing glycoconjugate **26** - the sugar unit could be useful to reach central nervous system avoiding previous degradation of the dopamine portion. Thus, the designed glycoconjugate could be relevant for more than one purpose.

Due to the failures to obtain the corresponding iododerivative of **7**, faced with the complexity to functionalize the position C-5 with good leaving groups, an alternative strategy to link a dopamine residue to the glycosyl donor was designed. Of note, substitution reactions are not an efficient synthetic procedure to obtain secondary amines. Thus, we chose, as alternative, the oxidation of the –OH group at C-5, followed by a reductive amination.

In details, we employed a mild and specific procedure to oxidise the -OH, namely a Swern oxidation, a selective reaction in which a primary or secondary alcohol is oxidized to aldehyde or ketone, respectively, using acetic anhydride and dimethyl sulfoxide (Scheme 8).



Scheme 8. Synthesis of the glycoconjugate **26**.

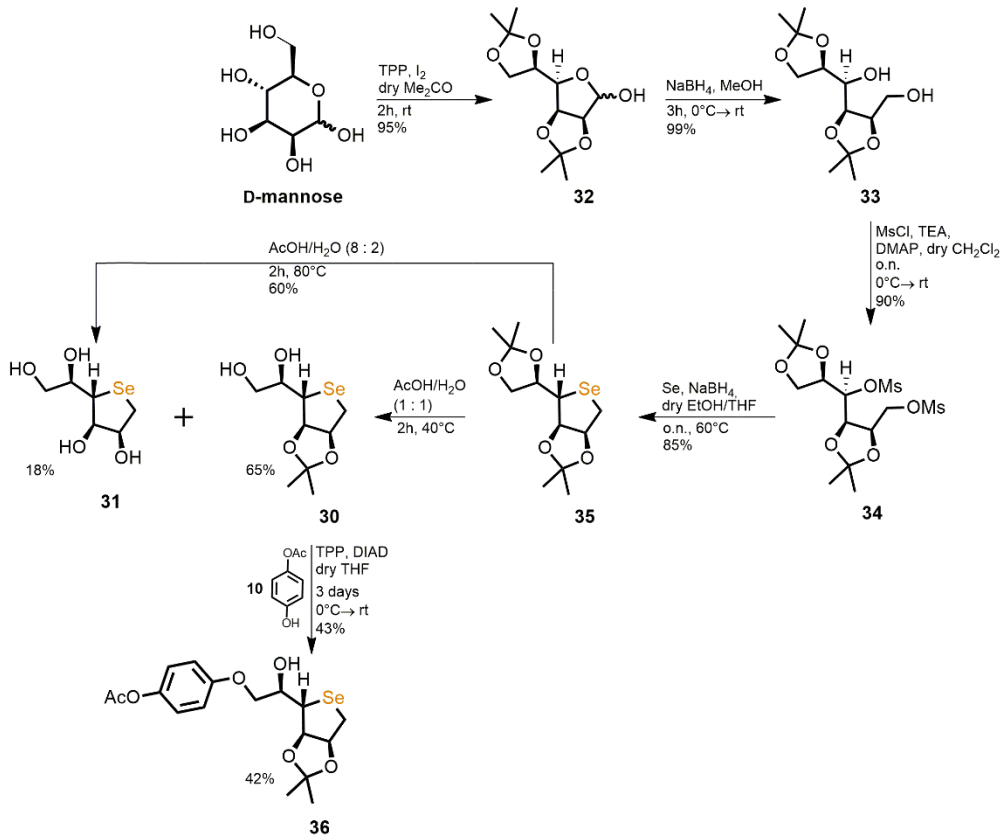
In our case the acetic anhydride was used as the Lewis' acid and the reaction gave the aldehyde **27**. Indeed, the ^{1}H NMR spectrum of the crude reaction mixture showed two characteristic signals at *ca* 10 ppm and *ca* 9 ppm. Besides, purification on silica gel unexpectedly afforded the only corresponding D-derivative **27**, evidently formed by acid catalyzed tautomerization of **28**, in favour of the most stable one (Scheme 8). The spectral data of the aldehyde **27** was identical with those reported in literature.[273]

Moreover, a preliminary reaction carried out at room temperature - gave the above mentioned product in low yield, while the increasing of temperature to 100°C led to a fair amount of the same product.

Then, the reductive amination was performed on dopamine chlorohydrate (**29**) in anhydrous MeOH. In addition, $NaBH_3CN$ was used as reductive agent. It is well known that $NaBH_3CN$ is a selective reducing agent of imine groups, thus it does not lead to the loss of the starting aldehyde. This route allowed us to carried out the reaction through a one-pot process without separation and purification of the intermediate imine. The procedure afforded the new glycoconjugate **26** in a good yield (42%).

3.1.2. Second approach

Considering the results of the activity assays executed so far (reported in the following sections), we focused our attention on new glycoconjugates containing a different selenosugar moiety. Precisely, we prepared the scaffold **30**, namely the O-2 O-3 protected analogue of the already reported 1,4-anhydro-D-4-seleno-tallitol (**31**, SeTal) (Scheme 9).[274] SeTal, indeed, is a "privileged structure" since its efficient biological activity is not only due to the presence of selenium atom, but also to the structural motif on its own.[18] To reach our purpose we modified an already reported procedure[275] starting with the commercially available monosaccharide D-mannose.



Scheme 9. Synthesis of second generation glycoconjugate.

As in the first approach, the synthesis firstly required the protection of the -OH groups. In this case we simultaneously obtained the protection of C-2/C-3 and C-4/C-5 positions exploiting the same reaction previously used. In details, the reaction is conducted with TPP polymer bound/I_2 in anhydrous acetone, as developed in the research group where this project was conducted.[260]

By treatment of **32** with $NaBH_4$ in MeOH, the diol **33** was obtained in excellent yield (99%). Then, it was converted into the corresponding dimesylate **34** (90% yield). In this compounds the two good leaving groups are necessary to gain the subsequent ring closing reaction. The following treatment of **34** with selenium in the presence of sodium borohydride in anhydrous EtOH-THF at 60°C, gave, in fact, the deoxy-selenosugar derivative **35** in excellent yield (90%). To allow the coupling reaction of

seleno derivative **30** with phenolic units, it was mandatory to selectively remove the isopropylidene group in C-5, C-6 positions from compound **35**; this was easily reached in 65% yield exploiting the highest reactivity of primary alcohol function in C-6 and performing the reaction under mild acid conditions (AcOH/H_2O 1:1) at 40°C. Actually, when compound **35** was treated with a mixture AcOH/H_2O 8:2 and the temperature was increased up to 80°C, the completely deprotected product **31** was obtained as main product (60%). The selectively removal of isopropylidene afforded the key intermediate six termed scaffold **30** in overall 45% yield.

With the aim to evaluate the possibility of a scaled-up chemical production, we carried out an alternative approach in which all the purification steps were skipped. This procedure gave the expected compound **30** in 35% overall yield, considered a fairly good yield in scaled-up approaches.

3.1.2.1. Coupling reaction

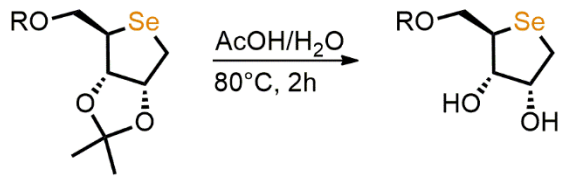
The obtained product **30** was used as donor in the glycoconjugate preparation exploiting the modified Mitsunobu reaction previously described, leading to the compound **36** in 42% yield (Scheme 9). This result confirms the versatility of our approach that will be employed to build a new library of potential antioxidant, six membered glycoconjugates.

3.1.3. Deprotection of the first generation glycoconjugates

With the aim to obtain compounds most similar to natural sugars usually taken with diet and to investigate the influence of sugar moiety on the biological activity, our new compounds (**7**, **21-24**) underwent removal of the isopropylidene group present at the C-2 and C-3 positions of selenosugar units.

Since the isopropylidene group requires acid conditions to be removed, we treated each glycoconjugate with a solution of CH_3COOH/H_2O (8:2 v/v). This procedure

allowed us to provide the corresponding derivatives **7a**, **21a-24a** (Scheme 10) which were purified by semi-preparative thin layer chromatography affording named compounds with high yields (68-80%).



7, 21-24 **7a, 21a-24a**

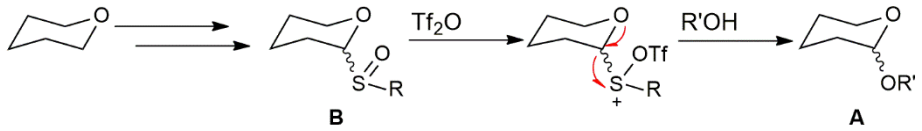
Scheme 10. Deprotection of the glycoconjugates **21a-24a**.

Indeed, the procedure failed for curcumin derivative as well as for dopamine containing glycoconjugates. In both cases the utilized conditions suffered of a low reproducibility in terms of yield. This is probably linked to the high and well-known lability of these polyphenols molecules. Therefore, different solutions should be proposed to the final steps of the designed synthesis.

3.1.4. Glycoside approach

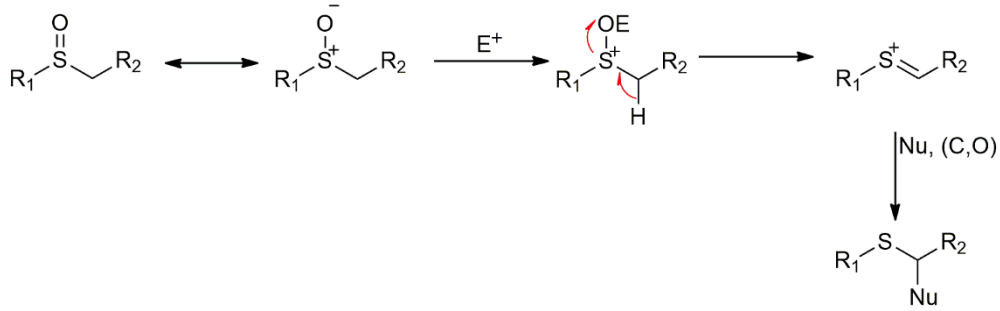
It is well known that the water solubility of phenolic natural compounds is limited by the presence and distribution of phenolic/hydrophobic functional groups which, in turn, also affect their antioxidant power. On this basis, to study the effect of hydrophilic function distribution on our new structures, we started to evaluate the influence of the covalent bond's position on the antioxidant activity of glycoconjugates. Namely, we investigated a new procedure to synthesise the *O*-glycoside version of our targets.

In the classical literature of thioglycosides it is known that it is possible to achieve the *O*-glycosides derivatives **A** through a remote activation of the corresponding sulfoxide **B** (Scheme 11).



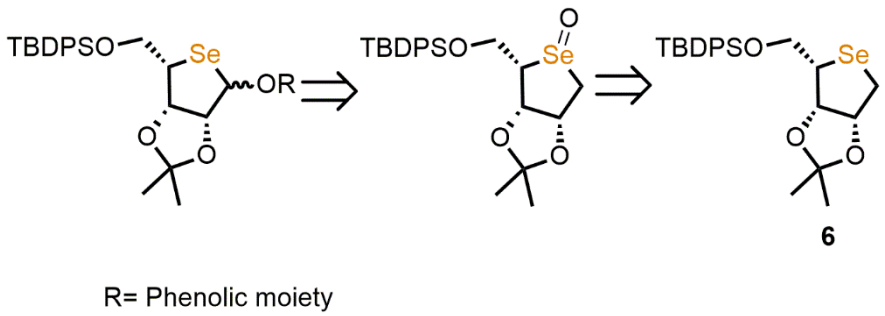
Scheme 11. Thioglycoside approach to glycosidation.

Moreover, we considered that the Pummerer reaction (Scheme 12) allows forming a new C-C or C-heteroatom bond through a highly polarized sulfoxide intermediate.



Scheme 12. Pummerer rearrangement

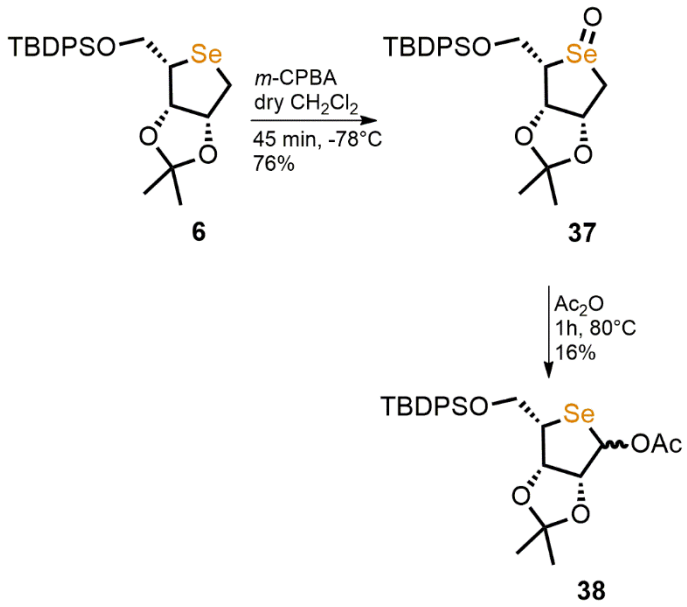
Taking this into account, with the aim of exploiting our selenosugar building blocks for the glycosylation reaction, we employed an intermediate of the aforementioned nature to form the *O*-glycoside of our seleno scaffold **6**, as described in the following retrosynthetic scheme (Scheme 13).



Scheme 13. Retrosynthetic scheme.

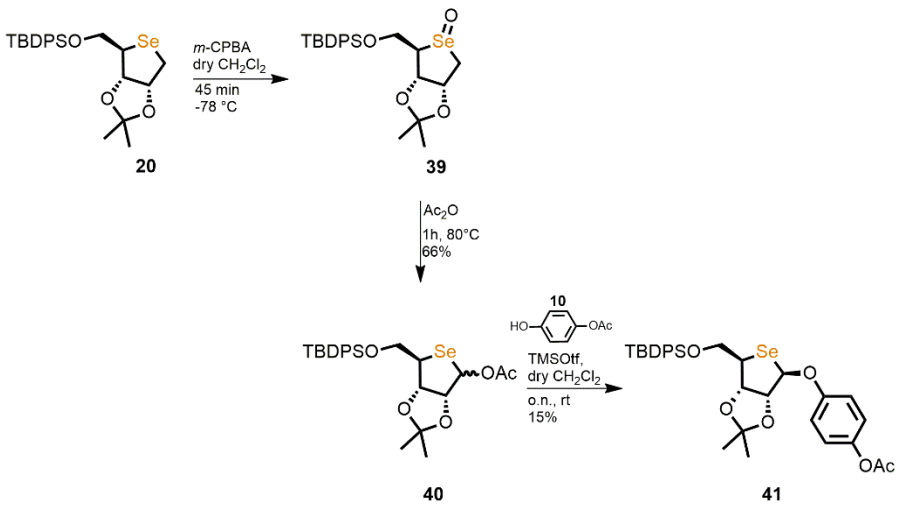
In the new synthetic route, based on the Pummerer rearrangement, the oxidation of Se atom in the sugar ring using *m*-chloroperoxybenzoic acid (*m*-CPBA) at -78°C led to the corresponding intermediate **37** as a single diasteroisomer in 76% yield. However,

the latter, that unlike the literature was found to be stable after purification by silica gel column chromatography, by treatment with anhydrous acetic anhydride at 80 °C, afforded the desired product **38** only in 16% yield, (Scheme 14). Unfortunately, beside a 10% of unreacted starting substrate, the main product is still under investigation. Actually, data analysis was not conclusive.



Scheme 14. Pummerer reaction.

On the contrary, as reported in literature, when the same procedure was employed starting from the corresponding D-donor **20**, the selenoxide **39** gave the glycosil acetil **40** in 66% overall yield (Scheme 15).



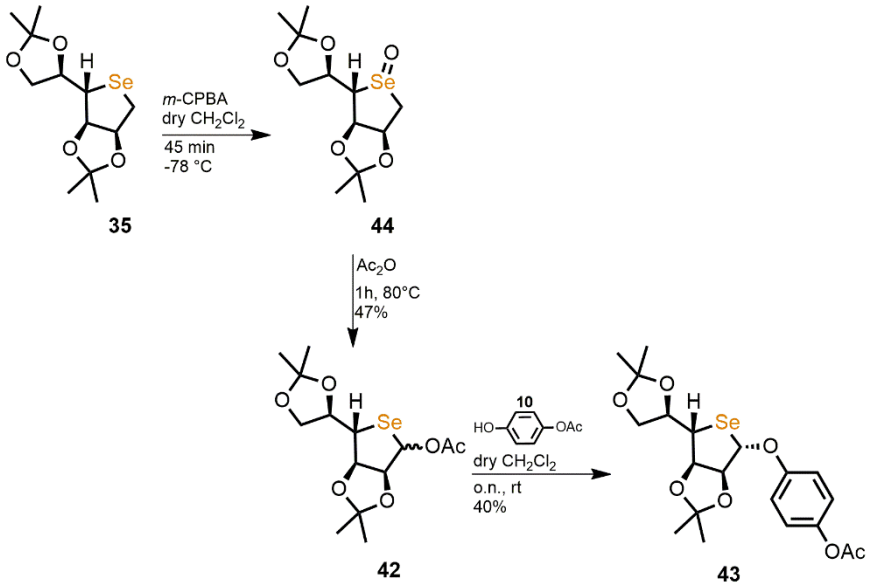
Scheme 15. Glycosilation

Also in this occasion, the unusual behaviour of selenium has not a simply rationalization. It is noteworthy that Pummerer rearrangement is likely to depend on the acidity of the α-proton.[276] As consequence, to control the regioselectivity (as the anomeric position or the 4-position) of the similar 1,4-anhydro-4-thio-D-ribitol, electron donating and sterical hindering protecting groups of OH in C-3 and C-5 positions were used. These modifications should be done in future, in order to elucidate the Pummerer pattern of the L-isomer **38**.

Concerning the glycosylation procedure, we explored different reaction conditions using our designed D-selenoglycosil donor **40** and monoacetylated hydroquinone **10** as glycosil acceptor, in a model reaction (Scheme 15). In these preliminary attempts, the *O*-glycoside formation was obtained in low but promising yield (15%). This result was achieved by treatment of a CH_2Cl_2 solution of donor **40** and phenol acceptor **10** in presence of TMSOTf at room temperature, under strict anhydrous conditions (4 Å MS). The coupling product **41** was obtained in full β-stereoselectivity as clearly indicated by the small $^3J_{1,2}$ value (<0.5 Hz).

This result prompted us to approach to the glycosylation reaction starting from the six membered selenosugar **35** (Scheme 16). Actually, that will allow us to compare the

biological activities of larger set of homologs. For this purpose, the Pummerer rearrangement described above was exploited to obtain the activation of the C-1 position by forming the selenoacetal intermediate **42**. The latter was achieved in 47% overall yield, confirming for the first time, to the best of our knowledge, the versatility of the transposition already observed in the five-term homologue. Next the introduction of the monoacetylated hydroquinone **10** at the anomeric center using TMSOTf in the presence of molecular sieve 4 Å was investigated, providing the expected glycoside **43** in 40% yield. Of note, the reaction was highly selective, giving only the α-isomer.



Scheme 16. Glycosilation of six membered selenosugar.

Therefore, both reactions of glycosylation yielded the corresponding glycoside with high selectivity. The presence of the isopropylidene group may be responsible for this outcome, however these findings are preliminary and although promising improved conditions should be found in future.

3.2. Activity assays

3.2.1. Chemical assays

Following synthesis and purification, the new glycoconjugates were investigated for their reducing and scavenging abilities, consistent with the mechanisms generally accepted for antioxidant compounds. In collaboration with Prof. Jacob's group at Saarland State University, we performed two different types of chemical antioxidant tests. Namely, FRAP (ferric reducing/antioxidant power) and DPPH (2,2-diphenyl-1-picrylhydrazyl) assays were carried out to verify the reducing and scavenging abilities, respectively.

3.2.1.1. DPPH assay

The DPPH· is a π-radical that is present in its monomeric form both in solid state and in solution. The radical is characterized by a very low reactivity and it is largely used in the evaluation of the antioxidant ability of chemicals.[277] DPPH· is soluble in various organic solvents, but not in water; indeed, it is typically dissolved in methanol or ethanol. In solution, DPPH· absorbs intensively in the visible region (513–528 nm), with ε values (L mol^{-1} cm^{-1}) being 10.800 (methanol, 515 nm) and 16.350 (ethanol,520 nm).[278] The representative deep purple colour of the DPPH solution is due to the unpaired electron and its partial spin density delocalization across the conjugated system of the aromatic rings. The principal mechanism of radical scavenging is the H atom transfer by a primary antioxidant, which leads to the formation of the hydrazine DPPH-H. This transformation causes a gradual change in the colour of the solution from violet to pale yellow upon formation of the respective hydrazine.[279]

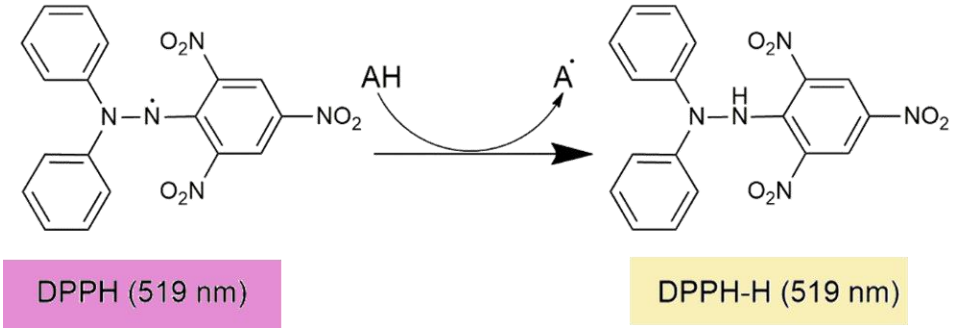


Figure 31. DPPH mechanism.

This change of colour, according to the principle of Blois,[280] is proportional to the concentration of radicals that are being scavenged, and it can be easily measured and monitored by UV-vis spectroscopy, since the reduction in radical concentration leads to a decrease in absorbance.

We tested our molecules at final concentrations included within 400 and 3.125 µM. In this range, the linearity between the amount of scavenged radical and absorbance signal is maintained. Furthermore, we measured the absorbance value after 30 minutes of reaction, when the absorbance resulted stable. We presented the related data as Efficient Concentration (EC_{50}), which is the concentration of antioxidant needed to scavenge 50% of the radicals present in the solution (Table 4). The EC_{50} is inversely correlated to the antioxidant capacity of the compound, thus, the lower the EC_{50}, the higher the antioxidant capacity. The EC_{50} of the compounds were compared to EC_{50} of the ascorbic acid (AA), a potent antioxidant, used as reference standard.

Table 4. EC_{50} of all the tested compounds.

Compound	*EC_{50} (μM)*
7	N.A.
21	N.A.
23	9.02
22	35.31
24	40.03
25	35.14
7a	N.A.
21a	N.A.
23a	9.57
22a	57.38
24a	38.16
26	8.09
AA	26.96

N.A.= not active

The highest DPPH scavenging capacity was given by sample **23, 23a** and **26**, thus the catecholic function appears as the most active functional group. This was confirmed by the activity of sample **24**, containing one -OMe instead of an -OH. Moreover, compounds where the polyphenolic moiety is not present, such as **7**, **7a**, **21** and **21a** are not active, evidencing that, as expected, Se does not exert its antioxidant effects through the investigated mechanism.

3.2.1.2. FRAP assay

The FRAP assay is a direct, relatively simple, quick, and inexpensive method of measuring the antioxidant activity of reductive (electron donating) antioxidants in a test sample.[281] It detects the antioxidant activity of a compound by measuring the

oxidant reduced form augmentation and the electron flow (actual or potential) from reductant to oxidant. FRAP assay has high sensitivity and reproducibility, since the stoichiometric factors of reacting antioxidants are constant over a wide range of concentrations; moreover, small differences in reaction conditions do not significantly affect the results. These characteristics help the reproducibility of the test prompting its large use in this field of research. In detail, in the FRAP assay, a Fe^{3+} containing complex, $[Fe^{3+}$-(2,4,6-Tris(2-pirydyl)-*s*-triazine$)_2]^{3+}$, is reduced by an electron donating antioxidant present in the reaction mixture.[281] The reduction of Fe^{3+} to Fe^{2+}, causes the formation of $[Fe^{2+}$-(2,4,6-Tris(2-pirydyl)-*s*-triazine$)_2]^{2+}$ and the shift of the solution colour from pale orange to blue; to measure this conversion, a low pH buffer is required.[282] The intensity of the blue colour depends on the amount of Fe^{3+} that is reduced to Fe^{2+} (electron transfer) and is measured as an increase in the characteristic absorption at 593 nm.[283]

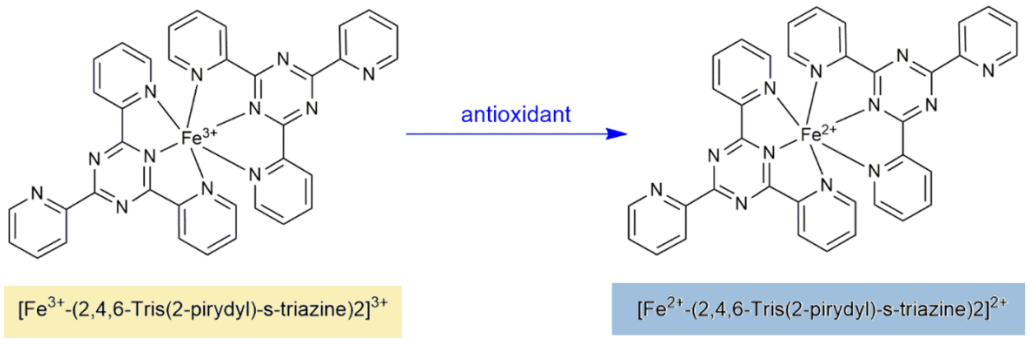


Figure 32. FRAP mechanism.

The conditions of the reaction (temperature, pH, reagent and sample volumes, reaction duration) are fixed. Results are expressed as EC_{50} values (

Table 5). EC_{50} of FRAP capacity is the concentration of sample or standard that can exhibit 50% of FRAP capacity. The EC_{50} is inversely correlated to the antioxidant capacity of the compound. The lower the EC_{50}, the higher the antioxidant activity of the compound is.[284] As above, AA was used as standard.

Table 5. EC_{50} of all the tested compounds.

Compound	*EC_{50} (µM)*
7	N.A.
21	N.A.
23	47.71
22	13.06
24	46.08
25	38.44
7a	N.A.
21a	N.A.
23a	24.10
22a	34.47
24a	11.97
26	35.80
AA	29.17

N.A.= not active

All not active compounds in DPPH were also not active in FRAP. Whereas, compounds **22** and **24a** -not active in DPPH - shown a good activity in this assay. But, of note, it's the data of compound **23a**, which was very efficient in DPPH, and kept a good activity also in this investigated mechanism, resulting better than AA.

3.2.2. Cellular Assays

3.2.2.1. MIC assay

With the aim to investigate the potential cytotoxic activity of our new compounds, a preliminary microbial toxicity assay was performed in simple cellular models. Specifically, the **minimum inhibitory concentration (MIC) assay** was executed in during the stay at the laboratory of Prof. Jacob. This assay is a low cost and rapid

screening and is widely used, since it allows determining the minimum concentration at which a molecule inhibits 100% of bacterial/yeast cellular growth. It consists of a bacterial inoculation in a liquid growth medium, in the presence of different concentrations of the compound under test. With the aim to investigate this activity in different models, three cellular lines were selected. Namely, a bacterial gram negative (*Escherichia coli*), a gram positive (*Lactobacillus plantarum*) and one eukaryotic cell line (*Saccharomyces cerevisiae*) were selected. As result, despite investigating an ample range of concentrations (within 1.25 mM and 19 μM), we did not find any MIC for all the examined compounds.

To support these findings, we calculated the percentage of growth inhibition (GI) for each compound, at the maximum concentration value. Again, we could not find a significant reduction in growth when comparing the treated cells with the positive control (cells with medium only). The GI value was in most cases no more than 15%. The hydroquinone conjugate showed just a modest inhibition (GI=60%) in the only *lactobacillus plantarum*. The latter result, matched with the previous antioxidant data, showing just a slight activity, prompted us to ruled out its use as reducing molecule and we did not investigate its properties further.

On the contrary, for the other first generation glycoconjugates, the non-toxicity in this kind of biological systems, allowed us to carry on the activity evaluation in more complex systems.

3.2.2.2. Biological human cell-based assays

With the aim to further investigate the biological activity of our compounds, we performed in vitro experiments in a human cell line, in collaboration with the group of Dr Manfra. Specifically, the human skin cell line HaCaT was used. HaCaT is a nontumorigenic monoclonal skin cell line, adapted to long term growth; it exhibits normal morphogenesis and is used as keratinocyte cellular model.[285] Keratinocytes represent 95% of the epidermal cells and are the first contact area between the body

and the external environment; as such, they can be exposed to a series of environmental assaults, which, in turn, can trigger inflammatory damage, oxidative stress, DNA damage, cellular as well as tissue injuries and ultimately cell death.[286] Thus, their role in the skin inflammatory and immunological responses, as well as in wound healing, is well recognized. HaCaT cell line is widely used as an in vitro model of epidermal cells. On this basis, we selected HaCaT as a valid cellular model for our experiments.

3.2.2.2.1. MTT assay

Firstly, we investigated the effect of our compounds on cellular viability by performing an **MTT assay** (following the Mosmann method).[287]

The MTT assay is based on the conversion of the water-soluble yellow dye MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to an insoluble purple formazan by the action of mitochondrial NAD(P)H-dependent oxidoreductase enzymes.[288] Formazan is then solubilized and the concentration determined by optical density at 570 nm. Since only living cells can do this conversion, this assay is broadly used to measure the in vitro cytotoxic effects of drugs on cell lines or primary cells isolated from patients. In the MTT assay, the linear relationship between metabolically active cells and the produced colour is established, thus allowing an accurate quantification of changes in the rate of cell death or proliferation.[289] MTT is the commonly applied method to evaluate cell viability and cytotoxicity for drugs' screening. We showed the related data (Table 6) as the mean value of percentage of dead cells, which was calculated as follow:

$$\% \text{ dead cells} = 100 - (\text{OD treated cells}/\text{OD control cells}) \times 100$$

Table 6.MTT assay's results.

Compound	*Mean ± SEM of % dead cells*
7 (100 µg/mL)	3.1± 0.8
(50 µg/mL)	0.0± 0.0
(25 µg/mL)	0.0± 0.0
(12.5 µg/mL)	0.0± 0.0
7a (100 µg/mL)	13.1± 0.5
(50 µg/mL)	7.2± 0.4
(25 µg/mL)	7.3± 0.3
(12.5 µg/mL)	5.0± 0.8
12 (100 µg/mL)	6.0± 0.2
(50 µg/mL)	0.0± 0.0
(25 µg/mL)	0.0± 0.0
(12.5 µg/mL)	0.0± 0.0
23 (100 µg/mL)	0.0± 0.0
(50 µg/mL)	0.0± 0.0
(25 µg/mL)	0.0± 0.0
(12.5 µg/mL)	0.0± 0.0
23a (100 µg/mL)	0.0± 0.0
(50 µg/mL)	0.0± 0.0
(25 µg/mL)	0.0± 0.0
(12.5 µg/mL)	0.0± 0.0

Compound	*Mean ± SEM of % dead cells*
24 (100 µg/mL)	8.0± 0.8
(50 µg/mL)	6.7± 0.3
(25 µg/mL)	0.0± 0.0
(12.5 µg/mL)	0.0± 0.0
24a (100 µg/mL)	11.0± 0.4
(50 µg/mL)	2.3± 0.3
(25 µg/mL)	3.3± 0.6
(12.5 µg/mL)	0.0± 0.0
25 (100 µg/mL)	13.4± 0.4
(50 µg/mL)	6.1± 0.5
(25 µg/mL)	6.2± 0.4
(12.5 µg/mL)	5.9± 0.1
26 (100 µg/mL)	1.8± 0.4
(50 µg/mL)	0.0± 0.0
(25 µg/mL)	0.0± 0.0
(12.5 µg/mL)	0.0± 0.0
DMSO (0.33%)	8.8± 0.0
(0.16%)	7.3± 0.2
(0.08%)	6.2± 0.0
(0.04%)	5.7± 0.0

In all cases the percentage value does not exceed 13%, supporting the general non-toxicity of the products. Of note, only two compounds, indeed, showed this value, in addition it does just at the highest concentration of 100 µg/mL, which is a very high value. If we consider the concentration value of 50 µg/mL, we observe a total non-inhibition in cellular growth.

Thus, we can conclude that our new glycoconjugates do not show any cytotoxic activity, and this finding is validated in different cellular models.

3.2.2.2.2. ROS assay

As stated above, with the aim to test the antioxidant power of all glycoconjugates in a cellular context, we carried out a fluorescence assay to indirectly measure the ROS release in HaCat cells. This assay is based on the use of a specific fluorochrome, the dichlorodihydrofluorescein diacetate (DCFH-DA). DCFH-DA is one of the most used probes for detecting intracellular H_2O_2 and oxidative stress conditions. This probe permeates the plasmatic membrane, entering into the cells, where it is hydrolysed to the DCFH carboxylate anion, that remains trapped into the cell. Two-electron oxidation of DCFH gives the formation of a fluorescent product, dichlorofluorescein (DCF), which can be measured. The resulting signal is directly related to the presence of ROS in the investigated system and it is largely used to evaluate the ROS presence.

As showed in the following plot (Figure 33), the DCF's fluorescence intensity was measured in:

- HaCaT cultured in standard conditions (c);
- HaCat stimulated with lipopolysaccharide (LPS) to induce oxidative stress (red bar);
- HaCaT incubated with both LPS and different concentrations of each glycoconjugate (50 and 25 µg/mL).

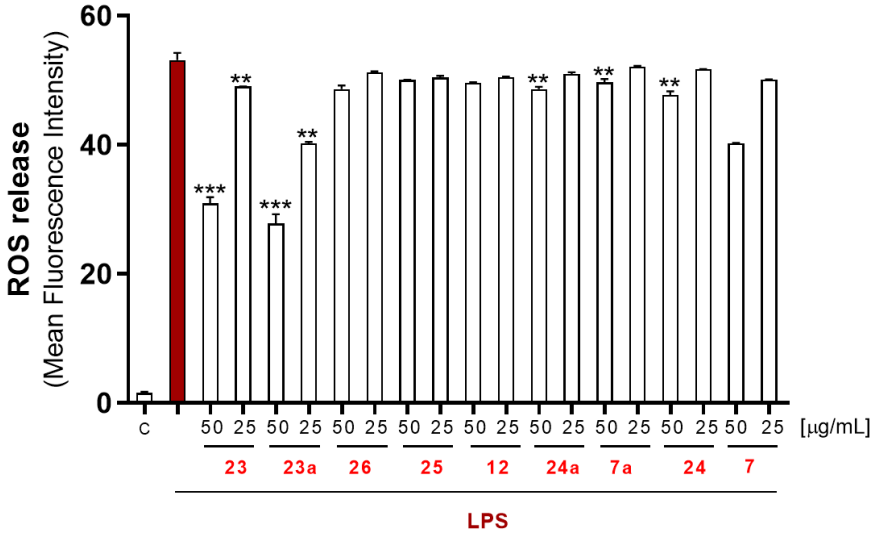


Figure 33. ROS assay results.

This graph shows that the release of ROS significantly decreases only when cells are incubated with caffeic containing compounds (**23** and **23a**). In the following table, the assessment of ROS release is numerically reported as percentage of released ROS.

Table 7. ROS assay results.

Compound	***Mean ± SEM of % ROS release***
23 (50 µg/mL)	31.7± 0.7
(25 µg/mL)	7.5± 0.2
23a (50 µg/mL)	38.2± 0.7
(25 µg/mL)	24.2± 0.4
26 (50 µg/mL)	9.1± 0.0
(25 µg/mL)	3.9± 0.1
25 (50 µg/mL)	5.8± 0.
(25 µg/mL)	5.0± 0.4

12 (50 µg/mL)	6.6± 0.1
(25 µg/mL)	5.3± 0.6
24a (50 µg/mL)	9.1± 0.6
(25 µg/mL)	3.7± 0.0
7a (50 µg/mL)	6.9± 0.3
(25 µg/mL)	2.3± 0.6
24 (50 µg/mL)	10.1± 0.0
(25 µg/mL)	2.6± 0.0
7 (50 µg/mL)	24.2± 0.1
(25 µg/mL)	5.7± 0.1

The data gathered so far allow concluding that at least one of the compounds synthesized, the conjugate with caffeic acid, is a good antioxidant. Furthermore, we are confident that additional investigation in diverse cellular context will shed light on the contribution of Se to the antioxidant activity of the glycoconjugate.

III APPLICATIONS IN TRANSDERMAL DRUG DELIVERY

4. APPLICATIONS IN TRANSDERMAL DRUG DELIVERY

It is well known that Se owns different and opposite biological and chemical proprieties, depending on the dose (hormesis). Due to this, it is considered a *Janus element*.[196] At low concentration, Se acts as a potent antioxidant, cytoprotective and chemoprotective agent;[12] furthermore, it is involved in a series of biological processes, as physiologically required element. By contrast, at high concentrations, selenium acts as a pro-oxidant, inducing thiol groups oxidation, and causing oxidative cellular damage. Indeed, this specific activity is used to induce cell death in certain kind of tumours.[197,198]

In this work, we firstly synthesised new selenium-containing glycoconjugates in order to investigate their antioxidant capacity. Then, in co-operation with the industrial partner of this PhD project - Avicenna Natural Institute – we performed a market research to hypothesise and identify a possible market niche for our products. Thus, exploring various applicative possibilities of the synthesised molecules, and considering the specific properties of selenosugars, which are our reference system, we focused on innovative drug delivery's platforms for wound healing and wound dressing. It has been proved, indeed, that selenosugars are involved in skin repair's mechanisms.[18]

The development of this device was possible thanks to the support and collaboration with the group of Dr Luca De Stefano from CNR-ISASI, Naples and it is actually subject of a patent application (n.102021000012806, 18/05/21).

In particular, taking into account the avowed advantages of using antioxidants in wound healing,[18,19] and the wide use of novel biocompatible hydrogels in wound dressing,[20] we developed a new device for transdermal delivery of antioxidants. In details, the system consists of a high biocompatible thin polymeric hydrogel membrane containing the previously synthesised organoselenium compounds.

4.1. Wound healing

Wounds' treatment is a relevant issue in health care and represents a prevalent condition. Wounds can be temporary, if they are caused by specific events (e.g., a surgery), but they can also represent a chronic condition, when they result from other physio-pathological circumstances such as diabetes[290] or prolonged bedrest. Depending on the cause, the Wound Healing Society differentiates chronic wounds into four categories: pressure ulcers, diabetic ulcers, venous ulcers and arterial insufficiency ulcers.[291] Although their prevalence and incidence are difficult to evaluate because of their various management across different regions in the world,[292,293] it is estimated that 1 to 2% of the population will experience a chronic wound during their lifetime in developed countries.[292] The incidence of chronic wounds is increased in the elderly population, in which also wound closure capacity usually declines.[294] This phenomenon has gained even more importance in the last decades during which the elderly population has grown steadily. Furthermore, the chronic wound repair is often impaired,[295] leading to subsequent complications that influence the required clinical assistance, as well as the necessary economic resources.[296] In the USA, for example, chronic wounds are reported to affect 6.5 million patients, with more than US$25 billion spent by the healthcare system each year, for the treatment of wound-related complications.[297]

Wound healing is a complex and multi-factorial process that involves a series of biological pathways,[298] including inflammation and redox regulation.[299] Wound healing can be divided in five different steps, such as haemostasis, inflammation, angiogenesis, cell proliferation and tissue remodelling. Redox signalling and increased oxidative stress are normally involved in all these steps (Figure 34).[300]

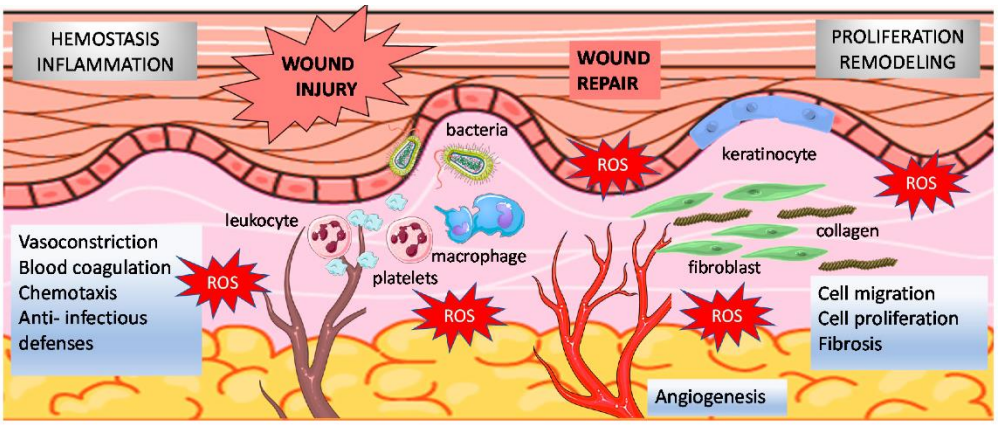


Figure 34. Wound's repairing phases and ROS involvement.
Sanchez at al.[19]

Haemostasis typically begins immediately after an injury occurs, in order to stop the bleeding. Platelets are key cells in this phase: they seal off the injured blood vessel – stopping the bleeding - and activate chemotactic recruiting of inflammatory cells like macrophages and leukocytes. Moreover, platelets release cytokines and growth factors to start the repairing of the injured area and promoting the inflammatory response.[301]

Migration of inflammatory cells at the site of injury participates to microorganism killing, preparing for successful healing.[19] Furthermore, the recruited and activated immune cells start releasing a series of growth and haemostatic factors that promote both the repairing of blood vessels and the inflammatory response. This environment increases ROS' production, and, in turn, oxidative stress. The proliferative phase generally follows and overlaps with the inflammatory phase. It is characterised by the macrophage switch from an inflammatory to a proliferative phenotype.[302] Thus, the subsequent production of specific growth factor stimulates the proliferation of involved cellular types leading to re-epithelialization and tissue rebuilding processes. Remodelling and maturation phases allow the termination of wound repair. During these processes, the components of extracellular matrix are restored and the proliferation is significantly reduced to the normal homeostatic equilibrium.[302]

4.2. Redox regulation in wounds

Rising data suggest that ROS are key regulating factors of healing processes. They are, indeed, physiologically required, but, when in excess, ROS cause oxidative damage that is considered the main cause of non-healing chronic wounds. At low level, ROS are involved in the killing of microorganism at the injured site and promote angiogenesis and re-epithelialization, favouring effective wound closure.[19] Moreover, ROS promote the activation of several transcription factors including nuclear factor kappa B (NF-kB), one of the main actors of inflammation.[19] By contrast, altered levels of ROS lead to the impairment of healing process. Among them, there is H_2O_2, which is mostly generated by neutrophils and macrophages via NADPH oxidase.[303]

Interestingly, restoring the redox balance – even if in chronic wounds, characterised by a persistent oxidative stress - has been shown to improve inflammatory skin conditions[300] and to restore the healing process.[304–307] In light of the above, different antioxidant approaches have been investigated to understand their potential beneficial effects in wound repair. For instance, intense research has been done to evaluate the impact of delivering antioxidant and anti-inflammatory molecules to the site of the wound, showing beneficial effects.[19]

With this aim, pharmaceutical and medical companies have been proposed and launched on the market a plethora of wearable devices that are more and more patient-friendly, comfortable and smart. In particular, a series of materials have been investigated and utilised for the fabrication of so-called “smart patches” which, rather than only isolating the wound, are also able to actively participate in the wound care process, thanks to the antioxidant substances they contain.[308]

4.3. Photo-crosslinkable hydrogels

Due to their properties, hydrogels are the most commonly used biomaterials in wound dressing (Figure 35). Indeed, they own a series of characteristics like good

biocompatibility and biodegradability, high water content and flexibility.[309,310] Furthermore, thanks to their hydrophilic polymeric 3D-configurations, hydrogels are water-swollen systems that are able to absorb tissue exudates, thus promoting fibroblast proliferation and keratinocyte migration, allowing oxygen to permeate and protecting wound from microbial invasion.[311]

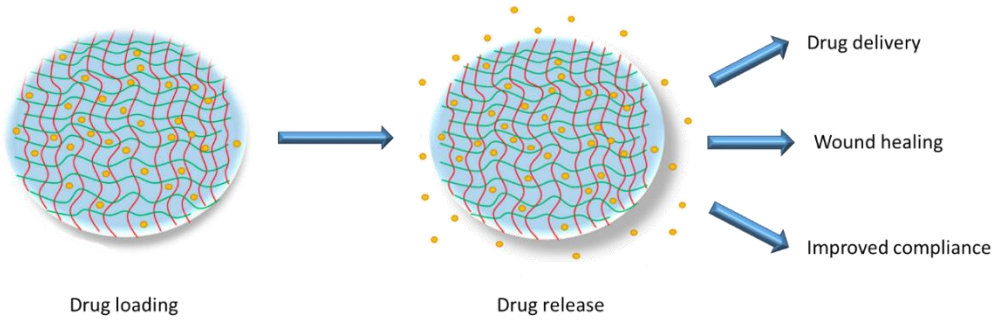


Figure 35. Polymeric delivery system.
Adapted from Harrison et al[311]

Hydrogels have been first introduced as crosslinked 2-hydroxyethylmethacrylate (HEMA) hydrogel by Wichterle and Lim.[312] Since then, they have been utilized in numerous biological biomedical applications,[313] like drug delivery, tissue engineering etc. Thanks to their properties, such as high biocompatibility, non-adhesion, good elasticity and similarity to biological tissues,[314] these polymers are used as constituent materials of different systems like thin membranes and scaffolds for cellular implantation.

According to their nature, hydrogels can be classified in:

- Natural: are the most biocompatible. They mimic the biological components of extra cellular matrix (ECM). An example of natural hydrogel is Matrigel™. It consists of a basement-membrane extract from Engelbrecht–Holm–Swarm (EHS) mouse sarcoma cells, and gels made from fibrin and hyaluronic acid. Despite their biocompatibility, natural hydrogels may present batches'

variability which affect the reproducibility of experiments and limits large scale production.

- Synthetic: they have more controllable mechanical and chemical properties, offer a higher reproducibility, but less biocompatibility. The most widely used is poly-etylene-glycole (PEG) and its derivatives.
- Hybrid: they are made of both natural and synthetic polymers. This combination joins the mechanical strength of a synthetic non-natural hydrogel with the biocompatibility and recoverability of natural hydrogels.

Hydrogels have been especially attractive for a wide range of biomedical applications, as they can crosslink under mild conditions and their biochemical, biophysical and mechanical properties can be modulated depending on requirements. For instance, flexibility can be tuned by the temperature or the crosslink degree within the structure.[311]Thanks to their chemical physical properties, hydrogels are ideal candidates for wound dressings, and some of them are currently commercially available.[315] Furthermore, hydrogels can be used to incorporate and deliver bioactive molecules such as antibiotics, drugs or natural compounds.[316] When placed on the wound surface, they can exchange embedded molecules with the absorbed exudates (Figure 36).[317]

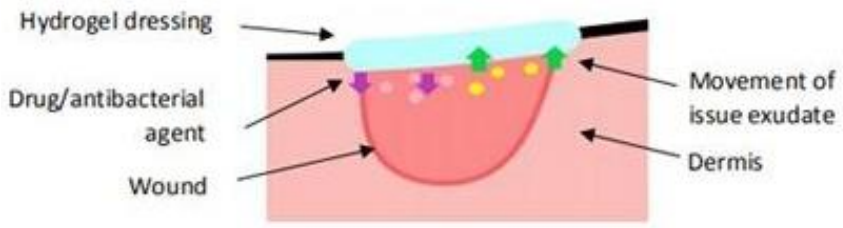


Figure 36. Exchange exudates/drugs

Taking into account the potential applications of hydrogels and the beneficial effects of antioxidant treatment in wound healing, we explored the idea of designing a new device for wound dressing which actively participate in the healing process, thanks to the antioxidant molecules therein embedded.

4.4. Results and discussion

Among biocompatible hydrogels, poly(hydroxyethyl methacrylate) (HEMA) and poly(ethylene glycol) (PEG) are particularly interesting since they allow to generate neutral synthetic polymers, exploiting their derivatives.[315] Besides, poly(ethylene glycol) diacrylate(PEGDA), used as crosslinking agent, owns a high biocompatibility and contributes to maintain the HEMA amphiphilic properties (Figure 37). Finally, it should be taken into account that PEGDA can be easily excreted via kidney and liver giving non-toxic metabolites.[318]

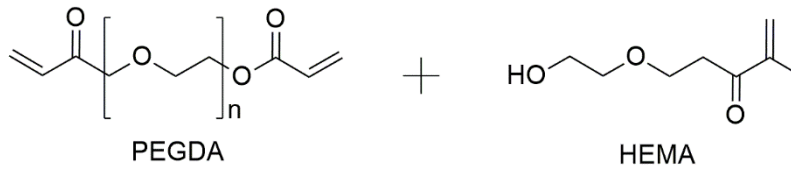


Figure 37. Synthetic monomers.

Hydrogels based on HEMA and PEGDA are, indeed, largely studied materials and widely utilised in biomedical applications for their aforementioned non-toxicity and non-immunogenic properties. In fact, Food and Drug Administration (FDA) approved their use for medical purpose, including human intravenous injection and oral and dermal applications.

Our work started with the fabrication of a series polymeric thin membranes constituted by different pre-polymers in various combination ratios. With the aim to get the best combination of mechanical and physical proprieties to allow a proper delivery of previously synthesised molecules, we selected the polymeric blend constituted by a diacrilic pre-polymer – PEGDA 250 (MW 250 Da) and a monoacrilic one –HEMA, in a 70:30 ratios.

The membranes were fabricated by photopolymerization: the UVA light exposure, in presence of a photoinitiator, induce the free-radical initiated chain reaction through a chain growth mechanism. Polymeric membranes were designed to control the delivery

of the previously synthesised organoselenium compounds: with this aim the molecules were mixed in preselected concentration in the prepolymeric blend, so directly incorporated in crosslinked solid film. The release kinetics of synthesised molecules in a simulated body fluid, constantly stirred at 37 °C, was monitored by HPLC. The dynamics of release of two different embedded molecules, SeTal **31** (Figure 38) and hydroquinone conjugate **22a** (Figure 39), are reported as percentage of release (weight) in time function. After the release, the structure integrity of used molecules was investigated by ^{1}H NMR; spectral data showed that both compounds preserved the characteristics of the structure.

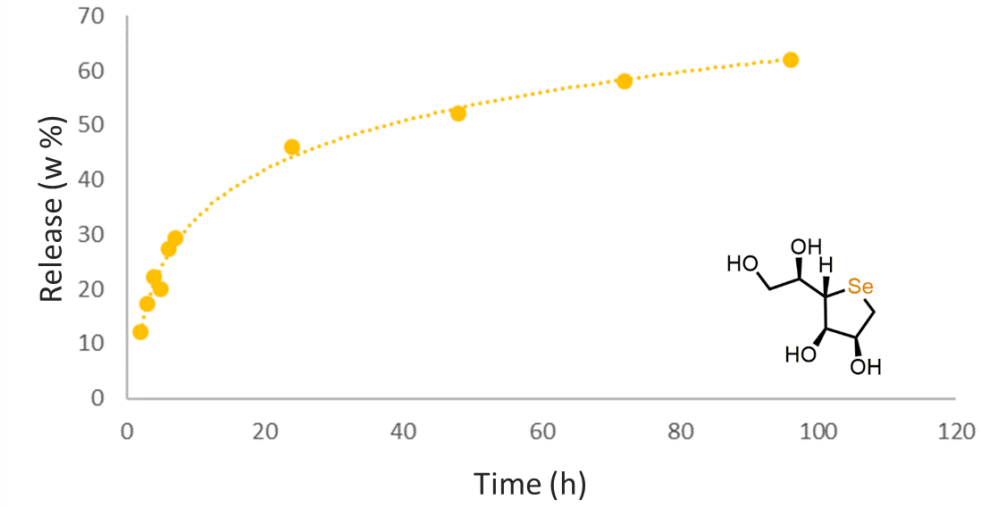


Figure 38. Release dynamics of compound **31**

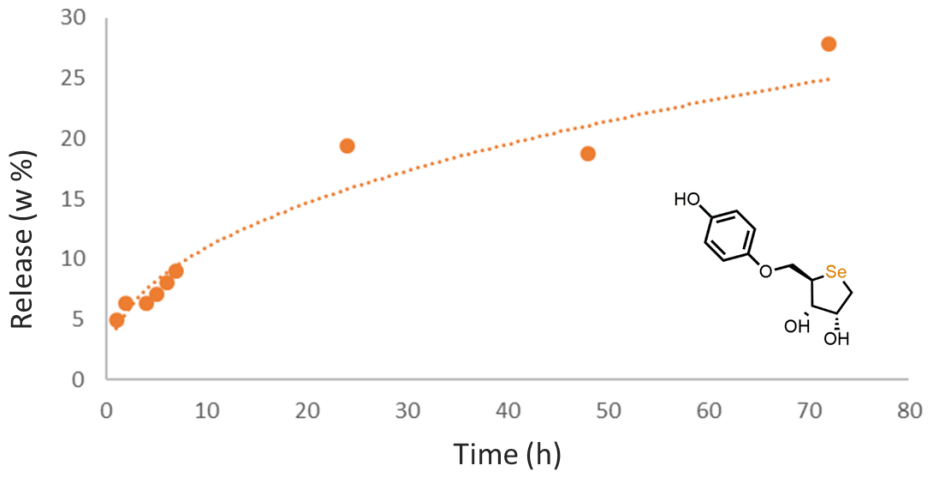


Figure 39. Release dynamics of compound **22a.**

As can be seen, both delivery systems release in a controlled manner under these conditions. However, when comparing the devices of the SeTal and the hydroquinone conjugate, the SeTal system exhibits a much faster release than the hydroquinone conjugate. The cumulative release reaches 62% after 96 hours for the SeTal, while 28% after 72 hours for the hydroquinone conjugate. This gap is evidently due to the relative difference in size and hydrophilicity of the hanging groups.

Therefore, a specific controlled release of sugar conjugates can be achieved choosing suitable polymers and mixture formulations, that result in a mesh size (ε) and an average molecular weight between crosslinks (Mc). More broadly, taking into account the aforementioned parameters, the nature and the structure of the incorporated molecule, active systems with the required delivery rate can be developed. In conclusion, a proper choice of hydrogel materials has the potential to provide the desired release pattern for a broad range of application fields.

CONCLUSIONS

5. CONCLUSIONS

In this Thesis new selenium containing glycoconjugates have been presented. The studies here reported provided valuable information for the use of these scaffolds as antioxidant molecules. Actually, the project leads to the following conclusions:

1. The pathway used for both, five and six membered, selenium sugars afforded the targets in high yields.
2. Efficient synthetic routes towards seleno containing glycoconjugates have been developed. These results pave the way for the design and synthesis of new antioxidants.
3. The versatility of Mitsunobu reaction mechanism in covalently link the primary alcoholic function of a seleno glycosyl donor (both 5 and 6 membered) and a phenolic moiety acceptor, to obtain the corresponding glycoconjugates, has been demonstrated. Moreover, the specific reaction conditions allowed to link with fairly yields both carboxylic and phenolic acceptor units.
4. Concerning the five membered seleno sugars, it has been found that the exploited Mitsunobu reaction affords only glycoconjugates with an *anti*-configuration at C-4 position, both if seleno donor is in L or D configuration. This unexpected result prompted us to exploit the more convenient and efficient L-route to obtain the selenoscaffold.
5. Preliminary coupling reactions have been evaluated to achieve *O*-glycoside compounds consisting of selenosugar scaffolds and monoacetylated hydroquinone. The synthetic procedure, based on Pummerer rearrangement followed by a glycosylation, gave two derivatives differing for the selenosugar part. Further studies are underway in our labs to elucidate mechanistic pathways and improve this simple and promising transformation.
6. It has been demonstrated, moreover, that the glycosylation reactions are highly stereoselective since they furnished only the α-anomer when the

glycosil donor was represented by SeTal derivative and, conversely, only the β ones, when the starting product was the D-seleno ribose. This finding suggests that the isopropylidene protecting group could play a role in the stereoselective control of the reaction, due to its steric hindering.

7. All the new synthetized glycoconjugates were tested for their antioxidant activity trough two different chemical assays (DPPH and FRAP), showing a general medium activity for both investigated mechanisms.
8. All the new synthetized glycoconjugates were tested for their potential cytotoxic activity in different biological models. They did not show any toxicity both in bacterial and eukaryotic cell lines.
9. Among them, the caffeic containing glycoconjugate showed a good antioxidant activity in human skin cell line HaCat, by counteracting the ROS release after LPS stimulation.
10. A new smart device for transdermal drug delivery was designed and fabricated. In details, a thin polymeric membrane containing antioxidants was fabricated.
11. Preliminary experiment of release dynamics of the compounds was performed and a controlled release was found, demonstrating the possible industrial application of our compound which consists to counteract oxidative stress in wound environment. Further investigations are ongoing on the use of this new and interesting approach.

EXPERIMENTAL PART

6. EXPERIMENTAL PART

General Information

The ^{1}H- and ^{13}C-NMR spectra were recorded at 500 and 125 MHz, respectively, on a Fourier Transform NMR Varian 500 Unity Inova spectrometer or at 400 and 100 MHz, respectively, on a Bruker DRX 400MHz spectrometer, using $CDCl_3$ as solvent unless otherwise specified. The proton couplings were evidenced by ^{1}H-^{1}H COSY experiments. The heteronuclear chemical shift correlations were determined by HMQC and HMBC pulse sequences. ^{1}H-^{1}H proximities through space within a molecule were determined by NOESY experiments. The thin-layer chromatography was performed with silica gel plates Merck 60 F_{254}, and the display of the products on TLC was accomplished with lighting UV lamp, molecular iodine, and in H_2SO_4–EtOH (95:5) and further heating until development of colour. The column chromatography was carried out using silica gel 70-230 mesh (Merck). Reagent-grade commercially available reagents and solvents were used (Aldrich, Fluka, Sigma). The TopSpin 4.0.6 software package was used for the analysis.

2,3-O-*Isopropylidene-D-ribofuranose (**2**)*

To a magnetically stirred suspension of anhydrous triphenylphosphine polymer-bound (525, mg, ca 2.0 mmol) in anhydrous acetone (6.0 mL) at r.t., a solution of I_2 (508 mg, 2.0 mmol) in the same solvent (18.0 mL) was added dropwise in the dark and under dry N_2 atmosphere. After 15 min, solid D-ribofuranose (150 mg, 1.0 mmol) was added in one portion to the suspension. TLC monitoring ($CHCl_3$–MeOH, 9:1) showed that the starting sugar was completely consumed within 30 min. The reaction mixture was then filtered, washed with acetone, and dried (Na_2SO_4). The evaporation of the solvent under reduced pressure gave a crude residue that was used directly in the next step without further purification (181 mg, 95%).

^{1}H NMR (500 MHz): δ 1.33 (s, 3 H, CH_3), 1.48 (s, 3 H, CH_3), 3.70 (dd, $J_{5a,5b}$ = 11.6, $J_{5a,4}$ = 1.5, 1H, H5a), 3.79 (dd, $J_{5b,5a}$ = 11.6, $J_{5b,4}$ = 1.8, 1 H, H5b), 4.30 (m, 1 H, H4), 4.51 (d, $J_{2,3}$ = 6.1 Hz, 1 H, H2), 4.84 (dd, $J_{3,2}$ = 6.1, $J_{3,4}$ = 1.0 Hz, 1 H, H3), 5.06 (bs, 1 H, H1$_\beta$). ^{13}C NMR (125 MHz): δ 24.8, 26.4, 65.2, 81.9, 86.5, 87.7, 106.7, 111.9. Anal. Calcd for $C_8H_{14}O_5$: C, 50.52; H, 7.42. Found: C, 50.60; H, 7.40. The NMR spectral data were identical to values in the literature.[260]

5-O-tert-*Butyldiphenylsilyl-2,3*-O-*isopropylidene-D-ribofuranose (**3**)*

TEA (0.28 mL, 2.0 mmol), DMAP (24 mg, 0.2 mmol), and TBDPSCl (0.26 mL, 1.5 mmol) at 0 °C were added to a magnetically stirred solution of **2** (190 mg, 1.0 mmol) in anhydrous CH_2Cl_2 (5.0 mL). The mixture was stirred at room temperature for 4 hours. As soon as the starting compound **2** was completely consumed (TLC monitoring), the organic layer was washed with brine and dried (Na_2SO_4). The evaporation of the solvent under reduced pressure gave a crude residue that was purified by silica gel column chromatography (hexane-ethyl acetate 97:3 (v/v)) to give the oily product **3** as a yellowish syrup (386 mg, 90%). The product was obtained as a mixture of both anomers, but the β anomer was the main product (α:β1:1.9).

^{1}H NMR (500 MHz): δ 1.08 (s, 9H$_\alpha$, CH_3), 1.12 (s, 9H, $CH_{3\beta}$), 1.34 (s, 3H, $CH_{3\beta}$), 1.42 (s, 3H, $CH_{3\alpha}$), 1.50 (s, 3H, $CH_{3\beta}$), 1.58 (s, 3H, $CH_{3\alpha}$), 3.71–3.63 (m, 2H, H5a$_{\alpha+\beta}$), 3.87–3.81 (m, 2H, H5b$_{\alpha+\beta}$), 3.96 (d, $J_{OH,1\alpha}$ = 11.4 Hz, 1H, OH$_\alpha$), 4.18 (m, 1H, H4$_\alpha$), 4.31 (m, 1H, H4$_\beta$),4.51 (d, $J_{OH,1\beta}$ = 10.6 Hz, 1H, OH$_\beta$), 4.63 (d, $J_{2,3}$ = 5.9 Hz, 1H, H2$_\beta$), 4.68 (dd, $J_{2,3}$ = 6.2 Hz, $J_{2,1}$ = 4.0 Hz, 1H, H2$_\alpha$), 4.74 (d, $J_{3,2}$ = 5.9 Hz, 1H, H3$_\beta$), 4.80 (d, $J_{3,2}$ = 6.2 Hz, 1H, H3$_\alpha$), 5.37 (d, $J_{1,OH}$ = 10.6 Hz, 1H, H1$_\beta$), 5.64 (dd, $J_{1,OH}$ = 11.4 Hz, $J_{1,2}$ = 4.0 Hz, 1H, H1$_\alpha$), 7.53–7.64 (m, 20H, Ar$_{\alpha+\beta}$); ^{13}C NMR (125 MHz): δ 19.1, 22.6, 25.0, 26.5, 27.0, 65.5, 66.1, 81.6, 87.1, 87.4, 98.0, 103.4, 127.7, 127.9, 128.0, 129.7, 130.2, 130.4, 134.8, 135.6, 135.7. Anal. Calcd. for $C_{24}H_{32}O_5Si$: C, 67.26; H, 7.53. Found: C, 67.20; H, 7.59.

5-O-tert-*Butyldiphenylsilyl-2,3*-O-*isopropylidene-D-ribitol (**4**)*

To a magnetically stirred solution of **3** (0.429 g, 1.0 mmol) in MeOH (10.0 mL), $NaBH_4$ (0.113 g, 4.0 mmol) at 0 °C was added. The mixture was stirred at room temperature for 2.5 hours. As soon as the starting compound **3** was completely consumed (TLC monitoring), the solvent was evaporated under reduced pressure and replaced with AcOEt. The organic layer was washed with brine, and dried (Na_2SO_4). The evaporation of the solvent under reduced pressure gave the pure product **4** as a colorless syrup (430 mg, >99%).

^{1}H NMR (500 MHz): δ 1.10 (s, 9H, CH_3), 1.31 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 3.10 (m, 2H, H4 and OH), 3.77–3.93 (m, 4H, H1 and H5), 4.14 (m, 1H, H2), 4.38 (m, 1H, H3), 7.40–7.46 (m, 6H, Ar), 7.67–7.70 (m, 4H, Ar); ^{13}C NMR (125 MHz): δ 19.3, 25.2, 26.9, 27.7, 60.9, 65.3, 69.7, 76.5, 77.6, 108.5, 127.8, 130.0, 132.8, 132.9, 135.6. Anal. Calcd for $C_{24}H_{34}O_5Si$: C, 66.94; H, 7.96. Found: C, 66.91; H, 8.02.

5-O-tert-*Butyldiphenylsilyl-2,3*-O-*isopropylidene-1,4-di*-O-*methanesulfonyl-D-ribitol (**5**)*

DMAP (2.4 mg, 0.02 mmol), TEA (1.1 mL, 8.0 mmol), and mesylchloride (0.309 mL, 4.0 mmol) at 0 °C were added to a magnetically stirred solution of **4** (430 mg, 1.0 mmol) in CH_2Cl_2 (14.0 mL), and the mixture was stirred at r.t. overnight. The organic layer was washed with brine and dried (Na_2SO_4). The evaporation of the solvent under reduced pressure gave a crude residue that was purified by silica gel column chromatography (hexane-ethyl acetate 6:4 (v/v)) to give the oily product **5** as a yellowish syrup (575 mg, 98%).

^{1}H NMR(500 MHz): δ 1.11 (s, 9H, CH_3), 1.38 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 3.06 (s, 6H, CH_3), 4.00 (dd, $J_{5a,5b}$ = 12.2 Hz, $J_{5a,4}$ = 3.8 Hz, 1H, H5a), 4.07 (dd, $J_{5b,5a}$ = 12.2 Hz, $J_{5b,4}$ = 2.7 Hz, 1H, H5b), 4.34 (dd, $J_{1a,1b}$ = 10.4 Hz, $J_{1a,2}$ = 6.4 Hz, 1H, H1a), 4.46–4.54 (m, 3H, H1b, H2, and H3), 4.85 (m, 1H, H4), 7.40–7.49 (m, 6H, Ar), 7.73–7.67 (m, 4H, Ar); ^{13}C NMR (125 MHz): δ 19.3, 25.4, 26.8, 27.4, 37.5, 39.3, 63.1, 68.3,

73.8, 74.9, 79.2, 109.5, 127.9, 130.1, 132.4, 132.5, 135.6. Anal. Calcd for $C_{26}H_{38}O_9S_2Si$: C, 53.22; H, 6.53. Found: C, 53.19; H, 6.60.

5-O-tert-*Butyldiphenylsilyl-1,4-deoxy-2,3*-O-*isopropylidene-4-seleno-L-lyxofuranose (**6**)*

$NaBH_4$ was added to a magnetically stirred suspension of selenium powder (166 mg, 2.1 mmol) in dry ethanol (13 mL) under N_2 atmosphere, and the mixture was stirred at r.t. until the color of the mixture changed from black to colourless. Then, compound **5** (586 mg, 1 mmol) in dry THF (8 mL) was added to the mixture which was heated at 60 °C overnight. Then, the solvent was removed and replaced with AcOEt. Next, the organic layer was washed with brine and dried (Na_2SO_4). The evaporation of the solvent under reduced pressure gave the pure seleno derivative **6** as a pale-yellow oil (470 mg, 99%).

^{1}H NMR (500 MHz): δ 1.08 (s, 9H, CH_3), 1.30 (s, 3H, CH_3), 1.48 (s, 3H, CH_3), 2.97 (dd, $J_{1a,1b}$ = 12.2 Hz, $J_{1a,2}$ = 1.5 Hz, 1H, H1a), 3.03 (dd, $J_{1b,1a}$ = 12.2 Hz, $J_{1b,2}$ = 4.7 Hz, 1H, H1b), 3.77 (m, 1H, H4), 3.93 (dd, $J_{5a,5b}$ = 10.2 Hz, $J_{5a,4}$ = 7.9 Hz, 1H, H5a), 4.15 (dd, $J_{5b,5a}$ = 10.2 Hz, $J_{5b,4}$ = 7.1 Hz, 1H, H5b), 4.77 (m, 1H, H2), 4.94 (dd, $J_{3,2}$ = 5.3 Hz, $J_{3,4}$ = 4.5 Hz, 1H, H3), 7.37–7.47 (m, 6H, Ar), 7.72 (ddd, J = 11.0, 7.9, 1.4 Hz, 4H, Ar); ^{13}C NMR (125 MHz): δ 19.3, 24.7, 26.1, 26.8, 29.8, 50.5, 63.0, 83.3, 84.9, 110.3, 127.6, 127.7, 129.6, 129.7, 133.5, 133.6, 135.7, 135.7. Anal. Calcd for $C_{24}H_{32}O_3SeSi$: C, 60.62; H, 6.78. Found: C, 60.59; H, 6.83.

1,4-Dideoxy-2,3-O-*isopropylidene-4-seleno-L-lyxofuranose (**7**)*

A solution of tetrabutylammonium fluoride (1.7 ml of a 1.1 M solution in THF, 2.0 mmol) was added to a magnetically stirred solution of seleno derivative **6** (475 mg, 1.0 mmol) in anhydrous THF (7.0 mL) at 0 °C under N_2, and the mixture was stirred at room temperature for 1 hour. The evaporation of the solvent under reduced pressure gave a crude residue that was purified by silica gel column chromatography (hexane-

ethyl acetate 6:4 (v/v)) to give the pure product **7** as a crystalline white solid (216 mg, 91%).

^{1}H NMR (500 MHz): δ 1.33 (s, 3H, CH_3), 1.53 (s, 3H, CH_3), 2.53 (brs, 1H, OH), 2.99–3.05 (m, 2H, H1), 3.66 (dt, $J_{4,5b}$ = 7.5 Hz, $J_{4,5a}$ = 5.0 Hz, $J_{4,3}$ = 4.2 Hz, 1H, H4), 3.91 (dd, $J_{5a,5b}$ = 11.5 Hz, $J_{5a,4}$ = 5.0 Hz, 1H, H5a), 4.00 (dd, $J_{5b,5a}$ = 11.5 Hz, $J_{5b,4}$ = 7.5 Hz, 1H, H5b), 4.87 (m, 1H, H3), 4.94 (ddd, $J_{2,3}$ = 6.1 Hz, $J_{2,1b}$ = 4.1 Hz, $J_{2,1a}$ = 2.0 Hz, 1H, H2); ^{13}C NMR (125 MHz): δ 24.5, 26.1, 29.0, 48.2, 62.2, 85.0, 85.1, 111.5.^{77}Se NMR (400 MHz): δ 200.40 Anal. Calcd for $C_8H_{14}O_3Se$: C, 40.52; H, 5.95. Found: C, 40.49; H, 5.98.

5-O-*Mesyl-2,3*-O-*isopropylidene-D-ribono-1,4-lactone (**16**)*

DMAP (1.2 mg, 0.01 mmol), TEA (2.2 mL, 3.9 mmol), and mesylchloride (0.06 mL, 0.75 mmol) at 0 °C were added to a magnetically stirred solution of 2,3-*O*-isopropylidene D-ribono-1,4-lactone **13** (188 mg, 1 mmol) in CH_2Cl_2 (3.4 mL), and the mixture was stirred at r.t. overnight. The organic layer was washed with brine and dried (Na_2SO_4). Then the solvent was removed under reduced pressure gave a crude residue that was used for the next reaction without further purification. The NMR spectral data were identical to values in the literature.[268]

*2,3-O-Isopropylidene-L-lyxono-1,4-lactone (**14**)*

To the crude **16**, a solution of potassium hydroxide (KOH) 2.65 M (1.3 mL) was added. The mixture was stirred for 4 hours at r.t. Then the pH was adjusted to 2.5-3.0 by adding hydrochloric acid (3 M, 0.60 mL). The acidic solution was concentrated in vacuum to afford a solid mass which was triturated with acetone (1.1 mL) and heated to reflux. The acetone was decanted, dried over anhydrous Na_2SO_4 and filtered. The clear filtrate was concentrated in vacuum to yield the crude product. The NMR spectral data were identical to values in the literature.[268]

5-O-tert-*Butyldiphenylsilyl-2,3-O-isopropylidene-L-lyxono-1,4-lactone* ***(15)***

To a magnetically stirred solution of **14** (0.188 g, 1.0 mmol) in anhydrous dichloromethane (5.0 mL) at 0 °C, TEA (0.28 mL, 2.0 mmol), DMAP (2 mg, 0.2 mmol), and TBDPSCl (0.26 mL, 1.5 mmol) were added. The mixture was stirred at r.t. for 4 hours. As soon as the starting compound **14** was completely consumed (TLC), a saturated aqueous solution of NH_4Cl was added (15 mL), and the mixture was kept under stirring for 10 min. The organic layer was separated, and the aqueous layer was extracted with dichloromethane (3 x 30 mL). The combined organic layer was then washed with brine, dried over anhydrous Na_2SO_4, and filtered. The solvent was removed under reduced pressure leading to a residue that was purified by silica gel column chromatography (*n*-hexane/ethyl acetate 98:2 (v/v)) affording the product **15** as a yellowish syrup (260 mg) with 61% yield.
^{1}H NMR; δ 1.07 (s, 9H, CH_3), 1.36 (s, 6H, CH_3), 3.99-4.04 (m, 2H, H5), 4.59 (td, $J_{4,3}$= 2.9, $J_{4,5}$= 6.5 Hz, 1H, H4), 4.82- 4.78 (m, 2H, H2 and H3), 7.47-7.37 (m, 6H, Ar,), 7.71-7.68 (m, 4H, Ar). ^{13}C NMR: δ 19.3, 25.9, 26.8, 60.4, 61.7, 76.0, 79.3, 79.4, 113.9, 127.7, 127.8, 127.8, 127.9, 129.8, 129.9, 130.0, 133.0, 133.1, 135.6, 135.7, 173.9. Anal. Calcd for $C_{24}H_{30}O_5Si$: C, 67.57; H, 7.09. Found: C, 67.55; H, 7.12.

5-O-tert-*Butyldiphenylsilyl-2,3*-O-*isopropylidene-L-lyxitol* ***(18)***

To a magnetically stirred solution of **15** (426 mg, 1.0 mmol) in methanol (10.0 mL) at 0 °C, $NaBH_4$ (113 mg, 4.0 mmol) was added. The mixture was stirred at r.t. for 2.5 h. As soon as the starting compound **15** was completely consumed (TLC), the solvent was evaporated under reduced pressure and replaced with ethyl acetate. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and filtered. The evaporation of the solvent under reduced pressure gave the pure product **18** as a colourless syrup (0.430 mg) with >99% yield.

^{1}H NMR: δ 1.07 (s, 9 H, CH_3), 1.37 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 3.70 (dd, $J_{5a,5b}$ = 10.0, $J_{5a,4}$ = 6.4, Hz, 1H, H5a), 3.80-3.76 (m, 3H, H5b and H1), 3.86 (td, $J_{4,5a}$ = 6.4, $J_{4,3}$ = 2.2 Hz, 1H, H4), 4.24-4.20 (m, 1H, H2), 4.31 (dd, $J_{3,2}$ = 7.0, $J_{3,4}$ = 2.2 Hz, 1H, H3), 7.46-7.37 (m, 6H, Ar), 7.68-7.65 (m, 4H, Ar); ^{13}C NMR; δ 19.6, 25.6, 27.1, 28.0, 50.4, 59.8, 66.1, 85.3, 90.2, 103.6, 112.4, 128.1, 128.2, 130.2, 130.3, 132.9, 133.2, 135.8, 135.9, 142.1, 150.1, 162.8. Anal. Calcd for $C_{24}H_{34}O_5Si$: C, 66.94; H, 7.96. Found: C, 66.90; H, 8.09.

5-O-tert-*Butyldiphenylsilyl-2,3*-O-*isopropylidene-1,4-di*-O-*methanesulfonyl-L-lyxitol* ***(19)***

To a magnetically stirred solution of **18** (430 mg, 1.0 mmol) in dichloromethane (14.0 mL) at 0 °C, DMAP (2 mg, 0.02 mmol), TEA (1.1 mL, 8.0 mmol), and methanesulfonyl chloride (0.309 mL, 4.0 mmol) were added, and the mixture was stirred at r.t. overnight. A saturated aqueous solution of NH_4Cl was added (15 mL), and the mixture was kept under stirring for 10 min. The organic layer was separated, and the aqueous layer was extracted with dichloromethane (3 x 30 mL). The combined organic layer was then washed with brine, dried over anhydrous Na_2SO_4, and filtered. The evaporation of the solvent under reduced pressure gave a crude residue that was purified by silica gel column chromatography (*n*-hexane/ethyl acetate 6:4 v/v) affording the product **19** as a yellowish syrup (575 mg) with 98% yield.

^{1}H NMR; δ 1.09 (s, 9H, CH_3), 1.37 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 3.00 (s, 3H, CH_3), 3.07 (s, 3H, CH_3), 3.92 (dd, $J_{1a,1b}$ = 11.0, $J_{1a,2}$ =5.2 Hz, 1H, H1), 3.95 (dd, $J_{1b,1a}$ = 11.0, $J_{1b,2}$ = 5.2 Hz, 1H, H1b), 4.33-4.27 (m, 2H, H5), 4.38 (m, 1H, H4), 4.53 (t, $J_{3,2}$ = 6.2 Hz, 1H, H3), 4.82 (m, 1H, H2), 7.46-7.40 (m, 6H, Ar), 7.70-7.66 (m, 4H, Ar); ^{13}C NMR; δ 19.2, 25.4, 26.8, 27.2, 37.5, 39.0, 63.3, 67.6, 74.3, 75.0, 79.1, 109.6, 128.1, 128.2, 130.2, 132.2, 132.3, 135.5, 135.6. Anal. Calcd for $C_{26}H_{38}O_9S_2Si$: C, 53.22; H, 6.53. Found: C, 53.32; H, 6.49.

5-O-tert-*butyldiphenylsilyl-1,4-deoxy-2,3*-O-*isopropylidene-4-seleno-D-ribofuranose* ***(20)***

To a magnetically stirred suspension of selenium powder (166 mg, 2.1 mmol) in dry ethanol (13 mL), $NaBH_4$ was added, and the mixture was stirred at r.t. until the colour of the mixture changed from black to colourless. To this mixture, compound **19** (586 mg, 1.0 mmol) in dry THF (8 mL) under N_2 atmosphere was added. The resulting mixture was heated at 60 °C overnight. Then the solvent was removed under reduced pressure and replaced with ethyl acetate. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and filtered. The solvent was removed under reduced pressure giving the pure seleno-derivative **20** as a pale yellow oil (456 mg) with 96% yield.

^{1}H NMR (400 MHz): δ 1.09 (s, 9H, CH_3), 1.33 (s, 3H, CH_3), 1.54 (s, 3H, CH_3), 2.96 (dd, $J_{1a,1b}$ = 11.8 Hz, $J_{1a,2}$ = 2.3 Hz, 1, H, H1a), 3.20 (dd, $J_{1b,1a}$ = 11.8 Hz, $J_{1b,2}$ = 5.1 Hz, 1, H, H1b), 3.67 (ddt, $J_{4,5a}$ = 7.3 Hz, $J_{4,5b}$ = 5.3 Hz, $J_{4,3}$ = 1.8 Hz, 1H, H4), 3.74 (dd, $J_{5a,5b}$ = 10.5 Hz, $J_{5a,4}$ = 7.0 Hz, 1H, H5a), 3.88 (dd, $J_{5b,5a}$ = 10.5 Hz, $J_{5b,4}$ = 5.4 Hz, 1H, H5b), 4.80 (dd, $J_{3,2}$ = 5.3 Hz, $J_{3,4}$ = 1.8 Hz, 1H, H3), 4.86 (ddd, $J_{2,1b}$ = 5.5 Hz, $J_{2,3}$ = 5.4 Hz, $J_{2,1a}$ = 2.3 Hz, 1H, H2), 7.38–7.47 (m, 6H, Ar), 7.65–7.72 (m, 4H, Ar); ^{13}C NMR (100 MHz): δ 19.2, 24.7, 26.9, 29.6, 50.1, 64.5, 85.1, 87.3, 110.3, 127.7, 129.8, 133.1, 135.6. Anal. Calcd for $C_{24}H_{32}O_3SeSi$: C, 60.62; H, 6.78. Found: C, 60.60; H, 6.80.

1,4-Dideoxy-2,3-O-*isopropylidene-4-seleno-D-ribofuranose* ***(12)***

To a magnetically stirred solution of seleno-derivative **20** (476 mg, 1.0 mmol) in anhydrous THF (7.0 mL) at 0 °C, a solution of tetrabutylammonium fluoride (1.7 ml of a 1.1 M solution in THF, 2.0 mmol) was added under N_2, and the mixture was stirred at r.t. for 1 hour. The solvent was removed under reduced pressure leading to a residue that was purified by silica gel column chromatography (*n*-hexane/ethyl

acetate 6:4 (v/v)) affording the pure product **12** (213 mg) as a crystalline yellow solid with 90% yield.

^{1}H NMR (500 MHz): δ 1.33 (s, 3H, CH_3), 1.54 (s, 3H, CH_3), 3.02 (dd, $J_{1a,1b}$ = 12.0, $J_{1a,2}$ = 2.1 Hz, 1H, H1a), 3.22 (dd, $J_{1b,1a}$ = 12.0 Hz, $J_{1b,2}$ = 5.2, 1H, H1b), 3.62–3.75 (m, 3H, H4 and H5), 4.73 (dd, $J_{3,2}$ = 5.5 Hz, $J_{3,4}$ = 1.4 Hz, 1H, H3), 4.99 (ddd, $J_{2,3}$ = 5.5 Hz, $J_{2,1b}$ = 5.2 Hz, $J_{2,1a}$ = 2.1 Hz, 1H, H2); ^{13}C NMR (125 MHz): δ 24.7, 26.8, 29.4, 52.2, 64.1, 85.1, 87.7, 111.6. Anal. Calcd for $C_8H_{14}O_3Se$: C, 40.52; H, 5.95. Found: C, 40.55; H, 6.00.

*4-Hydroxyphenyl acetate (**10**)*

To a magnetically stirred solution of hydroquinone (110 mg, 1.0 mmol) in acetic anhydride (1.0 mmol, 0.10 mL) and dry pyridine (0.90 mL), DMAP (10 mg, 0.091 mmol) was added under N_2. The reaction mixture was stirred overnight at r.t., and then diluted in ethyl acetate. The resulting solution was washed in sequence with HCl (1 M), a NH_4Cl solution and brine. Then the organic layer was dried over anhydrous Na_2SO_4 and filtered. The solvent was removed under reduced pressure leading to a crude residue which was purified by silica gel column chromatography ($CHCl_3$) affording the pure product **8** as a crystalline white solid (106 mg) with 70% yield. The NMR spectral data were identical to values in the literature.[319]

*1,4,5-Trideoxy-2,3-*O*-isopropylidene-5-oxo-4-seleno-D-ribofuranose (**27**)*

To a stirred solution of **7** (236 mg, 1.0 mmol) in dry dimethyl sulfoxide (2.7 mL), acetic anhydride (1.7 mL) was added. The solution was kept at 100 °C under Argon atmosphere. After 1 hour diethyl ether was added and the resulting solution was washed 5 times with distilled water. The organic layer was dried over anhydrous Na_2SO_4 and filtered. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel (*n*-hexane/acetone 99:1 v/v) giving compound **27**

as yellowish oil (103 mg) with 44% yield. The NMR spectral data were identical to values in the literature.[320]

5-O-tert-*butyldiphenylsilyl-1,4-dideoxy-2,3*-O-*isopropylidene-4-selenoxide-L-lyxofuranose (**37**).*

To a magnetically stirred solution of **6** (476 mg, 1.0 mmol) in anhydrous dichloromethane (3.5 mL) a solution of *m*-CPBA (247 mg, 77%) in anhydrous dichloromethane (3.5 mL) was added at -78 °C, under N_2, and the mixture was stirred at the same temperature for 45 min. After being stirred at –78 °C for 45 min, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (5 mL) and diluted with dichloromethane (20 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and filtered. The evaporation of the solvent under reduced pressure gave a crude residue that was purified by silica gel column chromatography (CH_2Cl_2:MeOH 9:1) to afford the oily product **37** as a pale yellow syrup (78.1 mg, 76%).

^{1}H NMR (500 MHz): δ 1.09 (s, 9H), 1.31 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 2.99 (dd, $J_{1a,1b}$= 13 Hz, $J_{1a,2}$= 6 Hz, 1H, H1a), 3.57 (dd, J= 11Hz, J= 8 Hz, 1H, H4), 3.69 (dd, $J_{1b,1a}$ = 12.5 Hz, $J_{1b,2}$=2.0 Hz, 1H, H1b), 4.21-4.26 (m, 2H, H5), 5.14 (t, $J_{3,2}$= 4.5 Hz, 1H, H3), 5.23 (dd, $J_{2,1}$= 5.5 Hz, $J_{2,3}$= 3.5 Hz, 1H, H2), 7.38-7.43 (m, 6H), 7.68-7.70 (m, 4H, TBDPS); ^{13}C NMR (125 MHz): δ 26.0, 26.6, 26.7, 55.2, 59.7, 72.8, 80.2, 110.7, 127.8, 128.0, 129.9, 132.8, 135.6, 135.7.

1-Acetyloxy-5-O-tert-*butyldiphenylsilyl-1,4-dideoxy-2,3*-O-*isopropylidene-4-seleno-L-lyxofuranose (**38**)*

A magnetically stirred solution of the **37** in anhydrous Ac_2O (13.2 mL), was heated at 80 °C for 1 hours, under N_2. Then the solvent was evaporated under reduced pressure and replaced with ethyl acetate. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and filtered. The crude residue was purified by silica gel column

chromatography (*n*-hexane/ethyl acetate 95:5 v/v) affording the pure product **38** (85.3 mg) as a pale yellow oil with 16% yield.

^{1}H NMR (500 MHz): δ 1.07 (s, CH_3, 9H,), 1.28 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 2.07 (s, 3H, Ac), 3.94 (m, 1H, H5b), 4.15 (m, 1H, H5a), 4.21 (m, 1H, H4), 4.86 (m, 1H, H2), 4.95 (m, 1H, H3), 6.21 (s, 1H, H1), 7.39-7.45 (m, 6H, Ar), 7.69-7.73 (m, 4H, Ar); ^{13}C NMR (125 MHz): δ 25.1, 26.6, 26.8, 49.6, 62.7, 81.3, 83.0, 87.9, 90.3, 111.0, 127.8, 129.9, 130.1, 132.5, 135.6.

*Under the same conditions 1-Acetyloxy-5-*O-tert-*butyldiphenylsilyl-1,4-dideoxy-2,3-*O-*isopropylidene-4-seleno-D-ribofuranose (**40**) was obtained in 66% yield starting from **20** skipping the purification of the intermediate **39**.*

^{1}H NMR; δ 1.09 (s, 9H, CH_3), 1.30 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 1.87 (s, 3H, CH_3), 3.87-3.69 (m, 3H, H4 and H5), 4.70 (dd, $J_{3,4}$ = 0.6, $J_{3,2}$ = 5.4 Hz, 1H, H3), 5.07 (dd, $J_{2,1}$ = 0.8, $J_{2,3}$ = 5.6 Hz, 1H, H2), 6.15 (d, $J_{1,2}$ = 1.2 Hz, 1H, H1), 7.45-7.38 (m, 6H, Ar), 7.70-7.66 (m, 4H, Ar). ^{13}C NMR; δ 19.5, 21.3, 24.8, 26.9, 27.1, 51.9, 66.8, 82.6, 86.4, 90.1, 110.6, 128.0, 130.06, 130.07, 133.3, 133.5, 135.82, 135.84, 169.4. Anal. Calcd for $C_{26}H_{34}O_5SeSi$: C, 58.52; H, 6.42. Found: C, 58.43; H, 6.43.

*2,3,5,6-Di-*O-*isopropylidene-D-mannofuranose (**32**)*

To a magnetically stirred suspension of anhydrous triphenylphosphine polymer-bound (525 mg, ca 2.0 mmol) in anhydrous acetone (6.0 mL) at r.t., a solution of I_2 (508 mg, 2.0 mmol) in the same solvent (18.0 mL) was added dropwise in the dark and under dry N_2 atmophere. After 15 min, solid D-mannose (180 mg, 1.0 mmol) was added in one portion to the suspension. TLC monitoring ($CHCl_3$–MeOH, 99:1) showed that the starting sugar was completely consumed within 30 min. The reaction mixture was then filtered, washed with acetone, and dried (Na_2SO_4). The evaporation of the solvent under reduced pressure gave a crude residue that was used directly in the next step without further purification (291 mg, 95%).

^{1}H NMR; δ 1.32 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 1.48 (s, 3H, CH_3), 4.03 (dd, $J_{6a,6b}$ = 10.2 Hz, $J_{6a,5}$ = 6.1 Hz, 1H, Ha-6), 4.08 (dd, $J_{6b,6a}$ = 10.2 Hz, $J_{6b,5}$ = 6.8 Hz, 1H, Hb-6), 4.20 (dd, $J_{4,3}$ = 7.2 Hz, $J_{4,5}$ = 3.7 Hz, 1H, H-4), 4.38-4.44 (m, 1H, H-5), 4.63 (d, $J_{2,3}$ = 6.2 Hz, 1H, H-2), 4.81 (dd, $J_{3,4}$ = 7.2 Hz, $J_{3,2}$ = 6.2 Hz, 1H, H3), 5.39 (s, 1H, H1); ^{13}C NMR; δ 24.4, 25.1, 25.8, 26.8, 66.5, 73.3, 79.6, 80.1, 85.5, 101.2, 109.12, 112.6. Calcd for $C_{12}H_{20}O_6$: C, 55.37; H, 7.75. Found: C, 55.41; H, 7.73.

2,3,5,6-Di-O-*isopropylidene-D-mannitol* ***(33)***

To a magnetically stirred solution of **32** (260 mg, 1.0 mmol) in methanol (10.0 mL) at 0°C, $NaBH_4$ (113 mg, 4.0 mmol) was added. The mixture was stirred at r.t. for 2 h. As soon as the starting compound **32** was completely consumed (TLC), the solvent was evaporated under reduced pressure and replaced with AcOEt. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and filtered. The evaporation of the solvent under reduced pressure gave the pure product **33** (259 mg) as a colorless syrup with 99% yield.

^{1}H NMR (DMSO-d6): δ 1.25 (s, 3H, CH_3), 1.26 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 3.43–3.48 (m, 1H, H4), 3.63-3.73 (m, 2H, H1), 3.83 (m, 1H, H6a), 3.92–3.99 (m, 2H, H5, H6b), 4.13–4.20 (m, 2H, H2, H3), 4.65 (d, $J_{OH,4}$ = 6.8 Hz, 1H, OH), 4.88-4.91 (m, 1H, OH). ^{13}C NMR (DMSO-d6): δ 25.2, 25.4, 26.6, 26.7, 59.7, 66.5, 69.3, 75.7, 75.8, 77.4, 107.2, 108.2. Calcd for $C_{12}H_{22}O_6$: C, 54.95; H, 8.45. Found: C, 54.95; H, 8.38.

2,3,5,6-Di-O-*isopropylidene-1,4-di*-O-*methanesulfonyl-D-mannitol* ***(34)***

To a magnetically stirred solution of **33** (262 mg, 1.0 mmol) in anhydrous dichloromethane (14.0 mL) at 0 °C, DMAP (2 mg, 0.02 mmol), TEA (1.1 mL, 8.0 mmol), and methanesulfonyl chloride (0.309 mL, 4.0 mmol) were added, and the mixture was stirred at rt overnight. The organic layer was separated, and the aqueous

layer was extracted with dichloromethane (3 x 30 mL). The combined organic layer was then washed with brine, dried over anhydrous Na_2SO_4, and filtered. The evaporation of the solvent under reduced pressure gave a crude residue that was purified by silica gel column chromatography (*n*-hexane/ethyl acetate 4:6 v/v) affording the product **34** (304 mg) as a yellowish syrup with 90% yield.
^{1}H NMR; δ 1.38 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 1.55 (s, 3H, CH_3), 3.11 (s, 3H, CH_3), 3.16 (s, 3H, CH_3), 4.05 (dd, $J_{6a,6b}$ = 8.5 $J_{6a,5}$ = 6.9 Hz, 1H, H6a), 4.18 (m, 1H, H5), 4.23 (dd, $J_{6b,6a}$ = 8.5, $J_{6b,5}$ = 6.2 Hz, 1H, H6b), 4.50-4.40 (m, 2H, H2 and H3), 4.48 (m, 2H, H1), 4.79 (t, $J_{4,3}$ = 7.7, $J_{4,5}$ =7.4 Hz, 1H, H4); ^{13}C NMR; δ 25.1, 25.7, 25.7, 26.0, 27.2, 37.7, 39.3, 46.1, 67.2, 67.9, 74.8, 78.7, 109.7, 110.7. Calcd for $C_{14}H_{26}O_{10}S_2$: C, 40.18; H, 6.26. Found: C, 40.02; H, 6.34.

*2,3,5,6-Di-O-isopropylidene-1,4-dideoxy-4-seleno-D-talofuranose (**35**)*

To magnetically stirred suspension of selenium powder (166 mg, 2.1 mmol) in dry ethanol (13 mL) at r.t., $NaBH_4$ was added and the mixture was stirred until the colour of the mixture changed from black to colourless. Compound **34** (418 mg, 1 mmol) in dry THF (8 mL) was added under N_2 atmosphere to the mixture which was heated at 60 °C overnight. Then the solvent was removed and replaced with AcOEt. Next the organic layer was washed with brine, dried over anhydrous Na_2SO_4 and filtered. The evaporation of the solvent under reduced pressure gave the pure seleno-derivative **35** as a pale yellow oil (261 mg) with 85% yield.
^{1}H NMR: δ 1.34 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 1.54 (s, 3H, CH_3), 2.98 (dd, $J_{1b,1a}$ = 11.5, $J_{1b,2}$ = 2.5 Hz, 1H, H1b), 3.25 (dd, $J_{1a,1b}$ = 11.5, $J_{1b,2}$ = 5.5 Hz, 1H, H1a), 3.61 (dd, $J_{4,5}$ = 4.8, $J_{4,3}$ = 3.3 Hz, 1H, H4), 3.76 (dd, $J_{6b,6a}$ = 8.2, $J_{6b,5}$ = 7.2 Hz, 1H, H6b), 4.10 (dd, $J_{6a,6b}$ = 8.2, $J_{6a,5}$ = 6.2 Hz, 1H, H6a), 4.27 (m, 1H, H5), 4.71 (dd, $J_{3,2}$ = 5.7, $J_{3,4}$ = 2.9 Hz, 1H, H3), 4.99 (ddd, $J_{2,3}$ = 5.9, $J_{2,1a}$ = 5.5, $J_{2,1b}$ = 2.5 Hz, 1H, H2). ^{13}C NMR: δ 24.8, 25.6, 26.3, 27.0, 29.5, 51.0, 69.0, 77.9, 85.1, 88.3, 109.6, 111.0. Calcd for $C_{12}H_{20}O_4Se$: C, 46.91; H, 6.56. Found: C, 46.99; H, 6.53.

2,3-O-*isopropylidene-1,4-dideoxy-4-seleno-D-talofuranose (**30**)*

To compound **35** (308 mg, 1.0 mmol) a solution (3.3 mL) of AcOH/H_2O (1:1) was added. The mixture was stirred at 80 °C for 2 hours. Then, the solvent was evaporated under reduced pressure and the organic layer was washed with diethyl ether. The crude residue was purified by silica gel column chromatography (chloroform/methanol 99:1 v/v) affording compound **30** as a pale yellow oil (173 mg) with 65% yield.
^{1}H NMR: δ 1.34 (s, 3H, CH_3), 1.54 (s, 3H, CH_3), 3.01 (dd, $J_{1b,1a}$ = 11.7, $J_{1b,2}$ = 2.6 Hz, $J_{H,Se}$ = 12.4 Hz, 1H, H1b), 3.24 (dd, $J_{1a,1b}$ = 11.7, $J_{1a,2}$ = 5.4 Hz, $J_{H,Se}$ = 12.4 Hz, 1H, H1a), 3.61 (dd, $J_{6a,6b}$ = 11.3, $J_{6a,5}$ = 6.5 Hz, 1H, H6a), 3.69 (dd, $J_{4,5}$ = 4.9, $J_{4,3}$ = 3.0 Hz, 1H, H4), 3.71 (dd, $J_{6a,6b}$ = 11.3, $J_{6b,5}$ = 3.9 Hz, 1H, H6a), 3.78 (m, 1H, H5), 4.77 (dd, $J_{3,2}$ = 5.6, $J_{3,4}$ = 2.9 Hz, 1H, H3), 4.99 (ddd, $J_{2,3}$ = 6.1, $J_{2,1a}$ = 5.5, $J_{2,1b}$ = 2.6 Hz, 1H, H2). ^{13}C NMR: δ 24.9, 27.1, 29.1, 52.9, 72.7, 84.9, 88.7, 111.1. Calcd for $C_9H_{16}O_4Se$: C, 40.46; H, 6.04. Found: C, 40.43; H, 6.03.

*1,4-dideoxy-4-seleno-D-talofuranose (**31**)*

To compound **35** (308 mg, 1.0 mmol) a solution (3.3 mL) of AcOH/H_2O (8:2) was added. The mixture was stirred at 80 °C for 2 hours. Then, the solvent was evaporated under reduced pressure and the organic layer was washed with diethyl ether. The crude residue was purified by silica gel column chromatography (chloroform/methanol 99:1 v/v) affording compound **31** as an amorphous solid (136 mg) with 60% yield.
^{1}H NMR (500 MHz, acetone-d6): δ 2.69 (dd, $J_{1b,1a}$ = 10.4, $J_{1b,2}$ = 3.3 Hz, 1H, H1b), 2.96 (dd, $J_{1a,1b}$ = 10.4, $J_{1a,2}$ = 4.5 Hz, 1H, H1a), 3.54 (m, 2H, H6), 3.67 (dd, $J_{4,3}$ = 7.4, $J_{4,5}$ = 5.3 Hz, 1H, H4), 3.76 (m, 1H, H5), 4.00-3.95 (m, OH), 4.06 (ddd, $J_{3,4}$ = 7.4, $J_{3,2}$ = 7.1, $J_{3,OH}$ = 3.4 Hz, 1H, H3), 4.17 (m, OH), 4.44 (ddd, $J_{2,3}$ = 7.5, $J_{2,1a}$ = 4.4, $J_{2,1b}$ = 3.5 Hz, 1H, H2). ^{13}C NMR (125 MHz, acetone-d6); δ 23.7, 49.1, 66.7, 72.5, 76.3, 79.0. Calcd for $C_6H_{12}O_4Se$: C, 31.73; H, 5.33. Found: C, 31.78; H, 5.22.

*2,3,5,6-Di-O-isopropylidene-1,4-dideoxy-4-selenoxide-D-talofuranose (**44**)*

To a magnetically stirred solution of **34** (268 mg, 1.0 mmol) in anhydrous dichloromethane (3.5 mL) at -78 °C, a solution of *m*-CPBA (189 mg, 77%) in anhydrous dicholoromethane (3.4 mL) was added under N_2, and the mixture was stirred at -78 °C for 45 min. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and filtered. The crude residue was used for the next reaction without any further purification.

*1-Acetyloxy-1,4-dideoxy-2,3,5,6-di-*O*-isopropylidene-4-seleno-D-talofuranose (**42**).*

A magnetically stirred solution of **44** in anhydrous Ac_2O (4.2 mL), was heated at 80 °C for 1 h, under N_2. As soon as the starting compound **44** was completely consumed (TLC), the solvent was evaporated under reduced pressure and replaced with ethyl acetate. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and filtered. The crude residue (a mixture of α:β, 2:1; by ^{1}H NMR) was purified by silica gel column chromatography (chloroform/methanol 95:5 (v/v)) gaving the pure α anomer **42** as an oil (172 mg) with 47% yield.
^{1}H NMR; δ 1.34 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 1.54 (s, 3H, CH_3), 2.09 (s, 3 H, CH_3), 3.75 (dd, $J_{6b,6a}$ = 8.4 Hz, $J_{6b,5}$ = 6.9 Hz, 1H, H6b), 3.82 (dd, $J_{4,5}$ = 6.3 Hz, $J_{4,3}$= 1.9 Hz, 1H, H4), 4.29 (m, 1H, H6a), 4.13 (m, 1H, H5), 4.83 (dd, $J_{3,2}$= 5.3 Hz, $J_{3,4}$ = 2.0 Hz, 1H, H3), 4.94 (bd, $J_{2,3}$ = 5.2 Hz, 1H, H2), 6.25 (bs, 1H, H1). ^{13}C NMR: δ 21.1, 25.0, 25.5, 26.5, 27.2, 51.8, 68.3, 77.6, 80.8, 86.5, 90.3, 109.8, 111.0, 169.5. Calcd for $C_{14}H_{22}O_6Se$: C, 46.03; H, 6.07. Found: C, 46.12; H, 6.08.

*Preparation of iododerivative (**8**)*

To a magnetically stirred solution of I_2 (0.140 g, 0.550 mmol) in anhydrous CH_2Cl_2 (5.0 mL) TPP (0.144 g, 0.550 mmol) was added under N_2. After 15 minutes imidazole (0.070 g, 1.05 mmol) was added; after 15 minutes dropwise a solution of seleno

derivative **10** (0.100 g, 0.422 mmol) in anhydrous CH_2Cl_2 (1.0 mL) was added. The reaction mixture was stirred at 60 °C for 1 hour. Then the organic layer was washed with $Na_2S_2O_3$, Brine and dried (Na_2SO_4). The evaporation of the solvent under reduced pressure gave a crude residue that was purified by silica gel column chromatography (hexane:ethyl acetate 8:2) to afford the unexpected product **8** (0.029 g, 0.063 mmol, 15%).
^{1}H NMR (500 MHz): δ 1.40 (s, 3H, CH_3), 1.52 (s, 3H, CH_3), 3.03-3.08 (m, 2H, H1a, H1b), 4.47 (m, 1H, H3), 4.65 (m, 1H, H2), 5.31 (m, 1H, H5a), 5.40 (m, 1H, H5b), 5.82 (m, 1H, H4). ^{13}C NMR (125 MHz): δ 25.5, 28.1, 31.3, 77.3, 79.3, 108.8, 119.1, 133.3.

Synthesis of glycoconjugates by Mitsunobu reaction.

General procedure

To a magnetically stirred solution of TPP (393 mg, 1.5 mmol) in anhydrous tetrahydrofuran (2.3 mL) at 0 °C, DIAD (0.303 mL, 1.5 mmol) was added under N_2. After 15 min a solution of seleno L-sugar **7** (238 mg, 1.0 mmol) and phenolic compound (1.5 mmol) in anhydrous THF (3.0 mL) was added dropwise. The reaction mixture was stirred at r.t. for 3 days. The solvent was evaporated under reduced pressure and replaced with ethyl acetate. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and filtered. The evaporation of the solvent under reduced pressure gave a crude residue that was purified by silica gel column chromatography (*n*-hexane/diethyl ether) to afford the pure D-sugar derivative.
The same reaction performed by using the seleno D-sugar **12** as the glycosyl donor lead to the same products having the D-configuration at the C-4 with similar yield.

*Under the above conditions, 5-*O*-(4-Acetyloxyphenyl)-1,4-dideoxy-2,3-*O*-isopropylidene-4-seleno-D-ribofuranose (***21***) was obtained as oil (43%) starting from the compound **7** and monoacetylated hydroquinone.*

^{1}H NMR (500 MHz): δ 1.36 (s, 3H, CH_3), 1.57 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 3.08 (dd, $J_{1a,1b}$ = 12.0 Hz, $J_{1a,2}$ = 1.5 Hz, 1H, H1a), 3.34 (dd, $J_{1b,1a}$ = 12.0 Hz, $J_{1b,2}$ = 5.0 Hz, 1H, H1b), 3.85 (m, 1H, H4), 4.03 (dd, $J_{5a,5b}$ = 9.5 Hz, $J_{5a,4}$ = 8.0 Hz, 1H, H5a), 4.21 (dd, $J_{5b,5a}$= 9.5 Hz, $J_{5b,4}$ = 5.5 Hz, 1H, H5b), 4.90 (dd, $J_{3,2}$ = 5.5 Hz, $J_{3,4}$ = 1.5 Hz, 1H, H3), 5.05 (dt, $J_{2,3}$ = 5.5 Hz, $J_{2,1b}$ = 5.0 Hz, $J_{2,1a}$ = 2.0 Hz, 1H, H2), 6.90 (d, J = 9.0 Hz, 2H, Ar), 7.00 (d, J = 9.0 Hz, 2H, Ar). ^{13}C NMR (125 MHz): δ 21.0, 24.7, 26.7, 30.2, 47.0, 70.9, 85.2, 87.6, 110.6, 115.3, 122.4, 144.6, 156.0, 169.8. ^{77}Se NMR (400 MHz): δ 226.54. Anal. Calcd for $C_{16}H_{20}O_5Se$: C, 51.76; H, 5.43. Found: C, 51.66; H, 5.49.

*Under the above conditions, (5-(4-hydroxyphenoxy)-1,4-deoxy-2,3-O-isopropylidene-4-seleno-D-ribofuranose (**22**) was obtained as oil (13%) starting from the compound **7** and hydroquinone.*

^{1}H NMR (500 MHz): δ 1.36 (s, 3H, CH_3), 1.56 (s, 3H, CH_3), 3.06 (dd, $J_{1a,1b}$ = 11.9 Hz, $J_{1a,2}$ = 1.9 Hz, 1H, H1a), 3.33 (dd, $J_{1b,1a}$ = 11.9 Hz, $J_{1b,2}$ = 5.1 Hz, 1H, H1b), 3.83 (m, 1H, H4), 3.99 (dd, $J_{5a,5b}$ = 9.9 Hz, $J_{5a,4}$ = 7.8 Hz, 1H, H5a), 4.16 (dd, $J_{5b,5a}$ = 9.9 Hz, $J_{5b,4}$ = 5.2 Hz, 1H, H5b), 4.91 (dd, $J_{3,2}$ = 5.6 Hz, $J_{3,4}$ = 1.7 Hz, 1H, H3), 5.05 (dt, $J_{2,3}$ = 5.6 Hz, $J_{2,1b}$ = 5.3 Hz, $J_{2,1a}$ = 1.9 Hz, 1H, H2), 6.77 (d, J = 9.2 Hz, 2H, Ar), 6.80 (d, J = 9.3 Hz, 2H, Ar); ^{13}C NMR (125 MHz): δ 24.7, 26.7, 30.2, 47.2, 71.3, 85.2, 87.7, 110.6, 115.9, 116.1, 150.0, 152.5. ^{77}Se NMR (400 MHz): δ 225.96. Anal. Calcd for $C_{14}H_{18}O_4Se$: C, 51.07; H, 5.51. Found: C, 51.01; H, 5.58.

*Under the above conditions, (E)-5-((3-(3,4-dihydroxyphenyl)acryloyl)oxy)-1,4-deoxy-2,3-O-isopropylidene-4-seleno-d-ribofuranose (**23**) was obtained as oil (70%) starting from the compound **7** and caffeic acid.*

^{1}H NMR (500 MHz): δ 1.34 (s, 3H, CH_3), 1.54 (s, 3H, CH_3), 3.06 (dd, $J_{1a,1b}$ = 12.1 Hz, $J_{1a,2}$ = 1.7 Hz, 1H, H1a), 3.26 (dd, $J_{1b,1a}$ = 12.1 Hz, $J_{1b,2}$ = 4.9 Hz, 1H, H1b), 3.76 (m, 1H, H4), 4.26 (dd, $J_{5a,5b}$ = 11.6 Hz, $J_{5a,4}$ = 8.9 Hz, 1H, H5a), 4.37 (dd, $J_{5b,5a}$ = 11.6 Hz, $J_{5b,4}$ = 5.8 Hz, 1H, H5b), 4.77 (dd, $J_{3,2}$ = 5.5 Hz, $J_{3,4}$ = 1.6 Hz, 1H, H3), 5.03 (ddd,

$J_{2,3}$ = 5.7 Hz, $J_{2,1b}$ = 5.0 Hz, $J_{2,1a}$ = 2.0 Hz, 1H, H2), 6.00 (brs, 2H, OH), 6.24 (d, J = 15.9 Hz, 1H, H2′), 6.87 (d, J = 8.2 Hz, 1H, H8′), 7.00 (dd, J = 8.2 Hz, J = 1.5 Hz, 1H, H9′), 7.07 (d, J = 1.5 Hz, 1H, H5′), 7.58 (d, J = 15.9 Hz, 1H, H3′); ^{13}C NMR (125 MHz): δ 24.7, 26.7, 29.7, 46.6, 65.7, 85.0, 87.3, 110.9, 114.5, 115.1, 115.6, 122.7, 127.6, 143.8, 145.5, 146.3, 167.0. ^{77}Se NMR (400 MHz): δ 225.52. Anal. Calcd for $C_{17}H_{20}O_6Se$: C, 51.14; H, 5.05. Found: C, 51.19; H, 5.03.

Under the above conditions, (E)-5-((3-(4-hydroxy-3-methoxyphenyl)acryloyl)oxy)-1,4-deoxy-2,3-O-isopropylidene-4-seleno-D-ribose ***(24)*** *was obtained as oil (68%) starting from the compound* ***7*** *and ferulic acid*

^{1}H NMR (500 MHz, acetone-d_6): δ 1.30 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 2.99 (dd, $J_{1a,1b}$ = 11.9 Hz, $J_{1a,2}$ = 1.3 Hz, 1H, H1a), 3.37 (dd, $J_{1b,1a}$ = 11.9 Hz, $J_{1b,2}$ = 4.9 Hz, 1H, H1b), 3.76 (ddd, $J_{4,5a}$ = 8.7 Hz, $J_{4,5b}$ = 7.4 Hz, $J_{4,3}$ = 1.3 Hz, 1H, H4), 3.95 (s, 3H, CH_3), 4.26 (dd, $J_{5a,5b}$ = 11.4 Hz, $J_{5a,4}$ = 8.8 Hz, 1H, H5a), 4.36 (dd, $J_{5b,5a}$= 11.4 Hz, $J_{5b,4}$ = 6.8 Hz, 1H, H5b), 4.86 (dd, $J_{3,2}$ = 5.8 Hz, $J_{3,4}$ = 1.5 Hz, 1H, H3), 5.11 (ddd, $J_{2,3}$= 6.1, $J_{2,1b}$= 5.9 Hz, $J_{2,1a}$ = 1.9 Hz, 1H, H2), 6.46 (d, J = 16.0 Hz, 1H, H2′), 6.90 (d, J = 8.0 Hz, 1H, H8′), 7.18 (dd, J = 8.5 Hz, J = 1.9 Hz, 1H, H9′), 7.39 (d, J= 1.5 Hz, 1H, H5′), 7.66 (d, J = 16.0 Hz, 1H, H3′); ^{13}C NMR (125 MHz): δ 24.7, 26.7, 29.3, 46.7, 56.0, 65.6, 85.0, 87.3, 109.4, 110.8, 114.8, 114.8, 123.3, 126.8, 145.7, 146.8, 148.2, 166.9. ^{77}Se NMR (400 MHz): δ 225.64. Anal. Calcd for $C_{18}H_{22}O_6Se$: C, 52.31; H, 5.37. Found: C, 52.25; H, 5.43.

Under the above conditions, 5-(4-((1E,6E)-7-(4-hydroxy-3-methoxyphenyl)-3,5-dioxohepta-1,6-dien-1-yl)-2-methoxyphenoxy)-1,4-deoxy-2,3-O-isopropylidene-4-seleno-D-ribose ***(25)*** *was obtained as oil (30 %) starting from the compound* ***7*** *and curcumin.*

^{1}H NMR (400 MHz): δ 1.36 (s, 3H, CH_3), 1.57 (s, 3H, CH_3), 3.04 (dd, $J_{1a,1b}$ = 12.0 Hz, $J_{1a,2}$ = 1.9 Hz, 1H, H1a), 3.55 (dd, $J_{1b,1a}$ = 12.0 Hz, $J_{1b,2}$ = 5.0 Hz, 1H, H1b), 3.93–

3.86 (m, 4H, H4 and CH_3), 3.96 (s, 3H, CH_3), 4.16 (dd, $J_{5a,5b}$ = 9.7 Hz, $J_{5a,4}$ = 8.0 Hz, 1H, H5a), 4.33 (dd, $J_{5b,5a}$ = 9.7 Hz, $J_{5b,4}$ = 5.2 Hz, 1H, H5b), 4.95 (dd, $J_{3,2}$ = 5.6 Hz, $J_{3,4}$ = 1.8 Hz, 1H, H3), 5.11 (ddd, $J_{2,3}$ = 5.6 Hz, $J_{2,1b}$ = 5.0 Hz, $J_{2,1a}$ = 1.9 Hz, 1H, H2), 5.82 (m, 2H, H10′), 5.98 (bs, 1H, OH), 6.52–6.46 (m, 2H, H8′ and H12′), 6.84 (d, J = 8.3 Hz, 1H, H6′), 6.94 (d, J = 8.3 Hz, 1H, H18′), 7.05 (d, J = 1.7 Hz, 1H, H15′), 7.07 (d, J = 1.9 Hz, 1H, H3′), 7.14–7.09 (m, 2H, H5′ and H19′), 7.65–7.561 (m, 2H, H7′ and H13′); ^{13}C NMR (125 MHz): δ 24.6, 26.7, 31.1, 47.1, 56.0, 72.1, 85.6, 88.2, 101.2, 109.6, 110.4, 113.1, 114.8, 121.8, 122.3, 122.9, 129.0, 140.2, 140.6, 146.8, 147.9, 149.7, 182.9, 183.6. ^{77}Se NMR (400 MHz): δ 229.42. Anal. Calcd for $C_{29}H_{32}O_8Se$: C, 59.29; H, 5.49. Found: C, 59.19; H, 5.55.

*Under the above conditions, 6-O-(4-Acetyloxyphenyl)-1-deoxy-2,3-O-isopropylidene-4-seleno-D-talofuranose (**36**) was obtained as oil (42 %) starting from the compound **30** and monoacetylated hydroquinone.*

^{1}H NMR (500 MHz); δ 1.31 (s, 3 H, CH_3), 1.53 (s, 3 H, CH_3), 2.28 (s, 3 H, CH_3), 3.04 (dd, $J_{1a,1b}$= 11.6, $J_{1a,2}$= 2.6 Hz, 1 H, H1a), 3.29 (dd, $J_{1b,1a}$= 11.6, $J_{1b,2}$= 5.7 Hz, 1H, H1b), 3.91 (m, 1H, H4), 4.51-4.43 (m, 2H, H6b, H5), 4.66 (dd, $J_{6a,6b}$= 11.6, $J_{6a,5}$= 8.7 Hz, 1H, H6a), 4.76 (dd, $J_{3,2}$= 5.5, $J_{3,4}$= 4.1 Hz, 1H, H3), 4.98 (ddd, $J_{2,H1b}$= 5.7, *J2,3*= 5.6, $J_{2,1a}$= 2.6 Hz, 1 H, H2), 6.97(d, J_{orto}= 10.0 Hz, 1H, Ar), 7.02 (d, J= 10.0 Hz, 1 H). ^{13}C NMR (125 MHz); δ 21.0, 24.7, 26.7, 30.2, 49.7, 72.7, 71.0, 85.3, 88.1, 110.7, 115.4, 122.4, 144.7, 156.0, 169.9.

*Synthesis of glycoconjugate **26** by reductive amination.*

*5-(3,4-Dihydroxyphenethylamino)-1,4,5-trideoxy-2,3-O-isopropylidene-4-seleno-D-ribofuranose (**26**)*

To a stirred solution of **27** (236 mg, 1.0 mmol) in anhydrous methanol (2.7 mL) $NaBH_3CN$ (40 mg, 0.59 mmol) and DOPA·HCl (**29**, 379 mg, 2.0 mmol) were added. The mixture was stirred at r.t. for 3 days. Then HCl was added until pH 1 (37%, 0.4

mL) and the solvent was removed under reduced pressure. The residue was dissolved in water and washed 5 times with diethyl ether. Then the aqueous solution was treated with $NaHCO_3$ until pH 9 and extracted with diethyl ether (3 x 10 mL). The organic layer was dried over anhydrous Na_2SO_4 and filtered. The solvent was removed under reduced pressure and the crude residue was chromatographed on silica gel (chloroform/methanol 9:1 (v/v)) giving compound **26** as beige amorphous solid (156 mg) with 42% yield.

^{1}H NMR; δ 1.30 (s, 3H, CH_3), 1.51 (s, 3H, CH_3), 2.66 (dd, $J_{5a,5b}$ = 12.0 Hz, $J_{5a,4}$= 9.1 Hz 1H, H5a), 2.73 (t, $J_{7,6}$ = 6.6 Hz, 2H, H7), 2.97-2.87 (m, 3H, H5b, H6), 2.99 (dd, $J_{1b,1a}$ = 12.0 Hz, $J_{1b,2}$ = 5.2 Hz, 1H, H1b), 3.08 (dd, $J_{1a,1b}$ = 12.0 Hz, $J_{1a,2}$= 2.6 Hz, 1H, H1a), 3.70 (ddt, $J_{4,5a}$ = 8.87 Hz, $J_{4,5b}$ = 6.33Hz, $J_{4,3}$ = 2.5 Hz, 1H, H4), 4.57 (dd, $J_{3,2}$ = 5.7 Hz, $J_{3,4}$= 2.5 Hz, 1H, H3), 4.93 (ddd, $J_{2,3}$= 5.5 $J_{2,1b}$= 5.3 $J_{2,1a}$= 2.6 Hz, 1H, H2) 6.62 (d, $J_{13,12}$= 8.0 Hz, 1H, H13), 6.66 (s, 1H, H9), 6.83 (d, $J_{12,13}$ = 8.0 Hz, 1H, H12); ^{13}C NMR; δ 24.6, 26.7, 28.4, 34.3, 48.4, 49.7, 51.1, 84.5, 88.1, 111.1, 115.4, 115.9, 119.5, 143.7, 144.7. ^{77}Se NMR (400 MHz): δ 221.28. Anal. Calcd. for $C_{16}H_{23}NO_4Se$: C, 51.62; H, 6.23. Found: C, 51.58; H, 6.28.

Synthesis of glycoconjugates by TMSOTf promoted coupling.

To a magnetically stirred solution of **40** (534 mg, 1.0 mmol) and 4-hydroxyphenyl acetate (**10**, 375 mg, 2.5 mmol) in anhydrous dichloromethane (3.0 mL), 4ÅMS (40 mg) was added and the mixture was stirred at room temperature for 30 min. Then trimethylsilyl trifluoromethanesulfonate (TMSOTf) (0,015 mL, 0,081 mmol) was added and the solution was stirred at r.t. overnight. Next, the solution was quenched with Et_3N and filtered. Then the solvent was evaporated under reduced pressure. The crude residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate 95:5 (v/v)) affording the pure products **41** (63 mg) with 15% yield.

^{1}H NMR; δ 1.09 (s, 9H,CH_3), 1.35 (s, 3H, CH_3), 1.56 (s, 3 H, CH_3), 2.27 (s, 3 H, CH_3), 3.87-3.78 (m, 3H, H4, H5), 4.91(d, $J_{3,2}$= 5.5 Hz, 1 H, H3), 5.18 (d, $J_{2,3}$ = 5.5 Hz, 1 H; H2), 5.88 (bd, $J_{H,Se}$= 15.0 Hz, 1H, H1), 6.63 (d, J_{orto}= 9.0 Hz, 2H, Ar), 6.97 (d, J_{orto}=

9.0 Hz, 2H, Ar), 7.48-7.35 (m, 6H, Ar), 7.72-7.63 (m, 4H, Ar); ^{13}C NMR; δ= 20.5, 24.1, 26.1, 26.7, 52.5, 66.1, 84.5, 88.0, 90.3, 91.9, 111.1, 116.4, 122.1, 129.5, 133.1, 136.9, 145.1, 153.6, 169.5. Anal. Calcd. for $C_{32}H_{38}O_6SeSi$: C, 61.43; H, 6.12. Found: C, 61.44; H, 6.10.

*Under the above conditions, 1-O-(4-Acetyloxyphenyl)-2,3,5,6-di-O-isopropylidene-4-seleno-D-talofuranose (**43**) was obtained as oil (40 %) starting from the compound **42** and 4-hydroxyphenyl acetate (**10**).*

^{1}H NMR; δ 1.35 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.57 (s, 3H, CH_3), 2.29 (bs, 3H, CH_3), 3.84 (dd, $J_{6b,6a}$=8.60 $J_{6b,5}$=6.7 Hz, 1H, H6b), 3.87 (dd, $J_{4,5}$= 6.7, $J_{4,3}$= 2.4 Hz, 1H, H4), 4.13 (dd, $J_{6a,6b}$= 8.7 $J_{6a,5}$=6.3 Hz, 1H, H6a), 4.31 (m, 1H, H5), 4.93 (dd, $J_{3,2}$= 5.4 $J_{3,4}$= 2.2 Hz, 1H, H3), 5.12 (bd, $J_{2,3}$= 5.4 Hz, 1H, H2), 6.02 (bd, $J_{H,Se}$= 15.0 Hz, 1H, H1), 6.86 (d, J_{orto}= 9.0 Hz, 1H, Ar), 7.01 (d, J_{orto}= 9.1 Hz, 1H, Ar); ^{13}C NMR; δ 21.1, 24.9, 25.3, 26.7, 27.1, 52.3, 68.0, 77.9, 85.9, 86.5, 91.0, 109.7, 111.0, 116.3, 122.6, 145.3, 153.9, 169.6. Anal. Calcd. for $C_{20}H_{26}O_7Se$: C, 52.52; H, 5.73. Found: C, 52.62; H, 5.65.

Removal of isopropylidene.

To the protected glycoconjugate **21** (1.0 mmol) a solution (3.3 mL) of AcOH/H_2O (8:2) was added. The mixture was stirred at 80 °C for 2 hours. Then, the solvent was evaporated under reduced pressure and next the organic layer was washed with diethyl ether. The crude residue was purified by preparative thin chromatography affording as amorphus solid the pure deprotected product **21a** (221.9 mg) with 67% yield.

^{1}H NMR (500 MHz, acetone-d6): δ 2.24 (s, 3H, CH_3), 2.85 (dd, $J_{1a,1b}$ = 10.0 Hz, $J_{1a,2}$ = 5.1 Hz, 1H, H1a), 3.06 (dd, $J_{1b,1a}$ = 10.0 Hz, $J_{1b,2}$ = 5.0 Hz, 1H, H1b), 3.82 (m, 1H, H4), 4.30-4.00 (m, 4H, OH, H5), 4.51-4.40 (m, 2H, H2, H3), 6.97 (d, J_{orto} = 9.0 Hz, 2H, Ar), 7.05 (d, J_{orto} = 9.0 Hz, 2H, Ar); ^{13}C NMR (125 MHz, acetone-d6): δ 21.2,

25.1, 42.8, 72.1, 75.5, 77.9, 115.6, 123.1, 144.6, 156.2, 170.0. ^{77}Se NMR (400 MHz): δ 221.28. 173.61Anal. Calcd for $C_{13}H_{16}O_5Se$: C, 47.14; H, 4.87. Found: C, 47.21; H, 4.77.

*Under the above conditions, 5-O-(4-Hydroxyphenyl)-1,4-dideoxy-4-seleno-D-ribofuranose (**22a**) was obtained as amorphous solid (71 %) starting from the compound **22**.*

^{1}H NMR (400 MHz, acetone-d6); δ 2.82 (dd, *J*= 10.0, 5.1 Hz, 1 H), 3.02 (dd, *J*= 10.0, 5.0 Hz, 1 H), 3.77 (m, 1 H), 3.99 (dd, *J*= 9.7, 8.3 Hz, 1 H), 4.10 (m, 1 H), 4.35 (dd, *J*= 9.7, 5.9 Hz, 1 H), 4.40 (m, 1 H), 6.75 (d, *J*= 9.2 Hz, 2 H), 6.79 (d, *J*= 9.2 Hz, 2 H); ^{13}C NMR (100 MHz, acetone-d_6); δ 23.9, 42.6, 72.4, 76.0, 78.4, 115.8, 151.6, 152.0. ^{77}Se NMR (400 MHz): δ 173.14. Anal. Calcd. for $C_{11}H_{14}O_4Se$: C, 45.69; H, 4.88. Found: C, 45.60; H, 4.92.

*Under the above conditions, (E)-5-((3-(3,4-dihydroxyphenyl)acryloyl)oxy)-1,4-dideoxy-4-seleno-D-ribofuranose (**23a**) was obtained as amorphus solid (68 %) starting from the compound **23**.*

^{1}H NMR (500 MHz, acetone-d_6): δ 2.84 (dd, $J_{1a,1b}$ = 9.9 Hz, $J_{1a,2}$ = 5.3 Hz, 1H, H1a), 3.06 (dd, $J_{1b,1a}$ = 9.9 Hz, $J_{1b,2}$ = 5.1 Hz, 1H, H1b), 3.74 (m, 1H, H4), 4.10 (dd, $J_{3,4}$ = 5.9 Hz, $J_{3,2}$ = 3.2 Hz, 1H, H3), 4.22 (dd, $J_{5a,5b}$ = 11.3 Hz, $J_{5a,4}$ = 7.6 Hz, 1H, H5a), 4.43 (ddt, $J_{2,1a}$ = 5.3 Hz, $J_{2,1b}$ = 5.1 Hz, $J_{2,3}$ = 3.1 Hz, 1H, H2), 4.57 (dd, $J_{5b,5a}$ = 11.3 Hz, $J_{5b,4}$ = 6.6 Hz, 1H, H5b), 6.32 (d, *J* = 15.9 Hz, 1H, H2′), 6.90 (d, *J* = 8.2 Hz, 1H, H5′), 7.09 (dd, *J* = 8.2 Hz, *J* = 2.0 Hz, 1H, H9′), 7.19 (d, *J* = 2.0 Hz, 1H, H8′), 7.58 (d, *J* = 15.6 Hz, 1H, H3′); ^{13}C NMR (125 MHz, acetone-d_6): δ 24.1, 41.9, 66.5, 76.0, 78.4, 114.4, 115.5, 121.7, 126.7, 145.2, 145.4, 148.0, 166.2. ^{77}Se NMR (400 MHz): δ 225.97. Anal. Calcd for $C_{14}H_{16}O_6Se$: C, 46.81; H, 4.49. Found: C, 46.81; H, 4.45.

*Under the above conditions, (E)-5-((3-(4-hydroxy-3-methoxyphenyl)acryloyl)oxy)-1,4-dideoxy-4-seleno-d-ribose (**24a**) was obtained as amorphus solid (80 %) starting from the compound **24**.*

^{1}H NMR (500 MHz, acetone-d_6): δ 2.85 (dd, $J_{1a,1b}$ = 10.0 Hz, $J_{1a,2}$ = 5.3 Hz, 1H, H1a), 3.06 (dd, $J_{1b,1a}$ = 10.0 Hz, $J_{1b,2}$ = 5.0 Hz, 1H, H1b), 3.74 (m, 1H, H4), 3.95 (s, 3H, CH_3), 4.09 (m, 1H, H3), 4.22 (dd, $J_{5a,5b}$ = 11.3 Hz, $J_{5a,4}$ = 7.8 Hz, 1H, H5a), 4.43 (m, 1H, H2), 4.58 (dd, $J_{5b,5a}$ = 11.3 Hz, $J_{5b,4}$ = 6.5 Hz, 1H, H5b), 6.43 (d, J = 15.9 Hz, 1H, H2′), 6.90 (d, J = 8.1 Hz, 1H, H8′), 7.18 (dd, J = 8.2 Hz, J = 1.7 Hz, 1H, H9′), 7.39 (bs, 1H, H5′), 7.64 (d, J = 15.9 Hz, 1H, H3′); ^{13}C NMR (125 MHz, acetone-d_6): δ 24.1, 41.9, 55.5, 66.6, 76.0, 78.4, 110.4, 114.6, 115.2, 123.3, 126.5, 145.2, 145.3, 147.9, 149.3, 166.3. ^{77}Se NMR (400 MHz): δ 176.99. Anal. Calcd for $C_{15}H_{18}O_6Se$: C, 48.27; H, 4.86. Found: C, 48.36; H, 4.82.

DPPH test: The radical scavenging activity of compounds towards the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was measured as described.[279] Stock solutions of each compound and standard were prepared in ethanol at a concentration of 4 mM (10 mL). Dissolutions (400– 3.125 µM) were prepared with serial dilutions from stock solution. After, Tris-HCl 0.1 M (800 µL), each dissolution (200 µL), and DPPH (1 mL) in ethanol (0.2 mM), resulting in a final concentration of 0.1 mM DPPH, were added in glass tubes. Furthermore, a solution with 200 µL of ethanol instead of sample was prepared. A solution of 800 µL of Tris-HCl buffer and 1.2 mL of ethanol was used as the blank. The mixtures were left for 30 min at room temperature, in dark, and the absorbances then were measured at 517 nm. Ascorbic acid was used as standard antioxidant. Analysis was done in triplet.

FRAP assay: The FRAP solution were prepared in acetate buffer pH 3.6. Stock solutions of each compound and standard were prepared in water at a concentration of 4 mM (10 mL). Dissolutions (400– 3.125 µM) were prepared with serial dilutions from stock solution. After, distilled water (1 mL), each dissolution (100 µL), and FRAP solution (1 mL) were added in glass tubes at 37°C. A solution of 1.1 ml of distilled water and 1 ml of buffer acetate was used as the blank. The mixtures were

left for 7 min at 37 °C and the absorbances then were measured at 593 nm. Ascorbic acid was used as standard. Analysis was done in duplet.

Bacterial strains: *Escherichia coli* (ATCC 25922); *Lactobacillus plantarum* (ATCC 8014). **Eucaryotic cells:** *saccharomyces cerevisiae* (ATCC 9673); HaCaT (HaCaT cells; PCS-200-001).

MIC assay: a published protocol was followed for MIC determination using microdilution assays in microtiter plates;[321] this protocol, in turn follows the guidelines of the Clinical and Laboratory Standards Institute(CLSI)[322] and the European Committee on Antimicrobial Susceptibility testing. MIC assays were prepared in 96-well polystyrene microtiter plates (Greiner) with 200 µl cylindrical wells. Bacterial strains were grown on Luria-Bertani (LB) agar or LB broth. Colonies, initially grown on an LB agar plate, were touched with a sterile loop and introduced to a 5 ml LB liquid culture, which were grown at 37 °C. After 24 h of growth, the suspensions were diluted in distilled water to obtain 2×10^6 cells/mL. Serial dilutions of bacteria and compounds were prepared in LB in 96-well plates (Greiner, 650161), 0.075 mL bacterial inoculum was added to each well on the microtiter plate containing 0.075 mL of the serial compound dilutions. Each condition was repeated in 3 wells, in three different plates. After incubation for 24 h at 37 °C, the MICs were determined with an ELISA reader (read at 600 nm Multiskan EX, Thermo Electron Corporation, France) as the lowest concentration of compound whose absorbance was comparable with the negative control wells (broth only or broth with compound, without inoculum). Bacterial strain susceptibility testing was performed during the time course experiments, using clinical reference agents (i.e. antibiotics): amoxicillin, ampicillin, oxacillin, penicillin G, ticarcillin and vancomycin (Fontanay et al., 2008). Yeast cells were grown in Yeast extract-Peptone-Destrose (YPD) medium, but the same procedure was followed for this cell line. The percentage of growth inhibition (GI), was calculated as follow:

$GI = [1- (O.D._{600}\,GC - O.D._{600}\,S) \times 100]$

GC= mean of 3 values corresponding to the 3 wells of the positive control – mean of 3 values corresponding to the 3 wells at of broth control;

S= mean of 3 values corresponding to the 3 wells at 1.25 mM + bacterium/yeast inoculum) – (mean of 3 values corresponding to the 3 wells of compound control).

MTT ASSAY: Cells were cultured in a humidified atmosphere containing 5% of CO_2 at 37°C in regular Dulbecco's Modified Eagle's Medium (DMEM) (DMEM - High Glucose, Euroclone S.P.A., Milan, Italy) supplemented with 10% of foetal calf serum (FCS), and 1% of penicillin-streptomycin (Euroclone S.P.A., Milan, Italy). Cells were seeded in a 96-well flat-bottom microliter plate at a density of 1×10^4 cells/well and allowed to adhere for 24 hours at 37°C. Then, culture medium was replaced with fresh medium and cells were treated with various concentrations of each compound (100-12.5 µg/mL) and incubated for further 24 hours at 37°C in a humidified atmosphere containing 5% of CO_2. After that, culture medium was replaced with fresh medium and 25 µL of MTT working solution (5 mg/mL in phosphate buffer solution) were added to each well and the plate was incubated for 3 hours in the same conditions. The medium was then aspirated, and the formed formazan crystals were solubilized by adding 100 µL DMSO per well. After 15 minutes, the intensity of the dissolved formazan crystals (purple colour) was quantified by measuring the optical density at 620 nm using a plate reader spectrophotometer (Titertek Multiskan MCC/340-DASIT, Cornaredo, Milano, Italia). Data, presented as percentage of dead cells, were calculated as follow:

% dead cells = [100-(OD treated cells/OD control cells) × 100]

ROS release assay: HaCat cells were plated at a density of 3.0×10^5 cells/well into 24-well plates. Cells were allowed to grow for 4 h; thereafter, the medium was replaced with fresh medium and cells were incubated with different concentrations of each compound (50 and 25 µg/mL) for 1 h and then co-exposed to LPS (1 µg/mL) for 24 h. Cells were then collected, washed twice with phosphate buffer saline (PBS) buffer and incubated in PBS containing H_2DCF-DA (10 µM) at 37 °C. After 45 min,

cells fluorescence was evaluated using a fluorescence-activated cell sorting (FACSscan; Becton Dickinson) and elaborated with Cell Quest software. Data are then expressed as mean fluorescence intensity.

Data analysis: data are reported as mean ± standard error mean (s.e.m.) values of independent experiments, done at least three times, with three or more independent observations in each. Statistical analysis was performed by analysis of variance test, and multiple comparisons were made by Bonferroni's test. A P-value less than 0.05 was considered significant.

Patch fabrication: HEMA monomer was mixed with PEGDA 250 in various rates. The mixture (50 mL) was magnetically stirred at r.t. for about 10 min, then Darocur 1173 (1% v/v) was added and the mixture was stirred for further 5 minutes. Next, a solution of 2.24 mg of organoselenium compound in 10 µL of solvent (water or ethanol) was added and the mixture was gently vortexed. This corresponds to a final compound's concentration - selenosugar or glycoconjugate - of 1 mg/cm^2 in the polymeric membrane. Finally, the resulting 60 µL were placed on a glass substrate (15mmx15mm) and then covered upside with another glass. The system was exposed for 2 minutes to UVA light (UVA exposure box, UV-Belichtungsgerät 2), resulting in a crosslinked polymer membrane of about 250 µm thick.

Compounds' release: Organoselenium compounds' release dynamics was performed placing the polymeric membranes – sealed with silicone on one side - in a PBS solution (10 mL) at pH 7.4. The silicone keeps the system afloat and allows the one-sided release. Then, periodically, withdrawals were executed from the release solution and analysed by HPLC (Shimadzu SPD-20A). Release data were reported as mass percentage of incorporated compound. Data analysis were executed by excel.

REFERENCES

8. REFERENCES

(1) Pizzino, G.; Irrera, N.; Cucinotta, M.; Pallio, G.; Mannino, F.; Arcoraci, V.; Squadrito, F.; Altavilla, D.; Bitto, A. Review Article Oxidative Stress : Harms and Benefits for Human Health. *Oxid. Med. Cell. Longev.* **2017**, *2017*. https://doi.org/10.1155/2017/8416763.

(2) Zhang, L.; Wang, X.; Cueto, R.; Effi, C.; Zhang, Y.; Tan, H.; Qin, X.; Ji, Y.; Yang, X.; Wang, H. Biochemical Basis and Metabolic Interplay of Redox Regulation. *Redox Biol.* **2019**, *26* (August), 101284. https://doi.org/10.1016/j.redox.2019.101284.

(3) Liguori, I.; Russo, G.; Curcio, F.; Bulli, G.; Aran, L.; Della-Morte, D.; Gargiulo, G.; Testa, G.; Cacciatore, F.; Bonaduce, D.; Abete, P. Oxidative Stress, Aging, and Diseases. *Clin. Interv. Aging* **2018**, *13*, 757–772. https://doi.org/10.2147/CIA.S158513.

(4) Wang, L.; Wang, X.; Wang, Q. Mulberry Seeds Perform High Hypoglycaemic Effect Partially by Inhibition of α-Glucosidase Activity. *Biomed. Res.* **2017**, *28* (8), 3568–3573.

(5) Münzel, T.; Gori, T.; Keaney, J. F.; Maack, C.; Daiber, A. Pathophysiological Role of Oxidative Stress in Systolic and Diastolic Heart Failure and Its Therapeutic Implications. *Eur. Heart J.* **2015**, *36* (38), 2555–2564. https://doi.org/10.1093/eurheartj/ehv305.

(6) Kiyuna, L. A.; Albuquerque, R. P. e.; Chen, C. H.; Mochly-Rosen, D.; Ferreira, J. C. B. Targeting Mitochondrial Dysfunction and Oxidative Stress in Heart Failure: Challenges and Opportunities. *Free Radic. Biol. Med.* **2018**, *129* (August), 155–168. https://doi.org/10.1016/j.freeradbiomed.2018.09.019.

(7) Rani, V.; Deep, G.; Singh, R. K.; Palle, K.; Yadav, U. C. S. Oxidative Stress and Metabolic Disorders: Pathogenesis and Therapeutic Strategies. *Life Sci.*

2016, *148*, 183–193. https://doi.org/10.1016/j.lfs.2016.02.002.

(8) Gerber, P. A.; Rutter, G. A. The Role of Oxidative Stress and Hypoxia in Pancreatic Beta-Cell Dysfunction in Diabetes Mellitus. *Antioxidants Redox Signal.* **2017**, *26* (10), 501–518. https://doi.org/10.1089/ars.2016.6755.

(9) Ighodaro, O. M. Molecular Pathways Associated with Oxidative Stress in Diabetes Mellitus. *Biomed. Pharmacother.* **2018**, *108* (August), 656–662. https://doi.org/10.1016/j.biopha.2018.09.058.

(10) Khansari, N.; Shakiba, Y.; Mahmoudi, M. Chronic Inflammation and Oxidative Stress as a Major Cause of Age- Related Diseases and Cancer. *Recent Pat. Inflamm. Allergy Drug Discov.* **2009**, *3* (1), 73–80. https://doi.org/10.2174/187221309787158371.

(11) Thanan, R.; Oikawa, S.; Hiraku, Y.; Ohnishi, S.; Ma, N.; Pinlaor, S.; Yongvanit, P.; Kawanishi, S.; Murata, M. Oxidative Stress and Its Significant Roles in Neurodegenerative Diseases and Cancer. *Int. J. Mol. Sci.* **2014**, *16* (1), 193–217. https://doi.org/10.3390/ijms16010193.

(12) Alhasan, R.; Nasim, M. J.; Jacob, C.; Gaucher, C. Selenoneine: A Unique Reactive Selenium Species From the Blood of Tuna With Implications for Human Diseases. *Curr. Pharmacol. Reports* **2019**, *5* (3), 163–173. https://doi.org/10.1007/s40495-019-00175-8.

(13) Jacob, C.; Giles, G. I.; Giles, N. M.; Sies, H. Sulfur and Selenium: The Role of Oxidation State in Protein Structure and Function. *Angew. Chemie Int. Ed.* **2003**, *42* (39), 4742–4758. https://doi.org/10.1002/anie.200300573.

(14) Storkey, C.; Pattison, D. I.; White, J. M.; Schiesser, C. H.; Davies, M. J. Preventing Protein Oxidation with Sugars: Scavenging of Hypohalous Acids by 5-Selenopyranose and 4-Selenofuranose Derivatives. *Chem. Res. Toxicol.* **2012**, *25* (11), 2589–2599. https://doi.org/10.1021/tx3003593.

(15) Mangiavacchi, F.; Dias, I. F. C.; Di Lorenzo, I.; Grzes, P.; Palomba, M.; Rosati, O.; Bagnoli, L.; Marini, F.; Santi, C.; Lenardao, E. J.; Sancineto, L. Sweet Selenium: Synthesis and Properties of Selenium-Containing Sugars and Derivatives. *Pharmaceuticals* **2020**, *13* (9), 1–28. https://doi.org/10.3390/ph13090211.

(16) Cutrim, C. S.; Cortez, M. A. S. A Review on Polyphenols: Classification, Beneficial Effects and Their Application in Dairy Products. *Int. J. Dairy Technol.* **2018**, *71* (3), 564–578. https://doi.org/10.1111/1471-0307.12515.

(17) Quideau, S.; Deffieux, D.; Douat-Casassus, C.; Pouységu, L. Plant Polyphenols: Chemical Properties, Biological Activities, and Synthesis. *Angew. Chemie Int. Ed.* **2011**, *50* (3), 586–621. https://doi.org/10.1002/anie.201000044.

(18) Davies, M. J.; Schiesser, C. H. 1,4-Anhydro-4-Seleno-d-Talitol (SeTal): A Remarkable Selenium-Containing Therapeutic Molecule. *New J. Chem.* **2019**, *43* (25), 9759–9765. https://doi.org/10.1039/c9nj02185j.

(19) Sanchez, M. C.; Lancel, S.; Boulanger, E.; Neviere, R. Targeting Oxidative Stress and Mitochondrial Dysfunction in the Treatment of Impaired Wound Healing: A Systematic Review. *Antioxidants* **2018**, *7* (8), 1–14. https://doi.org/10.3390/antiox7080098.

(20) Xu, Z.; Han, S.; Gu, Z.; Wu, J. Advances and Impact of Antioxidant Hydrogel in Chronic Wound Healing. *Adv. Healthc. Mater.* **2020**, *9* (5), 1901502. https://doi.org/10.1002/adhm.201901502.

(21) Sies, H. Oxidative Stress: Introductory Remarks. In *Oxidative Stress*; Elsevier, **1985**; pp 1–8. https://doi.org/10.1016/B978-0-12-642760-8.50005-3.

(22) Sies, H. Oxidative Stress: Concept and Some Practical Aspects. *Antioxidants* **2020**, *9* (9), 1–6. https://doi.org/10.3390/antiox9090852.

(23) Selye, H. A Syndrome Produced by Diverse Nocuous Agents. *Nature* **1936**, *138* (3479), 32–32. https://doi.org/10.1038/138032a0.

(24) Bhatti, J. S.; Bhatti, G. K.; Reddy, P. H. Mitochondrial Dysfunction and Oxidative Stress in Metabolic Disorders — A Step towards Mitochondria Based Therapeutic Strategies. *Biochim. Biophys. Acta - Mol. Basis Dis.* **2017**, *1863* (5), 1066–1077. https://doi.org/10.1016/j.bbadis.2016.11.010.

(25) Wisløff, U.; Najjar, S. M.; Ellingsen, Ø.; Haram, P. M.; Swoap, S.; Al-Share, Q.; Fernström, M.; Rezaei, K.; Lee, S. J.; Koch, L. G.; Britton, S. L. Cardiovascular Risk Factors Emerge after Artificial Selection for Low Aerobic Capacity. *Science (80-.).* **2005**, *307* (5708), 418–420. https://doi.org/10.1126/science.1108177.

(26) Wisløff, U.; Støylen, A.; Loennechen, J. P.; Bruvold, M.; Rognmo, Ø.; Haram, P. M.; Tjønna, A. E.; Helgerud, J.; Slørdahl, S. A.; Lee, S. J.; Videm, V.; Bye, A.; Smith, G. L.; Najjar, S. M.; Ellingsen, Ø.; Skjærpe, T. Superior Cardiovascular Effect of Aerobic Interval Training versus Moderate Continuous Training in Heart Failure Patients: A Randomized Study. *Circulation* **2007**, *115* (24), 3086–3094. https://doi.org/10.1161/CIRCULATIONAHA.106.675041.

(27) Surh, Y. J.; Kundu, J. K.; Li, M. H.; Na, H. K.; Cha, Y. N. Role of Nrf2-Mediated Heme Oxygenase-1 Upregulation in Adaptive Survival Response to Nitrosative Stress. *Arch. Pharm. Res.* **2009**, *32* (8), 1163–1176. https://doi.org/10.1007/s12272-009-1807-8.

(28) Ceaser, E. K.; Moellering, D. R.; Shiva, S.; Ramachandran, A.; Landar, A.; Venkartraman, A.; Crawford, J.; Patel, R.; Dickinson, D. A.; Ulasova, E.; Ji, S.; Darley-Usmar, V. M. Mechanisms of Signal Transduction Mediated by Oxidized Lipids: The Role of the Electrophile-Responsive Proteome. *Biochem. Soc. Trans.* **2004**, *32* (1), 151–155.

https://doi.org/10.1042/BST0320151.

(29) Noguchi, N. Role of Oxidative Stress in Adaptive Responses in Special Reference to Atherogenesis. *J. Clin. Biochem. Nutr.* **2008**, *43* (3), 131–138. https://doi.org/10.3164/jcbn.2008068.

(30) Oliveira-Marques, V.; Marinho, H. S.; Cyrne, L.; Antunes, F. Role of Hydrogen Peroxide in NF-KB Activation: From Inducer to Modulator. *Antioxidants Redox Signal.* **2009**, *11* (9), 2223–2243. https://doi.org/10.1089/ars.2009.2601.

(31) Sies, H. *Oxidative Eustress and Oxidative Distress: Introductory Remarks*; Elsevier Inc., **2019**. https://doi.org/10.1016/B978-0-12-818606-0.00001-8.

(32) Aschbacher, K.; O'Donovan, A.; Wolkowitz, O. M.; Dhabhar, F. S.; Su, Y.; Epel, E. Good Stress, Bad Stress and Oxidative Stress: Insights from Anticipatory Cortisol Reactivity. *Psychoneuroendocrinology* **2013**, *38* (9), 1698–1708. https://doi.org/10.1016/j.psyneuen.2013.02.004.

(33) Aschbacher, K.; O'Donovan, A.; Wolkowitz, O. M.; Dhabhar, F. S.; Su, Y.; Epel, E. Good Stress, Bad Stress and Oxidative Stress: Insights from Anticipatory Cortisol Reactivity. *Psychoneuroendocrinology* **2013**, *38* (9), 1698–1708. https://doi.org/10.1016/j.psyneuen.2013.02.004.

(34) Preiser, J. C. Oxidative Stress. *J. Parenter. Enter. Nutr.* **2012**, *36* (2), 147–154. https://doi.org/10.1177/0148607111434963.

(35) Sies, H. Hydrogen Peroxide as a Central Redox Signaling Molecule in Physiological Oxidative Stress: Oxidative Eustress. *Redox Biol.* **2017**, *11* (November 2016), 613–619. https://doi.org/10.1016/j.redox.2016.12.035.

(36) Mattson, M. P. Hormesis Defined. *Ageing Res. Rev.* **2008**, *7* (1), 1–7. https://doi.org/10.1016/j.arr.2007.08.007.

(37) Leri, M.; Scuto, M.; Ontario, M. L.; Calabrese, V.; Calabrese, E. J.; Bucciantini, M.; Stefani, M. Healthy Effects of Plant Polyphenols: Molecular Mechanisms. *Int. J. Mol. Sci.* **2020**, *21* (4), 1250. https://doi.org/10.3390/ijms21041250.

(38) Armstrong, D. Introduction to Free Radicals, Inflammation, and Recycling Donald. In *Oxidative Stress and Antioxidant Protection: The Science ofFree Radical Biology andDisease*; Donald Armstrong and Robert D. Stratton, Ed.; John Wiley & Sons, Inc., **2016**; pp 3–10.

(39) Osellame, L. D.; Blacker, T. S.; Duchen, M. R. Cellular and Molecular Mechanisms of Mitochondrial Function. *Best Pract. Res. Clin. Endocrinol. Metab.* **2012**, *26* (6), 711–723. https://doi.org/10.1016/j.beem.2012.05.003.

(40) Uif, B. O. E. L Bj. **1981**, *290* (April), 1–18.

(41) Shadel, G. S.; Horvath, T. L. Mitochondrial ROS Signaling in Organismal Homeostasis. *Cell* **2015**, *163* (3), 560–569. https://doi.org/10.1016/j.cell.2015.10.001.

(42) Murphy, M. P. How Mitochondria Produce Reactive Oxygen Species. *Biochem. J.* **2009**, *417* (1), 1–13. https://doi.org/10.1042/BJ20081386.

(43) Pagano, G.; Aiello Talamanca, A.; Castello, G.; Cordero, M. D.; D'Ischia, M.; Gadaleta, M. N.; Pallardó, F. V.; Petrović, S.; Tiano, L.; Zatterale, A. Oxidative Stress and Mitochondrial Dysfunction across Broad-Ranging Pathologies: Toward Mitochondria-Targeted Clinical Strategies. *Oxid. Med. Cell. Longev.* **2014**, *2014*. https://doi.org/10.1155/2014/541230.

(44) Cui, H.; Kong, Y.; Zhang, H. Oxidative Stress, Mitochondrial Dysfunction, and Aging. *J. Signal Transduct.* **2012**, *2012*, 1–13. https://doi.org/10.1155/2012/646354.

(45) Haas, R. H. Mitochondrial Dysfunction in Aging and Diseases of Aging.

Biology (Basel). **2019**, *8* (2), 1–5. https://doi.org/10.3390/biology8020048.

(46) Lin, M. T.; Beal, M. F. Mitochondrial Dysfunction and Oxidative Stress in Neurodegenerative Diseases. *Nature* **2006**, *443* (7113), 787–795. https://doi.org/10.1038/nature05292.

(47) Hussain, M.; Krishnamurthy, S.; Patel, J.; Kim, E.; Baptiste, B. A.; Croteau, D. L.; Bohr, V. A. Skin Abnormalities in Disorders with DNA Repair Defects, Premature Aging, and Mitochondrial Dysfunction. *J. Invest. Dermatol.* **2020**, 1–8. https://doi.org/10.1016/j.jid.2020.10.019.

(48) West, A. P.; Shadel, G. S. Mitochondrial DNA in Innate Immune Responses and Inflammatory Pathology. *Nat. Rev. Immunol.* **2017**, *17* (6), 363–375. https://doi.org/10.1038/nri.2017.21.

(49) Mittal, M.; Siddiqui, M. R.; Tran, K.; Reddy, S. P.; Malik, A. B. Reactive Oxygen Species in Inflammation and Tissue Injury. *Antioxidants Redox Signal.* **2014**, *20* (7), 1126–1167. https://doi.org/10.1089/ars.2012.5149.

(50) Di Meo, S.; Reed, T. T.; Venditti, P.; Victor, V. M. Role of ROS and RNS Sources in Physiological and Pathological Conditions. *Oxid. Med. Cell. Longev.* **2016**, *2016*. https://doi.org/10.1155/2016/1245049.

(51) Giles, G. I.; Jacob, C. Reactive Sulfur Species: An Emerging Concept in Oxidative Stress. *Biol. Chem.* **2002**, *383* (3–4), 375–388. https://doi.org/10.1515/BC.2002.042.

(52) Gruhlke, M. C. H.; Slusarenko, A. J. The Biology of Reactive Sulfur Species (RSS). *Plant Physiol. Biochem.* **2012**, *59*, 98–107. https://doi.org/10.1016/j.plaphy.2012.03.016.

(53) Poole, L. B. The Basics of Thiols and Cysteines in Redox Biology and Chemistry. *Free Radic. Biol. Med.* **2015**, *80*, 148–157. https://doi.org/10.1016/j.freeradbiomed.2014.11.013.

(54) Mano, J. Reactive Carbonyl Species: Their Production from Lipid Peroxides, Action in Environmental Stress, and the Detoxification Mechanism. *Plant Physiol. Biochem.* **2012**, *59*, 90–97. https://doi.org/10.1016/j.plaphy.2012.03.010.

(55) Kharma, A.; Misak, A.; Grman, M.; Brezova, V.; Kurakova, L.; Baráth, P.; Jacob, C.; Chovanec, M.; Ondrias, K.; Domínguez-Álvarez, E. Release of Reactive Selenium Species from Phthalic Selenoanhydride in the Presence of Hydrogen Sulfide and Glutathione with Implications for Cancer Research. *New J. Chem.* **2019**, *43* (29), 11771–11783. https://doi.org/10.1039/c9nj02245g.

(56) Kulkarni, A. C.; Kuppusamy, P.; Parinandi, N. Oxygen, the Lead Actor in the Pathophysiologic Drama: Enactment of the Trinity of Normoxia, Hypoxia, and Hyperoxia in Disease and Therapy. *Antioxidants Redox Signal.* **2007**, *9* (10), 1717–1730. https://doi.org/10.1089/ars.2007.1724.

(57) Davies, K. J. Oxidative Stress: The Paradox of Aerobic Life. *Biochem. Soc. Symp.* **1995**, *61*, 1–31. https://doi.org/10.1042/bss0610001.

(58) Halliwell, B. Redox Biology Is a Fundamental Theme of Aerobic Life. *Plant Physiol.* **2006**, *141* (June), 312–322. https://doi.org/10.1104/pp.106.077073.312.

(59) Gulcin, İ. Antioxidants and Antioxidant Methods: An Updated Overview. *Arch. Toxicol.* **2020**, *94* (3), 651–715. https://doi.org/10.1007/s00204-020-02689-3.

(60) D'Autréaux, B.; Toledano, M. B. ROS as Signalling Molecules: Mechanisms That Generate Specificity in ROS Homeostasis. *Nat. Rev. Mol. Cell Biol.* **2007**, *8* (10), 813–824. https://doi.org/10.1038/nrm2256.

(61) Murphy, M. P.; Holmgren, A.; Larsson, N. G.; Halliwell, B.; Chang, C. J.;

Kalyanaraman, B.; Rhee, S. G.; Thornalley, P. J.; Partridge, L.; Gems, D.; Nyström, T.; Belousov, V.; Schumacker, P. T.; Winterbourn, C. C. Unraveling the Biological Roles of Reactive Oxygen Species. *Cell Metab.* **2011**, *13* (4), 361–366. https://doi.org/10.1016/j.cmet.2011.03.010.

(62) Thannickal, V. J.; Fanburg, B. L. Reactive Oxygen Species in Cell Signaling. *Am. J. Physiol. - Lung Cell. Mol. Physiol.* **2000**, *279* (6 23-6). https://doi.org/10.1152/ajplung.2000.279.6.l1005.

(63) Collin, F. Chemical Basis of Reactive Oxygen Species Reactivity and Involvement in Neurodegenerative Diseases. *Int. J. Mol. Sci.* **2019**, *20* (10). https://doi.org/10.3390/ijms20102407.

(64) Finkel, T. Signal Transduction by Reactive Oxygen Species. *J. Cell Biol.* **2011**, *194* (1), 7–15. https://doi.org/10.1083/jcb.201102095.

(65) Meo, S. Di; Napolitano, G.; Venditti, P. Physiological and Pathological Role of ROS : Benefits and Limitations of Antioxidant Treatment. **2019**, *2*, 10–12.

(66) Valko, M.; Leibfritz, D.; Moncol, J.; Cronin, M. T. D.; Mazur, M.; Telser, J. Free Radicals and Antioxidants in Normal Physiological Functions and Human Disease. **2007**, *39*, 44–84. https://doi.org/10.1016/j.biocel.2006.07.001.

(67) Sies, H.; Jones, D. P. Reactive Oxygen Species (ROS) as Pleiotropic Physiological Signalling Agents. *Nat. Rev. Mol. Cell Biol.* **2020**. https://doi.org/10.1038/s41580-020-0230-3.

(68) Venditti, P.; Di Stefano, L.; Di Meo, S. Mitochondrial Metabolism of Reactive Oxygen Species. *Mitochondrion* **2013**, *13* (2), 71–82. https://doi.org/10.1016/j.mito.2013.01.008.

(69) Shigenaga, M. K.; Hagen, T. M.; Ames, B. N. Oxidative Damage and Mitochondrial Decay in Aging. *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91* (23),

10771–10778. https://doi.org/10.1073/pnas.91.23.10771.

(70) Carocho, M.; Ferreira, I. C. F. R. A Review on Antioxidants, Prooxidants and Related Controversy: Natural and Synthetic Compounds, Screening and Analysis Methodologies and Future Perspectives. *Food Chem. Toxicol.* **2013**, *51* (1), 15–25. https://doi.org/10.1016/j.fct.2012.09.021.

(71) Reybier, K.; Ayala, S.; Alies, B.; Rodrigues, J. V.; Bustos Rodriguez, S.; La Penna, G.; Collin, F.; Gomes, C. M.; Hureau, C.; Faller, P. Free Superoxide Is an Intermediate in the Production of H2O2 by Copper(I)-Aβ Peptide and O2. *Angew. Chemie - Int. Ed.* **2016**, *55* (3), 1085–1089. https://doi.org/10.1002/anie.201508597.

(72) Packer, M. A.; Porteous, C. M.; Murphy, M. P. Department of Biochemistry , University of Otago , PO Box 56 , Dunedin , New Zealand Phone + 64-3-479-7871 Facsimile + 64-3-479-7866 Email Murphy@sanger.Otago.Ac.Nz. **1996**, *40* (3), 527–534.

(73) Farhataziz, A. B. Selected Specific Rates of Reactions of Transients From Water in Aqueous Solution. Iii. Hydroxyl Radical and Perhydroxyl Radical and Their Radical Ions. *Natl Bur Stand Natl Stand Ref Data Ser* **1977**, No. 59.

(74) Cadet, J.; Delatour, T.; Douki, T.; Gasparutto, D.; Pouget, J. P.; Ravanat, J. L.; Sauvaigo, S. Hydroxyl Radicals and DNA Base Damage. *Mutat. Res. - Fundam. Mol. Mech. Mutagen.* **1999**, *424* (1–2), 9–21. https://doi.org/10.1016/S0027-5107(99)00004-4.

(75) Bielski, B. H. J.; Arudi, R. L.; Sutherland, M. W. A Study of the Reactivity of HO2/O2- with Unsaturated Fatty Acids. *J. Biol. Chem.* **1983**, *258* (8), 4759–4761. https://doi.org/10.1016/s0021-9258(18)32488-8.

(76) Homma, T.; Kobayashi, S.; Fujii, J. Induction of Ferroptosis by Singlet Oxygen Generated from Naphthalene Endoperoxide. *Biochem. Biophys. Res.*

Commun. **2019**, *518* (3), 519–525. https://doi.org/10.1016/j.bbrc.2019.08.073.

(77) Di Mascio, P.; Martinez, G. R.; Miyamoto, S.; Ronsein, G. E.; Medeiros, M. H. G.; Cadet, J. Singlet Molecular Oxygen Reactions with Nucleic Acids, Lipids, and Proteins. *Chem. Rev.* **2019**, *119* (3), 2043–2086. https://doi.org/10.1021/acs.chemrev.8b00554.

(78) Halliwell, B.; Clement, M. V.; Long, L. H. Hydrogen Peroxide in the Human Body. *FEBS Lett.* **2000**, *486* (1), 10–13. https://doi.org/10.1016/S0014-5793(00)02197-9.

(79) CAVE, A.; BREWER, A.; NARAYANAPANICKER, A.; RAY, R.; GRIEVE, D.; WALKER, S.; SHAH, A. Comprehensive Invited Review. *Ars* **2006**, *8*, 691.

(80) Rhee, S. G. Redox Signaling: Hydrogen Peroxide as Intracellular Messenger. *Exp. Mol. Med.* **1999**, *31* (2), 53–59. https://doi.org/10.1038/emm.1999.9.

(81) Haber, F.; Weiss, J. The Catalytic Decom Position o f Hydrogen Peroxide by Iron Salts *. **1932**, 332–351.

(82) Sies, H.; Jones, D. P. Reactive Oxygen Species (ROS) as Pleiotropic Physiological Signalling Agents. *Nat. Rev. Mol. Cell Biol.* **2020**, *21* (7), 363–383. https://doi.org/10.1038/s41580-020-0230-3.

(83) Pattison, D.; Davies, M. Reactions of Myeloperoxidase-Derived Oxidants with Biological Substrates:Gaining Chemical Insight into Human Inflammatory Diseases. *Curr. Med. Chem.* **2006**, *13* (27), 3271–3290. https://doi.org/10.2174/092986706778773095.

(84) Nussbaum, C.; Klinke, A.; Adam, M.; Baldus, S.; Sperandio, M. Myeloperoxidase: A Leukocyte-Derived Protagonist of Inflammation and Cardiovascular Disease. *Antioxidants Redox Signal.* **2013**, *18* (6), 692–713. https://doi.org/10.1089/ars.2012.4783.

(85) Ray, R. S.; Katyal, A. Myeloperoxidase: Bridging the Gap in Neurodegeneration. *Neurosci. Biobehav. Rev.* **2016**, *68*, 611–620. https://doi.org/10.1016/j.neubiorev.2016.06.031.

(86) Epstein, F. H.; Weiss, S. J. Tissue Destruction by Neutrophils. *N. Engl. J. Med.* **1989**, *320* (6), 365–376. https://doi.org/10.1056/NEJM198902093200606.

(87) Carrell Morris, J. The Acid Ionization Constant of HOCl from 5 to 35°. *J. Phys. Chem.* **1966**, *70* (12), 3798–3805. https://doi.org/10.1021/j100884a007.

(88) Prokopowicz, Z. M.; Arce, F.; Biedron, R.; Chiang, C. L.-L.; Ciszek, M.; Katz, D. R.; Nowakowska, M.; Zapotoczny, S.; Marcinkiewicz, J.; Chain, B. M. Hypochlorous Acid: A Natural Adjuvant That Facilitates Antigen Processing, Cross-Priming, and the Induction of Adaptive Immunity. *J. Immunol.* **2010**, *184* (2), 824–835. https://doi.org/10.4049/jimmunol.0902606.

(89) Fukuzaki, S. Mechanisms of Actions of Sodium Hypochlorite in Cleaning and Disinfection Processes. *Biocontrol Sci.* **2006**, *11* (4), 147–157. https://doi.org/10.4265/bio.11.147.

(90) Hawkins, C. L.; Pattison, D. I.; Davies, M. J. Hypochlorite-Induced Oxidation of Amino Acids, Peptides and Proteins. *Amino Acids* **2003**, *25* (3–4), 259–274. https://doi.org/10.1007/s00726-003-0016-x.

(91) Hazell, L. J.; Arnold, L.; Flowers, D.; Waeg, G.; Malle, E.; Stocker, R. Presence of Hypochlorite-Modified Proteins in Human Atherosclerotic Lesions. *J. Clin. Invest.* **1996**, *97* (6), 1535–1544. https://doi.org/10.1172/JCI118576.

(92) Hasegawa, T.; Malle, E.; Farhood, A.; Jaeschke, H. Generation of Hypochlorite-Modified Proteins by Neutrophils during Ischemia-Reperfusion Injury in Rat Liver: Attenuation by Ischemic Preconditioning. *Am. J. Physiol. - Gastrointest. Liver Physiol.* **2005**, *289* (4 52-4), 760–767.

https://doi.org/10.1152/ajpgi.00141.2005.

(93) Weitzman, S. A.; Gordon, L. I. Inflammation and Cancer: Role of Phagocyte-Generated Oxidants in Carcinogenesis. *Blood* **1990**, *76* (4), 655–663. https://doi.org/10.1182/blood.v76.4.655.655.

(94) Peskin, A. V.; Winterbourn, C. C. Kinetics of the Reactions of Hypochlorous Acid and Amino Acid Chloramines with Thiols, Methionine, and Ascorbate. *Free Radic. Biol. Med.* **2001**, *30* (5), 572–579. https://doi.org/10.1016/S0891-5849(00)00506-2.

(95) Storkey, C.; Davies, M. J.; Pattison, D. I. Reevaluation of the Rate Constants for the Reaction of Hypochlorous Acid (HOCl) with Cysteine, Methionine, and Peptide Derivatives Using a New Competition Kinetic Approach. *Free Radic. Biol. Med.* **2014**, *73*, 60–66. https://doi.org/10.1016/j.freeradbiomed.2014.04.024.

(96) Dawson, V. L.; Dawson, T. M. Nitric Oxide Neurotoxicity. *J. Chem. Neuroanat.* **1996**, *10* (3–4), 179–190. https://doi.org/10.1016/0891-0618(96)00148-2.

(97) Padmaja, S.; Huie, R. E. The Reaction of Nitric Oxide with Organic Peroxyl Radicals. *Biochem. Biophys. Res. Commun.* **1993**, *195* (2), 539–544. https://doi.org/10.1006/bbrc.1993.2079.

(98) Pacher, P.; Beckman, J. S.; Liaudet, L. Nitric Oxide and Peroxynitrite in Health and Disease. *Physiol. Rev.* **2007**, *87* (1), 315–424. https://doi.org/10.1152/physrev.00029.2006.

(99) Halliwell, Barry, J. M. C. G. Antioxidant Defences Synthesized in Vivo. In *Free Radicals in Biology and Medicine*; Oxford University Press: oxford, 2015; pp 77–78.

(100) Harwell, B. Biochemistry of Oxidative Stress. *Biochem. Soc. Trans.* **2007**, *35*

(5), 1147–1150. https://doi.org/10.1042/BST0351147.

(101) Halliwell, Barry, J. M. C. G. Repair of Oxidative DNA Damage. In *Free Radical Biology and Medicine*; Oxford University Press: oxford, **2015**; pp 247–252.

(102) Muftuoglu, M.; Mori, M. P.; Souza-Pinto, N. C. de. Formation and Repair of Oxidative Damage in the Mitochondrial DNA. *Mitochondrion* **2014**, *17*, 164–181. https://doi.org/10.1016/j.mito.2014.03.007.

(103) Tainer, J. A.; Getzoff, E. D.; Richardson, J. S.; Richardson, D. C. Structure and Mechanism of Copper, Zinc Superoxide Dismutase. *Nature* **1983**, *306* (5940), 284–287. https://doi.org/10.1038/306284a0.

(104) Sheng, Y.; Abreu, I. A.; Cabelli, D. E.; Maroney, M. J.; Miller, A. F.; Teixeira, M.; Valentine, J. S. Superoxide Dismutases and Superoxide Reductases. *Chem. Rev.* **2014**, *114* (7), 3854–3918. https://doi.org/10.1021/cr4005296.

(105) Murakami, K.; Murata, N.; Noda, Y.; Tahara, S.; Kaneko, T.; Kinoshita, N.; Hatsuta, H.; Murayama, S.; Barnham, K. J.; Irie, K.; Shirasawa, T.; Shimizu, T. SOD1 (Copper/Zinc Superoxide Dismutase) Deficiency Drives Amyloid β Protein Oligomerization and Memory Loss in Mouse Model of Alzheimer Disease. *J. Biol. Chem.* **2011**, *286* (52), 44557–44568. https://doi.org/10.1074/jbc.M111.279208.

(106) Ren, B.; Huang, W.; Åkesson, B.; Ladenstein, R. The Crystal Structure of Seleno-Glutathione Peroxidase from Human Plasma at 2.9 Å Resolution. *J. Mol. Biol.* **1997**, *268* (5), 869–885. https://doi.org/10.1006/jmbi.1997.1005.

(107) Sies, H. Ebselen, a Selenoorganic Compound as Glutathione Peroxidase Mimic. *Free Radic. Biol. Med.* **1993**, *14* (3), 313–323. https://doi.org/10.1016/0891-5849(93)90028-S.

(108) Thakare, R.; Kaul, G.; Shukla, M.; Kesharwani, P.; Srinivas, N.; Dasgupta, A.;

Chopra, S. Repurposing Nonantibiotic Drugs as Antibacterials. In *Drug Discovery Targeting Drug-Resistant Bacteria*; Elsevier, **2020**; pp 105–138. https://doi.org/10.1016/B978-0-12-818480-6.00005-9.

(109) Gill, S. S.; Anjum, N. A.; Hasanuzzaman, M.; Gill, R.; Trivedi, D. K.; Ahmad, I.; Pereira, E.; Tuteja, N. Glutathione and Glutathione Reductase: A Boon in Disguise for Plant Abiotic Stress Defense Operations. *Plant Physiol. Biochem.* **2013**, *70*, 204–212. https://doi.org/10.1016/j.plaphy.2013.05.032.

(110) Ighodaro, O. M.; Akinloye, O. A. First Line Defence Antioxidants-Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPX): Their Fundamental Role in the Entire Antioxidant Defence Grid. *Alexandria J. Med.* **2018**, *54* (4), 287–293. https://doi.org/10.1016/j.ajme.2017.09.001.

(111) Witkowska, A. M. Advances in Medical Sciences Endogenous Non-Enzymatic Antioxidants in the Human Body. **2018**, *63*, 68–78. https://doi.org/10.1016/j.advms.2017.05.005.

(112) Mirończuk-Chodakowska, I.; Witkowska, A. M.; Zujko, M. E. Endogenous Non-Enzymatic Antioxidants in the Human Body. *Adv. Med. Sci.* **2018**, *63* (1), 68–78. https://doi.org/10.1016/j.advms.2017.05.005.

(113) Eid, C.; Hémadi, M.; Ha-Duong, N. T.; El Hage Chahine, J. M. Iron Uptake and Transfer from Ceruloplasmin to Transferrin. *Biochim. Biophys. Acta - Gen. Subj.* **2014**, *1840* (6), 1771–1781. https://doi.org/10.1016/j.bbagen.2014.01.011.

(114) Hadzhieva, M.; Kirches, E.; Mawrin, C. Review: Iron Metabolism and the Role of Iron in Neurodegenerative Disorders. *Neuropathol. Appl. Neurobiol.* **2014**, *40* (3), 240–257. https://doi.org/10.1111/nan.12096.

(115) Taverna, M.; Marie, A.-L.; Mira, J.-P.; Guidet, B. Specific Antioxidant Properties of Human Serum Albumin. *Ann. Intensive Care* **2013**, *3* (1), 4.

https://doi.org/10.1186/2110-5820-3-4.

(116) Vincent, J.-L. Relevance of Albumin in Modern Critical Care Medicine. *Best Pract. Res. Clin. Anaesthesiol.* **2009**, *23* (2), 183–191. https://doi.org/10.1016/j.bpa.2008.11.004.

(117) Forman, H. J.; Zhang, H.; Rinna, A. Glutathione: Overview of Its Protective Roles, Measurement, and Biosynthesis. *Mol. Aspects Med.* **2009**, *30* (1–2), 1–12. https://doi.org/10.1016/j.mam.2008.08.006.

(118) Rahman, K. Studies on Free Radicals, Antioxidants, and Co-Factors. *Clinical interventions in aging*. **2007**, pp 219–236.

(119) Bentinger, M.; Tekle, M.; Dallner, G. Coenzyme Q - Biosynthesis and Functions. *Biochem. Biophys. Res. Commun.* **2010**, *396* (1), 74–79. https://doi.org/10.1016/j.bbrc.2010.02.147.

(120) Acosta, M. J.; Vazquez Fonseca, L.; Desbats, M. A.; Cerqua, C.; Zordan, R.; Trevisson, E.; Salviati, L. Coenzyme Q Biosynthesis in Health and Disease. *Biochim. Biophys. Acta - Bioenerg.* **2016**, *1857* (8), 1079–1085. https://doi.org/10.1016/j.bbabio.2016.03.036.

(121) Navas, P.; Villalba, J. M.; de Cabo, R. The Importance of Plasma Membrane Coenzyme Q in Aging and Stress Responses. *Mitochondrion* **2007**, *7* (SUPPL.), S34–S40. https://doi.org/10.1016/j.mito.2007.02.010.

(122) Block, G.; Patterson, B.; Subar, A. Fruit, Vegetables, and Cancer Prevention: A Review of the Epidemiological Evidence. *Nutr. Cancer* **1992**, *18* (1), 1–29. https://doi.org/10.1080/01635589209514201.

(123) Zujko, M. E.; Witkowska, A. M.; Waśkiewicz, A.; Mirończuk-Chodakowska, I. Dietary Antioxidant and Flavonoid Intakes Are Reduced in the Elderly. *Oxid. Med. Cell. Longev.* **2015**, *2015*. https://doi.org/10.1155/2015/843173.

(124) Naseri, R.; Farzaei, F.; Fakhri, S.; El-Senduny, F. F.; Altouhamy, M.; Bahramsoltani, R.; Ebrahimi, F.; Rahimi, R.; Farzaei, M. H. Polyphenols for Diabetes Associated Neuropathy: Pharmacological Targets and Clinical Perspective. *DARU J. Pharm. Sci.* **2019**, *27* (2), 781–798. https://doi.org/10.1007/s40199-019-00289-w.

(125) Hertog, M. G. L.; Feskens, E. J. M.; Kromhout, D.; Hertog, M. G. L.; Hollman, P. C. H.; Hertog, M. G. L.; Katan, M. B. Dietary Antioxidant Flavonoids and Risk of Coronary Heart Disease: The Zutphen Elderly Study. *Lancet* **1993**, *342* (8878), 1007–1011. https://doi.org/10.1016/0140-6736(93)92876-U.

(126) Yaribeygi, H.; Farrokhi, F. R.; Rezaee, R.; Sahebkar, A. Oxidative Stress Induces Renal Failure: A Review of Possible Molecular Pathways. *J. Cell. Biochem.* **2018**, *119* (4), 2990–2998. https://doi.org/10.1002/jcb.26450.

(127) Singh, S.; Verma, S. K.; Kumar, S.; Ahmad, M. K.; Nischal, A.; Singh, S. K.; Dixit, R. K. Evaluation of Oxidative Stress and Antioxidant Status in Chronic Obstructive Pulmonary Disease. *Scand. J. Immunol.* **2017**, *85* (2), 130–137. https://doi.org/10.1111/sji.12498.

(128) Akioyamen, L. E.; Genest, J.; Chu, A.; Inibhunu, H.; Ko, D. T.; Tu, J. V. Risk Factors for Cardiovascular Disease in Heterozygous Familial Hypercholesterolemia: A Systematic Review and Meta-Analysis. *J. Clin. Lipidol.* **2019**, *13* (1), 15–30. https://doi.org/10.1016/j.jacl.2018.10.012.

(129) Mozaffarian, D.; Wilson, P. W. F.; Kannel, W. B. Beyond Established and Novel Risk Factors Lifestyle Risk Factors for Cardiovascular Disease. *Circulation* **2008**, *117* (23), 3031–3038. https://doi.org/10.1161/CIRCULATIONAHA.107.738732.

(130) Senoner, T.; Dichtl, W. Oxidative Stress in Cardiovascular Diseases: Still a Therapeutic Target? *Nutrients* **2019**, *11* (9).

https://doi.org/10.3390/nu11092090.

(131) Chandra, A.; Surti, N.; Kesavan, S.; Agarwal, A. Significance of Oxidative Stress in Human Reproduction. *Arch Med Sci* **2009**, *5* (1A), S28–S42.

(132) Agarwal, A.; Gupta, S.; Sekhon, L.; Shah, R. Redox Considerations in Female Reproductive Function and Assisted Reproduction: From Molecular Mechanisms to Health Implications. *Antioxidants Redox Signal.* **2008**, *10* (8), 1375–1403. https://doi.org/10.1089/ars.2007.1964.

(133) Lu, J.; Wang, Z.; Cao, J.; Chen, Y.; Dong, Y. A Novel and Compact Review on the Role of Oxidative Stress in Female Reproduction. *Reprod. Biol. Endocrinol.* **2018**, *16* (1), 1–18. https://doi.org/10.1186/s12958-018-0391-5.

(134) Marí-Alexandre, J.; Carcelén, A. P.; Agababyan, C.; Moreno-Manuel, A.; García-Oms, J.; Calabuig-Fariñas, S.; Gilabert-Estellés, J. Interplay between MicroRNAs and Oxidative Stress in Ovarian Conditions with a Focus on Ovarian Cancer and Endometriosis. *Int. J. Mol. Sci.* **2019**, *20* (21). https://doi.org/10.3390/ijms20215322.

(135) Alahmar, A. Role of Oxidative Stress in Male Infertility: An Updated Review. *J. Hum. Reprod. Sci.* **2019**, *12* (1), 4. https://doi.org/10.4103/jhrs.JHRS_150_18.

(136) Bisht, S.; Faiq, M.; Tolahunase, M.; Dada, R. Oxidative Stress and Male Infertility. *Nat. Rev. Urol.* **2017**, *14* (8), 470–485. https://doi.org/10.1038/nrurol.2017.69.

(137) Riordan; Rommens, J.; Kerem, B.; Alon, N.; Rozmahel, R.; Grzelczak, Z.; Zielenski, J.; Lok, S.; Plavsic, N.; Chou, J.; Et, A. Identification of the Cystic Fibrosis Gene: Cloning and Characterization of Complementary DNA. *Science (80-.).* **1989**, *245* (4922), 1066–1073. https://doi.org/10.1126/science.2475911.

(138) Cohen, T. S.; Prince, A. Cystic Fibrosis: A Mucosal Immunodeficiency Syndrome. *Nat. Med.* **2012**, *18* (4), 509–519. https://doi.org/10.1038/nm.2715.

(139) Lezo, A.; Biasi, F.; Massarenti, P.; Calabrese, R.; Poli, G.; Santini, B.; Bignamini, E. Oxidative Stress in Stable Cystic Fibrosis Patients: Do We Need Higher Antioxidant Plasma Levels? *J. Cyst. Fibros.* **2013**, *12* (1), 35–41. https://doi.org/10.1016/j.jcf.2012.06.002.

(140) Wood, L. G.; Fitzgerald, D. A.; Lee, A. K.; Garg, M. L. Improved Antioxidant and Fatty Acid Status of Patients with Cystic Fibrosis after Antioxidant Supplementation Is Linked to Improved Lung Function. *Am. J. Clin. Nutr.* **2003**, *77* (1), 150–159. https://doi.org/10.1093/ajcn/77.1.150.

(141) Brown, R. K.; Wyatt, H.; Price, J. F.; Kelly, F. J. Pulmonary Dysfunction in Cystic Fibrosis Is Associated with Oxidative Stress. *Eur. Respir. J.* **1996**, *9* (2), 334–339. https://doi.org/10.1183/09031936.96.09020334.

(142) Magon, N. J.; Turner, R.; Gearry, R. B.; Hampton, M. B.; Sly, P. D.; Kettle, A. J. Oxidation of Calprotectin by Hypochlorous Acid Prevents Chelation of Essential Metal Ions and Allows Bacterial Growth: Relevance to Infections in Cystic Fibrosis. *Free Radic. Biol. Med.* **2015**, *86*, 133–144. https://doi.org/10.1016/j.freeradbiomed.2015.05.022.

(143) Stratton, M. R.; Campbell, P. J.; Futreal, P. A. Pan-Cancer Analysis of Whole Genomes. *Nature* **2020**, *578* (7793), 82–93. https://doi.org/10.1038/s41586-020-1969-6.

(144) Hanahan, D.; Weinberg, R. A. Hallmarks of Cancer: The Next Generation. *Cell* **2011**, *144* (5), 646–674. https://doi.org/10.1016/j.cell.2011.02.013.

(145) Stratton, M. R.; Campbell, P. J.; Futreal, & P. A. The Cancer Genome. **2009**. https://doi.org/10.1038/nature07943.

(146) Aggarwal, V.; Tuli, H. S.; Varol, A.; Thakral, F.; Yerer, M. B.; Sak, K.; Varol,

M.; Jain, A.; Khan, M. A.; Sethi, G. Role of Reactive Oxygen Species in Cancer Progression: Molecular Mechanisms and Recent Advancements. *Biomolecules* **2019**, *9* (11). https://doi.org/10.3390/biom9110735.

(147) Panieri, E.; Santoro, M. M. Ros Homeostasis and Metabolism: A Dangerous Liason in Cancer Cells. *Cell Death Dis.* **2016**, *7* (6), 1–12. https://doi.org/10.1038/cddis.2016.105.

(148) Puar, Y.; Shanmugam, M.; Fan, L.; Arfuso, F.; Sethi, G.; Tergaonkar, V. Evidence for the Involvement of the Master Transcription Factor NF-KB in Cancer Initiation and Progression. *Biomedicines* **2018**, *6* (3), 82. https://doi.org/10.3390/biomedicines6030082.

(149) Hayes, J. D.; Dinkova-Kostova, A. T.; Tew, K. D. Oxidative Stress in Cancer. *Cancer Cell* **2020**, *38* (2), 167–197. https://doi.org/10.1016/j.ccell.2020.06.001.

(150) Fox, D. B.; Garcia, N. M. G.; McKinney, B. J.; Lupo, R.; Noteware, L. C.; Newcomb, R.; Liu, J.; Locasale, J. W.; Hirschey, M. D.; Alvarez, J. V. NRF2 Activation Promotes the Recurrence of Dormant Tumour Cells through Regulation of Redox and Nucleotide Metabolism. *Nat. Metab.* **2020**, *2* (4), 318–334. https://doi.org/10.1038/s42255-020-0191-z.

(151) Nieto, M. A. The Ins and Outs of the Epithelial to Mesenchymal Transition in Health and Disease. *Annu. Rev. Cell Dev. Biol.* **2011**, *27*, 347–376. https://doi.org/10.1146/annurev-cellbio-092910-154036.

(152) Craene, B. De; Berx, G. Regulatory Networks Defining EMT during Cancer Initiation and Progression. *Nat. Rev. Cancer* **2013**, *13* (2), 97–110. https://doi.org/10.1038/nrc3447.

(153) Ushio-Fukai, M.; Nakamura, Y. Reactive Oxygen Species and Angiogenesis: NADPH Oxidase as Target for Cancer Therapy. *Cancer Lett.* **2008**, *266* (1),

37–52. https://doi.org/10.1016/j.canlet.2008.02.044.

(154) De Sá Junior, P. L.; Câmara, D. A. D.; Porcacchia, A. S.; Fonseca, P. M. M.; Jorge, S. D.; Araldi, R. P.; Ferreira, A. K. The Roles of ROS in Cancer Heterogeneity and Therapy. *Oxid. Med. Cell. Longev.* **2017**, *2017*. https://doi.org/10.1155/2017/2467940.

(155) Dewhirst, M. W.; Cao, Y.; Moeller, B. Cycling Hypoxia and Free Radicals Regulate Angiogenesis and Radiotherapy Response. *Nat. Rev. Cancer* **2008**, *8* (6), 425–437. https://doi.org/10.1038/nrc2397.

(156) Dai, X.; Wang, L.; Deivasigamni, A.; Looi, C. Y.; Karthikeyan, C.; Trivedi, P.; Chinnathambi, A.; Alharbi, S. A.; Arfuso, F.; Dharmarajan, A.; Goh, B. C.; Hui, K. M.; Kumar, A. P.; Mustafa, M. R.; Sethi, G. A Novel Benzimidazole Derivative, MBIC Inhibits Tumor Growth and Promotes Apoptosis via Activation of ROS-Dependent JNK Signaling Pathway in Hepatocellular Carcinoma. *Oncotarget* **2017**, *8* (8), 12831–12842. https://doi.org/10.18632/oncotarget.14606.

(157) Simon, H. U.; Haj-Yehia, A.; Levi-Schaffer, F. Role of Reactive Oxygen Species (ROS) in Apoptosis Induction. *Apoptosis* **2000**, *5* (5), 415–418. https://doi.org/10.1023/A:1009616228304.

(158) Redza-Dutordoir, M.; Averill-Bates, D. A. Activation of Apoptosis Signalling Pathways by Reactive Oxygen Species. *Biochim. Biophys. Acta - Mol. Cell Res.* **2016**, *1863* (12), 2977–2992. https://doi.org/10.1016/j.bbamcr.2016.09.012.

(159) Khan, M. A.; Tania, M.; Fu, S.; Fu, J. Thymoquinone, as an Anticancer Molecule: From Basic Research to Clinical Investigation. *Oncotarget* **2017**, *8* (31), 51907–51919. https://doi.org/10.18632/oncotarget.17206.

(160) Khan, M. A.; Chen, H.-C.; Wan, X.-X.; Tania, M.; Xu, A.-H.; Chen, F.-Z.;

Zhang, D.-Z. Erratum to "Regulatory Effects of Resveratrol on Antioxidant Enzymes: A Mechanism of Growth In-Hibition and Apoptosis Induction in Cancer Cells)] Erratum Molecules and Cells. *Mol. Cells* **2013**, *35*, 219–225. https://doi.org/10.1007/s10059-013-1259-3.

(161) Ozben, T. Oxidative Stress and Apoptosis: Impact on Cancer Therapy. *J. Pharm. Sci.* **2007**, *96* (9), 2181–2196. https://doi.org/10.1002/jps.20874.

(162) Jin, K.; Simpkins, J. W.; Ji, X.; Leis, M.; Stambler, I. The Critical Need to Promote Research of Aging and Aging-Related Diseases to Improve Health and Longevity of the Elderly Population. *Aging Dis.* **2015**, *6* (1), 1–5. https://doi.org/10.14336/AD.2014.1210.

(163) Flatt, T. A New Definition of Aging? *Front. Genet.* **2012**, *3*. https://doi.org/10.3389/fgene.2012.00148.

(164) López-Otín, C.; Blasco, M. A.; Partridge, L.; Serrano, M.; Kroemer, G. The Hallmarks of Aging. *Cell* **2013**, *153* (6), 1194. https://doi.org/10.1016/j.cell.2013.05.039.

(165) Islam, M. T. Oxidative Stress and Mitochondrial Dysfunction-Linked Neurodegenerative Disorders. *Neurol. Res.* **2017**, *39* (1), 73–82. https://doi.org/10.1080/01616412.2016.1251711.

(166) Eckmann, J.; Eckert, S. H.; Leuner, K.; Muller, W. E.; Eckert, G. P. Mitochondria: Mitochondrial Membranes in Brain Ageing and Neurodegeneration. *Int. J. Biochem. Cell Biol.* **2013**, *45* (1), 76–80. https://doi.org/10.1016/j.biocel.2012.06.009.

(167) Tan, B. L.; Norhaizan, M. E.; Liew, W. P. P.; Rahman, H. S. Antioxidant and Oxidative Stress: A Mutual Interplay in Age-Related Diseases. *Front. Pharmacol.* **2018**, *9* (OCT), 1–28. https://doi.org/10.3389/fphar.2018.01162.

(168) García-Sánchez, A.; Miranda-Díaz, A. G.; Cardona-Muñoz, E. G. The Role of

Oxidative Stress in Physiopathology and Pharmacological Treatment with Pro- and Antioxidant Properties in Chronic Diseases. *Oxid. Med. Cell. Longev.* **2020**, *2020*, 1–16. https://doi.org/10.1155/2020/2082145.

(169) Trist, B. G.; Hare, D. J.; Double, K. L. Oxidative Stress in the Aging Substantia Nigra and the Etiology of Parkinson's Disease. *Aging Cell* **2019**, *18* (6), 1–23. https://doi.org/10.1111/acel.13031.

(170) Simpson, D. S. A.; Oliver, P. L. ROS Generation in Microglia: Understanding Oxidative Stress and Inflammation in Neurodegenerative Disease. *Antioxidants* **2020**, *9* (8), 743. https://doi.org/10.3390/antiox9080743.

(171) Scuderi, S. A.; Ardizzone, A.; Paterniti, I.; Esposito, E.; Campolo, M. Antioxidant and Anti-Inflammatory Effect of Nrf2 Inducer Dimethyl Fumarate in Neurodegenerative Diseases. *Antioxidants* **2020**, *9* (7), 1–15. https://doi.org/10.3390/antiox9070630.

(172) Zuo, L.; Hemmelgarn, B. T.; Chuang, C. C.; Best, T. M. The Role of Oxidative Stress-Induced Epigenetic Alterations in Amyloid-β Production in Alzheimer's Disease. *Oxid. Med. Cell. Longev.* **2015**, *2015*. https://doi.org/10.1155/2015/604658.

(173) Tönnies, E.; Trushina, E. Oxidative Stress, Synaptic Dysfunction, and Alzheimer's Disease. *J. Alzheimer's Dis.* **2017**, *57* (4), 1105–1121. https://doi.org/10.3233/JAD-161088.

(174) Zhao, Y.; Zhao, B. Review Article Oxidative Stress and the Pathogenesis of Alzheimer ' s Disease. *Oxid. Med. Cell. Longev.* **2013**, *2013*.

(175) Ansari, M. A.; Scheff, S. W. Oxidative Stress in the Progression of Alzheimer Disease in the Frontal Cortex. *J. Neuropathol. Exp. Neurol.* **2010**, *69* (2), 155–167. https://doi.org/10.1097/NEN.0b013e3181cb5af4.

(176) Haynes, W. N. "Properties of the Elements and Inorganic Compounds." In

Handbook of Chemistry and Physics,; Hoboken : CRC Press, 2014; pp 115–120.

(177) Kieliszek, M.; Błazejak, S. Current Knowledge on the Importance of Selenium in Food for Living Organisms: A Review. *Molecules* **2016**, *21* (5). https://doi.org/10.3390/molecules21050609.

(178) Hu, T.; Liang, Y.; Zhao, G.; Wu, W.; Li, H.; Guo, Y. Selenium Biofortification and Antioxidant Activity in Cordyceps Militaris Supplied with Selenate, Selenite, or Selenomethionine. *Biol. Trace Elem. Res.* **2019**, *187* (2), 553–561. https://doi.org/10.1007/s12011-018-1386-y.

(179) Fordyce, F. Selenium Geochemistry and Health. *https://doi.org/10.1579/0044-7447(2007)36[94:SGAH]2.0.CO;2* **2007**, *36* (1), 94–97. https://doi.org/https://www.jstor.org/stable/i399783.

(180) Dumont, E.; Vanhaecke, F.; Cornelis, R. Selenium Speciation from Food Source to Metabolites: A Critical Review. *Anal. Bioanal. Chem.* **2006**, *385* (7), 1304–1323. https://doi.org/10.1007/s00216-006-0529-8.

(181) Kipp, A. P.; Strohm, D.; Brigelius-Flohé, R.; Schomburg, L.; Bechthold, A.; Leschik-Bonnet, E.; Heseker, H. Revised Reference Values for Selenium Intake. *J. Trace Elem. Med. Biol.* **2015**, *32*, 195–199. https://doi.org/10.1016/j.jtemb.2015.07.005.

(182) Kieliszek, M. Selenium–Fascinating Microelement, Properties and Sources in Food. *Molecules* **2019**, *24* (7). https://doi.org/10.3390/molecules24071298.

(183) Liu, H.; Yu, F.; Shao, W.; Ding, D.; Yu, Z.; Chen, F.; Geng, D.; Tan, X.; Lammi, M. J.; Guo, X. Associations Between Selenium Content in Hair and Kashin-Beck Disease/Keshan Disease in Children in Northwestern China: A Prospective Cohort Study. *Biol. Trace Elem. Res.* **2018**, *184* (1), 16–23. https://doi.org/10.1007/s12011-017-1169-x.

(184) Keshan Disease Research Group. Observations on Effect of Sodium Selenite in Prevention of Keshan Disease. *Chin. Med. J.* **1979**, *92*, 471–477.

(185) Chen, J. An Original Discovery: Selenium Deficiency and Keshan Disease (an Endemic Heart Disease). *Asia Pac. J. Clin. Nutr.* **2012**, *21* (3), 320–326.

(186) Steinbrenner, H.; Sies, H. Selenium Homeostasis and Antioxidant Selenoproteins in Brain: Implications for Disorders in the Central Nervous System. *Arch. Biochem. Biophys.* **2013**, *536* (2), 152–157. https://doi.org/10.1016/j.abb.2013.02.021.

(187) Cardoso, B. R.; Roberts, B. R.; Bush, A. I.; Hare, D. J. Selenium, Selenoproteins and Neurodegenerative Diseases. *Metallomics* **2015**, *7* (8), 1213–1228. https://doi.org/10.1039/c5mt00075k.

(188) Solovyev, N.; Drobyshev, E.; Bjørklund, G.; Dubrovskii, Y.; Lysiuk, R.; Rayman, M. P. Selenium, Selenoprotein P, and Alzheimer's Disease: Is There a Link? *Free Radic. Biol. Med.* **2018**, *127*, 124–133. https://doi.org/10.1016/j.freeradbiomed.2018.02.030.

(189) Loef, M.; Schrauzer, G. N.; Walach, H. Selenium and Alzheimer's Disease: A Systematic Review. *J. Alzheimer's Dis.* **2011**, *26* (1), 81–104. https://doi.org/10.3233/JAD-2011-110414.

(190) Zhang, D. W.; Yan, H. L.; Xu, X. S.; Xu, L.; Yin, Z. H.; Chang, S.; Luo, H. The Selenium-Containing Drug Ebselen Potently Disrupts LEDGF/P75-HIV-1 Integrase Interaction by Targeting LEDGF/P75. *J. Enzyme Inhib. Med. Chem.* **2020**, *35* (1), 906–912. https://doi.org/10.1080/14756366.2020.1743282.

(191) Seale, L. A.; Torres, D. J.; Berry, M. J.; Pitts, M. W. A Role for Selenium-Dependent GPX1 in SARS-CoV-2 Virulence. *Am. J. Clin. Nutr.* **2020**, *112* (2), 447–448. https://doi.org/10.1093/ajcn/nqaa177.

(192) Alexander, J.; Tinkov, A.; Strand, tor a; Alehagen, U.; Skalny, A.; Aaseth, J. Early Nutritional Interventions with Zinc , Selenium Against Progressive COVID-19. *Nutrients* **2020**, *12* (8), 1–12.

(193) Editor, D.; Commission, N. H.; Province, H.; Table, S.; Software, S. S.; Province, H.; Table, S.; Province, H.; Table, S.; Table, S.; Province, H.; Plain, S.; Table, S. Letters to the Editor. **2020**, 1297–1299.

(194) Kielczykowska, M.; Kocot, J.; Pazdzior, M.; Musik, I. Selenium - A Fascinating Antioxidant of Protective Properties. *Adv. Clin. Exp. Med.* **2018**, *27* (2), 245–255. https://doi.org/10.17219/acem/67222.

(195) Hira, C. K.; Partal, K.; Dhillon, K. Dietary Selenium Intake by Men and Women in High and Low Selenium Areas of Punjab. *Public Health Nutr.* **2004**, *7* (1), 39–43. https://doi.org/10.1079/phn2003513.

(196) Álvarez-Pérez, M.; Ali, W.; Marć, M. A.; Handzlik, J.; Domínguez-Álvarez, E. Selenides and Diselenides: A Review of Their Anticancer and Chemopreventive Activity. *Molecules* **2018**, *23* (3). https://doi.org/10.3390/molecules23030628.

(197) Gandin, V.; Khalkar, P.; Braude, J.; Fernandes, A. P. Organic Selenium Compounds as Potential Chemotherapeutic Agents for Improved Cancer Treatment. *Free Radic. Biol. Med.* **2018**, *127* (April), 80–97. https://doi.org/10.1016/j.freeradbiomed.2018.05.001.

(198) Bartolini, D.; Sancineto, L.; Fabro de Bem, A.; Tew, K. D.; Santi, C.; Radi, R.; Toquato, P.; Galli, F. Selenocompounds in Cancer Therapy: An Overview. *Adv. Cancer Res.* **2017**, *136*, 259–302. https://doi.org/10.1016/bs.acr.2017.07.007.

(199) Tinggi, U. Selenium: Its Role as Antioxidant in Human Health. *Environ. Health Prev. Med.* **2008**, *13* (2), 102–108. https://doi.org/10.1007/s12199-

007-0019-4.

(200) Tapiero, H.; Townsend, D. M.; Tew, K. D. The Antioxidant Role of Selenium and Seleno-Compounds. *Biomed. Pharmacother.* **2003**, *57* (3), 134–144. https://doi.org/10.1016/S0753-3322(03)00035-0.

(201) Zimmerman, M. T.; Bayse, C. A.; Ramoutar, R. R.; Brumaghim, J. L. Sulfur and Selenium Antioxidants: Challenging Radical Scavenging Mechanisms and Developing Structure-Activity Relationships Based on Metal Binding. *J. Inorg. Biochem.* **2015**, *145*, 30–40. https://doi.org/10.1016/j.jinorgbio.2014.12.020.

(202) Kryukov, G. V.; Castellano, S.; Novoselov, S. V.; Lobanov, A. V.; Zehtab, O.; Guigó, R.; Gladyshev, V. N. Characterization of Mammalian Selenoproteomes. *Science (80-.).* **2003**, *300* (5624), 1439–1443. https://doi.org/10.1126/science.1083516.

(203) Steinbrenner, H.; Sies, H. Protection against Reactive Oxygen Species by Selenoproteins. *Biochim. Biophys. Acta - Gen. Subj.* **2009**, *1790* (11), 1478–1485. https://doi.org/10.1016/j.bbagen.2009.02.014.

(204) Burk, R. F.; Hill, K. E. Selenoprotein P—Expression, Functions, and Roles in Mammals. *Biochim. Biophys. Acta - Gen. Subj.* **2009**, *1790* (11), 1441–1447. https://doi.org/10.1016/j.bbagen.2009.03.026.

(205) Saito, Y. Selenoprotein P as an *in Vivo* Redox Regulator: Disorders Related to Its Deficiency and Excess. *J. Clin. Biochem. Nutr.* **2020**, *66* (1), 1–7. https://doi.org/10.3164/jcbn.19-31.

(206) Saito, Y.; Takahashi, K. Selenoprotein P. Its Structure and Functions. *J. Heal. Sci.* **2000**, *46* (6), 409–413. https://doi.org/10.1248/jhs.46.409.

(207) Saito, Y.; Yoshida, Y.; Akazawa, T.; Takahashi, K.; Niki, E. Cell Death Caused by Selenium Deficiency and Protective Effect of Antioxidants. *J. Biol.*

Chem. **2003**, *278* (41), 39428–39434. https://doi.org/10.1074/jbc.M305542200.

(208) Gao, S.; Jin, Y.; Hall, K. S.; Liang, C.; Unverzagt, F. W.; Ma, F.; Cheng, Y.; Shen, J.; Cao, J.; Matesan, J.; Li, P.; Bian, J.; Hendrie, H. C.; Murrell, J. R. Selenium Level Is Associated with ApoE E4 in Rural Elderly Chinese. *Public Health Nutr.* **2009**, *12* (12), 2371–2376. https://doi.org/10.1017/S1368980009005102.

(209) Wenstrup, D.; Ehman, W. D.; Markesbery, W. R. Trace Element Imbalances in Isolated Subcellular Fractions of Alzheimer's Disease Brains. *Brain Res.* **1990**, *533* (1), 125–131. https://doi.org/10.1016/0006-8993(90)91804-P.

(210) Storkey, C.; Davies, J. (12) Patent Application Publication (10) Pub. No.: US 2014/0206658 A1. **2014**, *1* (19).

(211) Classification, P. (12) Patent Application Publication (10) Pub . No .: US 2015 / 0191446 A1 SCHESSER et A. **2015**, *1* (19).

(212) Aaseth, J.; Alexander, J.; Bjørklund, G.; Hestad, K.; Dusek, P.; Roos, P. M.; Alehagen, U. Treatment Strategies in Alzheimer's Disease: A Review with Focus on Selenium Supplementation. *BioMetals* **2016**, *29* (5), 827–839. https://doi.org/10.1007/s10534-016-9959-8.

(213) Cai, Z.; Zhang, J.; Li, H. Selenium, Aging and Aging-Related Diseases. *Aging Clin. Exp. Res.* **2019**, *31* (8), 1035–1047. https://doi.org/10.1007/s40520-018-1086-7.

(214) Alfthan, G.; Eurola, M.; Ekholm, P.; Venäläinen, E.-R.; Root, T.; Korkalainen, K.; Hartikainen, H.; Salminen, P.; Hietaniemi, V.; Aspila, P.; Aro, A. Effects of Nationwide Addition of Selenium to Fertilizers on Foods, and Animal and Human Health in Finland: From Deficiency to Optimal Selenium Status of the Population. *J. Trace Elem. Med. Biol.* **2015**, *31*, 142–147.

https://doi.org/10.1016/j.jtemb.2014.04.009.

(215) Pérez-Corona, M. T.; Sánchez-Martínez, M.; Valderrama, M. J.; Rodríguez, M. E.; Cámara, C.; Madrid, Y. Selenium Biotransformation by Saccharomyces Cerevisiae and Saccharomyces Bayanus during White Wine Manufacture: Laboratory-Scale Experiments. *Food Chem.* **2011**, *124* (3), 1050–1055. https://doi.org/10.1016/j.foodchem.2010.07.073.

(216) Lippman, S. M.; Goodman, P. J.; Klein, E. A.; Parnes, H. L.; Thompson, I. M.; Kristal, A. R.; Santella, R. M.; Probstfield, J. L.; Moinpour, C. M.; Albanes, D.; Taylor, P. R.; Minasian, L. M.; Hoque, A.; Thomas, S. M.; Crowley, J. J.; Gaziano, J. M.; Stanford, J. L.; Cook, E. D.; Fleshner, N. E.; Lieber, M. M.; Walther, P. J.; Khuri, F. R.; Karp, D. D.; Schwartz, G. G.; Ford, L. G.; Coltman, C. A. Designing the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *JNCI J. Natl. Cancer Inst.* **2005**, *97* (2), 94–102. https://doi.org/10.1093/jnci/dji009.

(217) Duffield-Lillico, A. J.; Reid, M. E.; Turnbull, B. W.; Combs, G. F.; Slate, E. H.; Fischbach, L. A.; Marshall, J. R.; Clark, L. C. Baseline Characteristics and the Effect of Selenium Supplementation on Cancer Incidence in a Randomized Clinical Trial: A Summary Report of the Nutritional Prevention of Cancer Trial 1. *AACR* **2002**, *11* (7), 630–639.

(218) Asbaghi, O.; Saboori, S.; Hekmatdoost, A.; Abdollahpour, F.; Yousefi Rad, E.; Salehpour, S. Effects of Selenium Supplementation on Serum C Reactive Protein Level: A Systematic Review and Meta-Analysis of Randomized Controlled Clinical Trials. *Obes. Med.* **2020**, *17* (January), 100182. https://doi.org/10.1016/j.obmed.2020.100182.

(219) Zakeri, N.; kelishadi, M. R.; Asbaghi, O.; Naeini, F.; Afsharfar, M.; Mirzadeh, E.; Naserizadeh, S. kasra. Selenium Supplementation and Oxidative Stress: A Review. *PharmaNutrition* **2021**, 100263.

https://doi.org/10.1016/j.phanu.2021.100263.

(220) Kieliszek, M.; Lipinski, B. Selenium Supplementation in the Prevention of Coronavirus Infections (COVID-19). *Med. Hypotheses* **2020**, *143* (May), 109878. https://doi.org/10.1016/j.mehy.2020.109878.

(221) Fakhrolmobasheri, M.; Nasr-Esfahany, Z.; Khanahmad, H.; Zeinalian, M. Selenium Supplementation Can Relieve the Clinical Complications of COVID-19 and Other Similar Viral Infections. *Int. J. Vitam. Nutr. Res.* **2021**, *91* (3–4), 197–199. https://doi.org/10.1024/0300-9831/a000663.

(222) Han, X.; Shen, T.; Lou, H. Dietary Polyphenols and Their Biological Significance. *Int. J. Mol. Sci.* **2007**, *8* (9), 950–988. https://doi.org/10.3390/i8090950.

(223) Baião, D.; de Freitas, C.; Gomes, L.; da Silva, D.; Correa, A.; Pereira, P.; Aguila, E.; Paschoalin, V. Polyphenols from Root, Tubercles and Grains Cropped in Brazil: Chemical and Nutritional Characterization and Their Effects on Human Health and Diseases. *Nutrients* **2017**, *9* (9), 1044. https://doi.org/10.3390/nu9091044.

(224) Momtaz, S.; Salek-Maghsoudi, A.; Abdolghaffari, A. H.; Jasemi, E.; Rezazadeh, S.; Hassani, S.; Ziaee, M.; Abdollahi, M.; Behzad, S.; Nabavi, S. M. Polyphenols Targeting Diabetes via the AMP-Activated Protein Kinase Pathway; Future Approach to Drug Discovery. *Crit. Rev. Clin. Lab. Sci.* **2019**, *56* (7), 472–492. https://doi.org/10.1080/10408363.2019.1648376.

(225) Mileo, A. M.; Nisticò, P.; Miccadei, S. Polyphenols: Immunomodulatory and Therapeutic Implication in Colorectal Cancer. *Front. Immunol.* **2019**, *10* (APR), 729. https://doi.org/10.3389/fimmu.2019.00729.

(226) Thyagarajan, A.; Forino, A. S.; Konger, R. L.; Sahu, R. P. Dietary Polyphenols in Cancer Chemoprevention: Implications in Pancreatic Cancer. *Antioxidants*

2020, *9* (8), 651. https://doi.org/10.3390/antiox9080651.

(227) Sajadimajd, S.; Bahramsoltani, R.; Iranpanah, A.; Kumar Patra, J.; Das, G.; Gouda, S.; Rahimi, R.; Rezaeiamiri, E.; Cao, H.; Giampieri, F.; Battino, M.; Tundis, R.; Campos, M. G.; Farzaei, M. H.; Xiao, J. Advances on Natural Polyphenols as Anticancer Agents for Skin Cancer. *Pharmacol. Res.* **2020**, *151*, 104584. https://doi.org/10.1016/j.phrs.2019.104584.

(228) Silveira, A. C.; Dias, J. P.; Santos, V. M.; Oliveira, P. F.; Alves, M. G.; Rato, L.; Silva, B. M. The Action of Polyphenols in Diabetes Mellitus and Alzheimer's Disease: A Common Agent for Overlapping Pathologies. *Curr. Neuropharmacol.* **2018**, *17* (7), 590–613. https://doi.org/10.2174/1570159x16666180803162059.

(229) Giuliano, C.; Cerri, S.; Blandini, F. Potential Therapeutic Effects of Polyphenols in Parkinson's Disease: In Vivo and in Vitro Pre-Clinical Studies. *Neural Regen. Res.* **2021**, *16* (2), 234. https://doi.org/10.4103/1673-5374.290879.

(230) Singh, A.; Tripathi, P.; Kumar Yadawa, A.; Singh, S. Promising Polyphenols in Parkinson's Disease Therapeutics. *Neurochem. Res.* **2020**, *45*, 1731–1745. https://doi.org/10.1007/s11064-020-03058-3.

(231) Kujawska, M.; Jodynis-Liebert, J. Polyphenols in Parkinson's Disease: A Systematic Review of In Vivo Studies. *Nutrients* **2018**, *10* (5), 642. https://doi.org/10.3390/nu10050642.

(232) Singla, R. K.; Dubey, A. K.; Garg, A.; Sharma, R. K.; Fiorino, M.; Ameen, S. M.; Haddad, M. A.; Al-Hiary, M. Natural Polyphenols: Chemical Classification, Definition of Classes, Subcategories, and Structures. *J. AOAC Int.* **2019**, *102* (5), 1397–1400. https://doi.org/10.5740/jaoacint.19-0133.

(233) Kawai, Y. Development of Antibodies To Flavonoids. **2018**, *65*, 162–165.

(234) Lagiou, P.; Samoli, E.; Lagiou, A.; Tzonou, A.; Kalandidi, A.; Peterson, J.; Dwyer, J.; Trichpoulos, D. Intake of Specific Flavonoid Classes and Coronary Heart Disease - A Case-Control Study in Greece. *Eur. J. Clin. Nutr.* **2004**, *58* (12), 1643–1648. https://doi.org/10.1038/sj.ejcn.1602022.

(235) Le Marchand, L. Cancer Preventive Effects of Flavonoids - A Review. *Biomed. Pharmacother.* **2002**, *56* (6), 296–301. https://doi.org/10.1016/S0753-3322(02)00186-5.

(236) Liskova, A.; Koklesova, L.; Samec, M.; Smejkal, K.; Samuel, S. M.; Varghese, E.; Abotaleb, M.; Biringer, K.; Kudela, E.; Danko, J.; Shakibaei, M.; Kwon, T. K.; Büsselberg, D.; Kubatka, P. Flavonoids in Cancer Metastasis. *Cancers (Basel).* **2020**, *12* (6), 1498. https://doi.org/10.3390/cancers12061498.

(237) Reddy, V. P.; Aryal, P.; Robinson, S.; Rafiu, R.; Obrenovich, M.; Perry, G. Polyphenols in Alzheimer ' s Disease and in the Gut – Brain Axis. **2020**, *1*.

(238) Tsao, R. Chemistry and Biochemistry of Dietary Polyphenols. *Nutrients* **2010**, *2* (12), 1231–1246. https://doi.org/10.3390/nu2121231.

(239) Bravo, L. Polyphenols: Chemistry, Dietary Sources, Metabolism, and Nutritional Significance. *Nutr. Rev.* **2009**, *56* (11), 317–333. https://doi.org/10.1111/j.1753-4887.1998.tb01670.x.

(240) Brglez Mojzer, E.; Knez Hrnčič, M.; Škerget, M.; Knez, Ž.; Bren, U. Polyphenols: Extraction Methods, Antioxidative Action, Bioavailability and Anticarcinogenic Effects. *Molecules* **2016**, *21* (7). https://doi.org/10.3390/molecules21070901.

(241) Kim, K. H.; Tsao, R.; Yang, R.; Cui, S. W. Phenolic Acid Profiles and Antioxidant Activities of Wheat Bran Extracts and the Effect of Hydrolysis Conditions. *Food Chem.* **2006**, *95* (3), 466–473. https://doi.org/10.1016/j.foodchem.2005.01.032.

(242) Tomøs-Barberøn, F. A.; Clifford, M. N. Dietary Hydroxybenzoic Acid Derivatives - Nature, Occurrence and Dietary Burden. *J. Sci. Food Agric.* **2000**, *80* (7), 1024–1032. https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7<1024::AID-JSFA567>3.0.CO;2-S.

(243) Razzaghi-Asl, N.; Garrido, J.; Khazraei, H.; Borges, F.; Firuzi, O. Antioxidant Properties of Hydroxycinnamic Acids: A Review of Structure- Activity Relationships. *Curr. Med. Chem.* **2013**, *20* (36), 4436–4450.

(244) Oomah, B. D. Flaxseed as a Functional Food Source. *J. Sci. Food Agric.* **2001**, *81* (9), 889–894. https://doi.org/10.1002/jsfa.898.

(245) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure-Antioxidant Activity Relationships of Flavonoids and Phenolic Acids. *Free Radic. Biol. Med.* **1996**, *20* (7), 933–956. https://doi.org/10.1016/0891-5849(95)02227-9.

(246) Massimo D'Archivio, Carmela Filesi, Roberta Di Benedetto, Raffaella Gargiulo, C. G. and R. M. Polyphenols, Dietary Sources and Bioavailability. **2007**, *43* (4), 348–361.

(247) Vuolo, M. M.; Lima, V. S.; Maróstica Junior, M. R. Phenolic Compounds. In *Bioactive Compounds*; Elsevier, **2019**; pp 33–50. https://doi.org/10.1016/B978-0-12-814774-0.00002-5.

(248) Kotra, V. S. R.; Satyabanta, L.; Goswami, T. K. A Critical Review of Analytical Methods for Determination of Curcuminoids in Turmeric. *J. Food Sci. Technol.* **2019**, *56* (12), 5153–5166. https://doi.org/10.1007/s13197-019-03986-1.

(249) Nelson, K. M.; Dahlin, J. L.; Bisson, J.; Graham, J.; Pauli, G. F.; Walters, M. A. The Essential Medicinal Chemistry of Curcumin. *J. Med. Chem.* **2017**, *60* (5), 1620–1637. https://doi.org/10.1021/acs.jmedchem.6b00975.

(250) Peram, M. R.; Jalalpure, S. S.; Joshi, S. A.; Palkar, M. B.; Diwan, P. V. Single

Robust RP-HPLC Analytical Method for Quantification of Curcuminoids in Commercial Turmeric Products, Ayurvedic Medicines, and Nanovesicular Systems. *J. Liq. Chromatogr. Relat. Technol.* **2017**, *40* (10), 487–498. https://doi.org/10.1080/10826076.2017.1329742.

(251) Meng, F.-C.; Zhou, Y.-Q.; Ren, D.; Wang, R.; Wang, C.; Lin, L.-G.; Zhang, X.-Q.; Ye, W.-C.; Zhang, Q.-W. Turmeric: A Review of Its Chemical Composition, Quality Control, Bioactivity, and Pharmaceutical Application. In *Natural and Artificial Flavoring Agents and Food Dyes*; Elsevier, **2018**; pp 299–350. https://doi.org/10.1016/B978-0-12-811518-3.00010-7.

(252) Rahman, T.; Hosen, I.; Islam, M. M. T.; Shekhar, H. U. Oxidative Stress and Human Health. *Adv. Biosci. Biotechnol.* **2012**, *03* (07), 997–1019. https://doi.org/10.4236/abb.2012.327123.

(253) Collins, C. A.; Fry, F. H.; Holme, A. L.; Yiakouvaki, A.; Al-qenaei, A.; Jacob, C. Towards Multifunctional Antioxidants: Synthesis, Electrochemistry,. **2005**, 1541–1546.

(254) Li, F.; Li, T.; Sun, C.; Xia, J.; Jiao, Y.; Xu, H. Selenium-Doped Carbon Quantum Dots for Free-Radical Scavenging. *Angew. Chemie - Int. Ed.* **2017**, *56* (33), 9910–9914. https://doi.org/10.1002/anie.201705989.

(255) Nacca, F. G.; Monti, B.; Lenardão, E. J.; Evans, P.; Santi, C. A Simple Zinc-Mediated Method for Selenium Addition to Michael Acceptors. *Molecules* **2020**, *25* (9), 2018. https://doi.org/10.3390/molecules25092018.

(256) Inagaki, Y.; Minakawa, N.; Matsuda, A. Synthesis of 4'-Selenoribonucleosides. *Nucleic Acids Symp. Ser. (Oxf).* **2007**, *51* (51), 139–140. https://doi.org/10.1093/nass/nrm070.

(257) Jayakanthan, K.; Johnston, B. D.; Pinto, B. M. Stereoselective Synthesis of 4′-Selenonucleosides Using the Pummerer Glycosylation Reaction. *Carbohydr.*

Res. **2008**, *343* (10–11), 1790–1800. https://doi.org/10.1016/j.carres.2008.02.014.

(258) Jeong, L. S.; Tosh, D. K.; Kim, H. O.; Wang, T.; Hou, X.; Yun, H. S.; Kwon, Y.; Lee, S. K.; Choi, J.; Zhao, L. X. First Synthesis of 4‘-Selenonucleosides Showing Unusual Southern Conformation. *Org. Lett.* **2008**, *10* (2), 209–212. https://doi.org/10.1021/ol7025558.

(259) Yu, J.; Kim, J. H.; Lee, H. W.; Alexander, V.; Ahn, H. C.; Choi, W. J.; Choi, J.; Jeong, L. S. New RNA Purine Building Blocks, 4’-Selenopurine Nucleosides: First Synthesis and Unusual Mixture of Sugar Puckerings. *Chem. - A Eur. J.* **2013**, *19* (18), 5528–5532. https://doi.org/10.1002/chem.201300741.

(260) Pedatella, S.; Guaragna, A.; D’Alonzo, D.; De Nisco, M.; Palumbo, G. Triphenylphosphine Polymer-Bound/Iodine Complex: A Suitable Reagent for the Preparation of O-Isopropylidene Sugar Derivatives. *ChemInform* **2006**, *37* (22). https://doi.org/10.1002/chin.200622194.

(261) Jayakanthan, K.; Johnston, B. D.; Pinto, B. M. Stereoselective Synthesis of 4′-Selenonucleosides Using the Pummerer Glycosylation Reaction. *Carbohydr. Res.* **2008**, *343* (10–11), 1790–1800. https://doi.org/10.1016/j.carres.2008.02.014.

(262) Lak, S. J.; Tosh, D. K.; Hea, O. K.; Wang, T.; Hou, X.; Ho, S. Y.; Kwon, Y.; Sang, K. L.; Choi, J.; Long, X. Z. First Synthesis of 4′-Selenonucleosides Showing Unusual Southern Conformation. *Org. Lett.* **2008**, *10* (2), 209–212. https://doi.org/10.1021/ol7025558.

(263) Caputo, R.; Kunz, H.; Mastroianni, D.; Palumbo, G.; Pedatella, S.; Solla, F. Mild Synthesis of Protected α-D-Glycosyl Iodides. *European J. Org. Chem.* **1999**, No. 11, 3147–3150. https://doi.org/10.1002/(sici)1099-

0690(199911)1999:11<3147::aid-ejoc3147>3.0.co;2-i.

(264) Serpico, L.; De Nisco, M.; Cermola, F.; Manfra, M.; Pedatella, S. Stereoselective Synthesis of Selenium-Containing Glycoconjugates via the Mitsunobu Reaction. *Molecules* **2021**, *26* (9), 2541. https://doi.org/10.3390/molecules26092541.

(265) Taniguchi, T.; Nakano, K.; Baba, R.; Monde, K. Analysis of Configuration and Conformation of Furanose Ring in Carbohydrate and Nucleoside by Vibrational Circular Dichroism. *Org. Lett.* **2017**, *19* (2), 404–407. https://doi.org/10.1021/acs.orglett.6b03626.

(266) Taha, H. A.; Richards, M. R.; Lowary, T. L. Conformational Analysis of Furanoside-Containing Mono- and Oligosaccharides. *Chem. Rev.* **2013**, *113* (3), 1851–1876. https://doi.org/10.1021/cr300249c.

(267) Altona, C.; Sundaralingam, M. *Conformational Analysis of the Sugar Ring in Nucleosides and Nucleotides. Improved Method for the Interpretation of Proton Magnetic Resonance Coupling Constants*; **2021**.

(268) Batra, H.; Moriarty, R. M.; Penmasta, R.; Sharma, V.; Stanciuc, G.; Staszewski, J. P.; Tuladhar, S. M.; Walsh, D. A.; Datla, S.; Krishnaswamy, S. A Concise, Efficient and Production-Scale Synthesis of a Protected L-Lyxonolactone Derivative: An Important Aldonolactone Core. *Org. Process Res. Dev.* **2006**, *10* (3), 484–486. https://doi.org/10.1021/op050222n.

(269) Inagaki, Y.; Minakawa, N.; Matsuda, A. Synthesis of 4'selenoribo Nucleosides. *Nucleic Acids Symp. Ser.* **2007**, *51* (1), 139–140. https://doi.org/10.1093/nass/nrm070.

(270) Taniike, H.; Inagaki, Y.; Matsuda, A.; Minakawa, N. Practical Synthesis of 4′-Selenopyrimidine Nucleosides Using Hypervalent Iodine. *Tetrahedron* **2011**, *67* (41), 7977–7982. https://doi.org/10.1016/j.tet.2011.08.020.

(271) Appendino, G.; Minassi, A.; Daddario, N.; Bianchi, F.; Tron, G. C. Chemoselective Esterification of Phenolic Acids and Alcohols. *Org. Lett.* **2002**, *4* (22), 3839–3841. https://doi.org/10.1021/ol0266471.

(272) Nobili, A.; Latagliata, E. C.; Viscomi, M. T.; Cavallucci, V.; Cutuli, D.; Giacovazzo, G.; Krashia, P.; Rizzo, F. R.; Marino, R.; Federici, M.; Bartolo, P. De; Aversa, D.; Dell'Acqua, M. C.; Cordella, A.; Sancandi, M.; Keller, F.; Petrosini, L.; Puglisi-Allegra, S.; Mercuri, N. B.; Coccurello, R.; Berretta, N.; D'Amelio, M. Dopamine Neuronal Loss Contributes to Memory and Reward Dysfunction in a Model of Alzheimer's Disease. *Nat. Commun. 2017 81* **2017**, *8* (1), 1–14. https://doi.org/10.1038/ncomms14727.

(273) Sahu, P. K.; Kim, G.; Yu, J.; Ahn, J. Y.; Song, J.; Choi, Y.; Jin, X.; Kim, J. H.; Lee, S. K.; Park, S.; Jeong, L. S. Stereoselective Synthesis of 4′-Selenonucleosides via Seleno-Michael Reaction as Potent Antiviral Agents. *Org. Lett.* **2014**, *16* (21), 5796–5799. https://doi.org/10.1021/ol502899b.

(274) Storkey, C.; Pattison, D. I.; White, J. M.; Schiesser, C. H.; Davies, M. J. Preventing Protein Oxidation with Sugars: Scavenging of Hypohalous Acids by 5-Selenopyranose and 4-Selenofuranose Derivatives. *Chem. Res. Toxicol.* **2012**, *25* (11), 2589–2599. https://doi.org/10.1021/tx3003593.

(275) Storkey, C.; Davies, M. J.; White, J. M.; Schiesser, C. H. Synthesis and Antioxidant Capacity of 5-Selenopyranose Derivatives. *Chem. Commun.* **2011**, *47* (34), 9693–9695. https://doi.org/10.1039/c1cc13652f.

(276) Naka, T.; Minakawa, N.; Abe, H.; Kaga, D.; Matsuda, A. The Stereoselective Synthesis of 4'-β-Thioribonucleosides via the Pummerer Reaction. *J. Am. Chem. Soc.* **2000**, *122* (30), 7233–7243. https://doi.org/10.1021/ja000541o.

(277) Xie, J.; Schaich, K. M. Re-Evaluation of the 2,2-Diphenyl-1-Picrylhydrazyl Free Radical (DPPH) Assay for Antioxidant Activity. *J. Agric. Food Chem.*

2014, *62* (19), 4251–4260. https://doi.org/10.1021/jf500180u.

(278) Foti, M. C. Use and Abuse of the DPPH • Radical. *J. Agric. Food Chem.* **2015**, *63* (40), 8765–8776. https://doi.org/10.1021/acs.jafc.5b03839.

(279) Shimamura, T.; Sumikura, Y.; Yamazaki, T.; … A. T.-A.; 2014, U. Applicability of the DPPH Assay for Evaluating the Antioxidant Capacity of Food Additives–Inter-Laboratory Evaluation Study–. *Anal. Sci.* **2014**, *30*, 717–721.

(280) Blois, M. S. Antioxidant Determinations by the Use of a Stable Free Radical. *Nature* **1958**, *181*, 1199–1200.

(281) Benzie, I. F. F.; Strain, J. J. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "'Antioxidant Power'": The FRAP Assay. *Anal. Biochem.* **1996**, *239*, 70–76.

(282) Wojtunik-Kulesza, K. A. Approach to Optimization of FRAP Methodology for Studies Based on Selected Monoterpenes. *Molecules* **2020**, *25* (22), 5267. https://doi.org/10.3390/molecules25225267.

(283) Vignoli, J.; Bassoli, D.; Benassi, M. T. Antioxidant Activity, Polyphenols, Caffeine and Melanoidins in Soluble Coffee: The Influence of Processing Conditions and Raw Material. *Food Chem.* **2011**, *124* (3), 863–868. https://doi.org/10.1016/j.foodchem.2010.07.008.

(284) Villaño, D.; Fernández-Pachón, M.; Moyá, M. L.; Troncoso, A. M.; García-Parrilla, M. C. Radical Scavenging Ability of Polyphenolic Compounds towards DPPH Free Radical. *Talanta* **2007**, *71* (1), 230–235. https://doi.org/10.1016/j.talanta.2006.03.050.

(285) Colombo, I.; Sangiovanni, E.; Maggio, R.; Mattozzi, C.; Zava, S.; Corbett, Y.; Fumagalli, M.; Carlino, C.; Corsetto, P. A.; Scaccabarozzi, D.; Calvieri, S.; Gismondi, A.; Taramelli, D.; Dell'Agli, M. HaCaT Cells as a Reliable in Vitro

Differentiation Model to Dissect the Inflammatory/Repair Response of Human Keratinocytes. *Mediators Inflamm.* **2017**, *2017*. https://doi.org/10.1155/2017/7435621.

(286) Nichols, J. A.; Katiyar, S. K. Skin Photoprotection by Natural Polyphenols: Anti-Inflammatory, Antioxidant and DNA Repair Mechanisms. *Arch. Dermatol. Res.* **2010**, *302* (2), 71–83. https://doi.org/10.1007/s00403-009-1001-3.

(287) Mosmann, T. *Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays*; **1983**; Vol. 65.

(288) Berridge, M. V.; Herst, P. M.; Tan, A. S. Tetrazolium Dyes as Tools in Cell Biology: New Insights into Their Cellular Reduction. In *Biotechnology Annual Review*; Elsevier, 2005; Vol. 11, pp 127–152. https://doi.org/10.1016/S1387-2656(05)11004-7.

(289) van de Loosdrecht, A. A.; Beelen, R. H. J.; Ossenkoppele, G. J.; Broekhoven, M. G.; Langenhuijsen, M. M. A. C. A Tetrazolium-Based Colorimetric MTT Assay to Quantitate Human Monocyte Mediated Cytotoxicity against Leukemic Cells from Cell Lines and Patients with Acute Myeloid Leukemia. *J. Immunol. Methods* **1994**, *174* (1–2), 311–320. https://doi.org/10.1016/0022-1759(94)90034-5.

(290) Kim, J. H.; Yang, B.; Tedesco, A.; Lebig, E. G. D.; Ruegger, P. M.; Xu, K.; Borneman, J.; Martins-Green, M. High Levels of Oxidative Stress and Skin Microbiome Are Critical for Initiation and Development of Chronic Wounds in Diabetic Mice. *Sci. Rep.* **2019**, *9* (1), 1–16. https://doi.org/10.1038/s41598-019-55644-3.

(291) Frykberg, R. G.; Banks, J. Challenges in the Treatment of Chronic Wounds. *Adv. Wound Care* **2015**, *4* (9), 560–582.

https://doi.org/10.1089/wound.2015.0635.

(292) Järbrink, K.; Ni, G.; Sönnergren, H.; Schmidtchen, A.; Pang, C.; Bajpai, R.; Car, J. Prevalence and Incidence of Chronic Wounds and Related Complications: A Protocol for a Systematic Review. *Syst. Rev.* **2016**, *5* (1), 1–6. https://doi.org/10.1186/s13643-016-0329-y.

(293) Martinengo, L.; Olsson, M.; Bajpai, R.; Soljak, M.; Upton, Z.; Schmidtchen, A.; Car, J.; Järbrink, K. Prevalence of Chronic Wounds in the General Population: Systematic Review and Meta-Analysis of Observational Studies. *Ann. Epidemiol.* **2019**, *29*, 8–15. https://doi.org/10.1016/j.annepidem.2018.10.005.

(294) Blair, M. J.; Jones, J. D.; Woessner, A. E.; Quinn, K. P. Skin Structure–Function Relationships and the Wound Healing Response to Intrinsic Aging. *Adv. Wound Care* **2020**, *9* (3), 127–143. https://doi.org/10.1089/wound.2019.1021.

(295) Moura, J.; Madureira, P.; Leal, E. C.; Fonseca, A. C.; Carvalho, E. Immune Aging in Diabetes and Its Implications in Wound Healing. *Clin. Immunol.* **2019**, *200* (January), 43–54. https://doi.org/10.1016/j.clim.2019.02.002.

(296) Han, G.; Ceilley, R. Chronic Wound Healing: A Review of Current Management and Treatments. *Adv. Ther.* **2017**, *34* (3), 599–610. https://doi.org/10.1007/s12325-017-0478-y.

(297) Sen, C. K.; Gordillo, G. M.; Roy, S.; Kirsner, R.; Lambert, L.; Hunt, T. K.; Gottrup, F.; Gurtner, G. C.; Longaker, M. T. Human Skin Wounds: A Major and Snowballing Threat to Public Health and the Economy. *Wound Repair Regen.* **2009**, *17* (6), 763–771. https://doi.org/10.1111/j.1524-475X.2009.00543.x.

(298) Janis, J. E.; Harrison, B. Wound Healing: Part I. Basic Science. *Plast.*

Reconstr. Surg. **2016**, *138* (3), 9S-17S. https://doi.org/10.1097/PRS.0000000000002773.

(299) Sen, C. K.; Roy, S. Redox Signals in Wound Healing. *Biochim. Biophys. Acta - Gen. Subj.* **2008**, *1780* (11), 1348–1361. https://doi.org/10.1016/j.bbagen.2008.01.006.

(300) Dunnill, C.; Patton, T.; Brennan, J.; Barrett, J.; Dryden, M.; Cooke, J.; Leaper, D.; Georgopoulos, N. T. Reactive Oxygen Species (ROS) and Wound Healing: The Functional Role of ROS and Emerging ROS-Modulating Technologies for Augmentation of the Healing Process. *Int. Wound J.* **2017**, *14* (1), 89–96. https://doi.org/10.1111/iwj.12557.

(301) Demidova-Rice, T. N.; Hamblin, M. R.; Herman, I. M. Acute and Impaired Wound Healing. *Adv. Skin Wound Care* **2012**, *25* (7), 304–314. https://doi.org/10.1097/01.ASW.0000416006.55218.d0.

(302) Landén, N. X.; Li, D.; Ståhle, M. Transition from Inflammation to Proliferation: A Critical Step during Wound Healing. *Cell. Mol. Life Sci.* **2016**, *73* (20), 3861–3885. https://doi.org/10.1007/s00018-016-2268-0.

(303) Zhu, G.; Wang, Q.; Lu, S.; Niu, Y. Hydrogen Peroxide: A Potential Wound Therapeutic Target. *Med. Princ. Pract.* **2017**, *26* (4), 301–308. https://doi.org/10.1159/000475501.

(304) Tao, S.; Justiniano, R.; Zhang, D. D.; Wondrak, G. T. The Nrf2-Inducers Tanshinone I and Dihydrotanshinone Protect Human Skin Cells and Reconstructed Human Skin against Solar Simulated UV. *Redox Biol.* **2013**, *1* (1), 532–541. https://doi.org/10.1016/j.redox.2013.10.004.

(305) Luo, J. D.; Wang, Y. Y.; Fu, W. L.; Wu, J.; Chen, A. F. Gene Therapy of Endothelial Nitric Oxide Synthase and Manganese Superoxide Dismutase Restores Delayed Wound Healing in Type 1 Diabetic Mice. *Circulation* **2004**,

110 (16), 2484–2493. https://doi.org/10.1161/01.CIR.0000137969.87365.05.

(306) Fadini, G. P.; Albiero, M.; Menegazzo, L.; Boscaro, E.; Pagnin, E.; Iori, E.; Cosma, C.; Lapolla, A.; Pengo, V.; Stendardo, M.; Agostini, C.; Pelicci, P. G.; Giorgio, M.; Avogaro, A. The Redox Enzyme P66Shc Contributes to Diabetes and Ischemia-Induced Delay in Cutaneous Wound Healing. *Diabetes* **2010**, *59* (9), 2306–2314. https://doi.org/10.2337/db09-1727.

(307) Weinstein, A. L.; Lalezarzadeh, F. D.; Soares, M. A.; Saadeh, P. B.; Ceradini, D. J. Normalizing Dysfunctional Purine Metabolism Accelerates Diabetic Wound Healing. *Wound Repair Regen.* **2015**, *23* (1), 14–21. https://doi.org/10.1111/wrr.12249.

(308) Zhao, X.; Wu, H.; Guo, B.; Dong, R.; Qiu, Y.; Ma, P. X. Antibacterial Anti-Oxidant Electroactive Injectable Hydrogel as Self-Healing Wound Dressing with Hemostasis and Adhesiveness for Cutaneous Wound Healing. *Biomaterials* **2017**, *122*, 34–47. https://doi.org/10.1016/j.biomaterials.2017.01.011.

(309) Lih, E.; Lee, J. S.; Park, K. M.; Park, K. D. Rapidly Curable Chitosan-PEG Hydrogels as Tissue Adhesives for Hemostasis and Wound Healing. *Acta Biomater.* **2012**, *8* (9), 3261–3269. https://doi.org/10.1016/j.actbio.2012.05.001.

(310) Wu, S.; Deng, L.; Hsia, H.; Xu, K.; He, Y.; Huang, Q.; Peng, Y.; Zhou, Z.; Peng, C. Evaluation of Gelatin-Hyaluronic Acid Composite Hydrogels for Accelerating Wound Healing. *J. Biomater. Appl.* **2017**, *31* (10), 1380–1390. https://doi.org/10.1177/0885328217702526.

(311) Harrison, I. P.; Spada, F. Hydrogels for Atopic Dermatitis and Wound Management: A Superior Drug Delivery Vehicle. *Pharmaceutics* **2018**, *10* (2). https://doi.org/10.3390/pharmaceutics10020071.

(312) Islands, F.; Survey, D. Industrial Research Associations: Some Taxation Problems. *Nature* **1960**, *185* (4706), 63–64. https://doi.org/10.1038/185063a0.

(313) Kamoun, E. A.; Kenawy, E.-R. S.; Chen, X. A Review on Polymeric Hydrogel Membranes for Wound Dressing Applications: PVA-Based Hydrogel Dressings. *J. Adv. Res.* **2017**, *8* (3), 217–233. https://doi.org/10.1016/j.jare.2017.01.005.

(314) Tyeb, S.; Kumar, N.; Kumar, A.; Verma, V. Flexible Agar-Sericin Hydrogel Film Dressing for Chronic Wounds. *Carbohydr. Polym.* **2018**, *200*, 572–582. https://doi.org/10.1016/j.carbpol.2018.08.030.

(315) González-Henríquez, C. M.; Pizarro, G. del C.; Sarabia-Vallejos, M. A.; Terraza, C. A.; López-Cabaña, Z. E. In Situ-Preparation and Characterization of Silver-HEMA/PEGDA Hydrogel Matrix Nanocomposites: Silver Inclusion Studies into Hydrogel Matrix. *Arab. J. Chem.* **2019**, *12* (7), 1413–1423. https://doi.org/10.1016/j.arabjc.2014.11.012.

(316) Gong, C. Y.; Wu, Q. J.; Wang, Y. J.; Zhang, D. D.; Luo, F.; Zhao, X.; Wei, Y. Q.; Qian, Z. Y. A Biodegradable Hydrogel System Containing Curcumin Encapsulated in Micelles for Cutaneous Wound Healing. *Biomaterials* **2013**, *34* (27), 6377–6387. https://doi.org/10.1016/j.biomaterials.2013.05.005.

(317) Xiang, J.; Shen, L.; Hong, Y. Status and Future Scope of Hydrogels in Wound Healing: Synthesis, Materials and Evaluation. *Eur. Polym. J.* **2020**, *130*, 109609. https://doi.org/10.1016/j.eurpolymj.2020.109609.

(318) Browning, M. B.; Cereceres, S. N.; Luong, P. T.; Cosgriff-Hernandez, E. M. Determination of the in Vivo Degradation Mechanism of PEGDA Hydrogels. *J. Biomed. Mater. Res. - Part A* **2014**, *102* (12), 4244–4251. https://doi.org/10.1002/jbm.a.35096.

(319) Cepanec, I.; Litvić, M. Simple and Efficient Synthesis of Arbutin. *ARKIVOC*

2008, *2*, 19–24.

(320) Sahu, P. K.; Kim, G.; Yu, J.; Ahn, J. Y.; Song, J.; Choi, Y.; Jin, X.; Kim, J. H.; Lee, S. K.; Park, S.; Jeong, L. S. Stereoselective Synthesis of 4′-Selenonucleosides via Seleno-Michael Reaction as Potent Antiviral Agents. *Org. Lett.* **2014**, *16* (21), 5796–5799. https://doi.org/10.1021/ol502899b.

(321) Wiegand, I.; Hilpert, K.; Hancock, R. E. W. Agar and Broth Dilution Methods to Determine the Minimal Inhibitory Concentration (MIC) of Antimicrobial Substances. *nature.com* **2008**. https://doi.org/10.1038/nprot.2007.521.

(322) Mary Jane Ferraro. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. In *NCCLS*; 2013.

(323) Wu, C. T.; Morris, J. R. Genes, Genetics, and Epigenetics: A Correspondence. *Science (80-.).* **2001**, *293* (5532), 1103–1105. https://doi.org/10.1126/science.293.5532.1103.

(324) Saha, S.; Panigrahi, D. P.; Patil, S.; Bhutia, S. K. Autophagy in Health and Disease: A Comprehensive Review. *Biomed. Pharmacother.* **2018**, *104*, 485–495. https://doi.org/10.1016/j.biopha.2018.05.007.

(325) D'Arcy, M. S. Cell Death: A Review of the Major Forms of Apoptosis, Necrosis and Autophagy. *Cell Biol. Int.* **2019**, *43* (6), 582–592. https://doi.org/10.1002/cbin.11137.

(326) Bellomo, D.; Lander, A.; Harragan, I.; Brown, N. A. Cell Proliferation in Mammalian Gastrulation: The Ventral Node and Notochord Are Relatively Quiescent. *Dev. Dyn.* **1996**, *205* (4), 471–485. https://doi.org/10.1002/(SICI)1097-0177(199604)205:4<471::AID-AJA10>3.0.CO;2-4.

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