Scavengome of an Antioxidant

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ABSTRACT

The term 'scavengome' refers to the chemical space of all the metabolites that may be formed from an antioxidant upon scavenging reactive oxygen or nitrogen species (ROS/RNS). This chemical space is very rich in structures representing an increased chemical complexity as compared to the parent antioxidant: a wide range of unusual heterocyclic structures, new C-C bonds, etc. may be formed. Further, in a biological environment, this increased chemical complexity is directly translated from the localized conditions of oxidative stress that determines the amounts and types of ROS/RNS present. Biomimetic oxidative chemistry provides an excellent tool to model chemical reactions between antioxidants and ROS/RNS. In this chapter, we provide an overview on the known metabolites obtained by biomimetic oxidation of a few selected natural antioxidants, i.e., a stilbene (resveratrol), a pair of hydroxycinnamates (caffeic acid and methyl caffeate), and a flavonol (quercetin), and discuss the drug discovery perspectives of the related chemical space.

Keywords: Scavengome, antioxidant-based drug discovery, chemical space, oxidative metabolism, diversity-oriented synthesis, biomimetic oxidation, resveratrol, caffeic acid, methyl caffeate, quercetin

1. ANTIOXIDANTS AND THEIR MECHANISM OF ACTION: INTRODUCTION OF THE SCAVENGOME CONCEPT

Plant polyphenols are among the best-studied dietary antioxidants, and their research keeps on the rise. In PubMed, the search term 'antioxidant AND polyphenol' gives over 30,000 hits in total, and over 3,300 only for 2021. This everlasting popularity is undoubtedly due to the plethora of beneficial bioactivities attributed to such compounds in connection with a wide range of chronic diseases such as diabetes, malignant tumors, and degenerative central nervous system (CNS) pathologies. This is no wonder, considering the well-known key role of oxidative stress in the pathomechanism and progression of such diseases.

Oxidative stress and its role in health and disease has been thoroughly reviewed over the years, and the field has recently gone through a paradigm shift towards recognizing the physiological importance of oxidative processes and re-defining oxidative stress as a loss of control and balance. The biologically most important reactive oxygen species (ROS) are hydrogen peroxide (H_2O_2) (Gough & Cotter, 2011), superoxide anion radical ($O_2^{\bullet-}$) (Hayyan et al., 2016), hypochlorous acid (HOCl) (Bauer, 2018), singlet oxygen ($^1O_2$) (Bauer, 2016), hydroxyl, alkoxyl and peroxyl radicals ($^{\bullet}OH$, $RO^{\bullet}$, and $ROO^{\bullet}$, respectively) (Dickinson & Chang, 2011), and major reactive nitrogen species (RNS) are nitric oxide (NO), nitrogen dioxide (NO_2), and peroxynitrite ($ONOO^-$) (Nimse & Pal, 2015). The reactivity of these species varies; primary ROS/RNS, like H_2O_2, $O_2^{\bullet-}$, and NO are much less reactive than toxic secondary species, particularly $^{\bullet}OH$, $ONOO^-$, and HOCl, whose increasing amounts highly contribute to oxidative stress and related damage of macromolecules, cells, and tissues (Weidinger & Kozlov, 2015). The formation of ROS/RNS, and particularly the secondary species is determined by transition metal catalysis; in biological environment this is primarily connected to enzymatic processes, i.e., mitochondrial electron transport chain (ETC) complexes I and II, lipoxygenases, cyclooxygenases, xanthine oxidase, and cytochrome P monoxygenases (Dickinson & Chang, 2011; Weidinger & Kozlov, 2015).

There is now wide a consensus about it that small molecule antioxidants decrease oxidative stress mainly through modulating the intrinsic enzymatic defense that maintains the redox balance in the body (Forman et al., 2014; Ruskovska et al., 2020; Sies, 1993). However, while direct free radical scavenging is generally thought to have little *in vivo* relevance in decreasing the levels of toxic ROS/RNS, the interaction between such reactive species and oxidizable dietary antioxidants should also not be ignored. Naturally, such an interaction has a major impact on both reaction partners. The reactive intermediate that is formed from the antioxidant may then be reduced back in a redox cycle, couple with an appropriate small-molecule or macromolecule reaction partner or get stabilized by intramolecular rearrangements. Because reactive antioxidant intermediates have a much more complex structure then ROS/RNS, their binding to macromolecules is also more specific, therefore such a "damage" may not necessarily cause toxic effects

but can also be translated to pharmacological activity. A good example to this is the covalent binding of quinones to the thiol groups of cysteine residues, which then leads to complex signal transduction processes (e.g., through the activation of Keap1/Nrf2 signaling) and may result in beneficial effects through an adaptive antioxidant response (Kato & Suga, 2018; Kerimi & Williamson, 2018). Further, the many possible intra- or intermolecular rearrangements stabilizing reactive antioxidant intermediates unravel a rich chemical space of small-molecule metabolites that may then exert a non-covalent action on any druggable targets in the microenvironment where they have been formed. Even though some antioxidants, e.g., curcumin, may undergo oxidative fragmentation (Shen & Ji, 2009), free radical scavenging by an antioxidant more frequently yields more complex chemical structures; this is due to the many possibilities for radical coupling reactions forming unique new rings or ring systems, various heterocycles, etc. In our recent review, we outlined the potential drug discovery perspectives of this novel and orthogonal chemical space and named it as the 'scavengome' (Hunyadi, 2019).

Diversity-oriented synthesis is a major drug discovery strategy. A wide variety of success-oriented chemical approaches have been pursued over the years to turn plain chemical diversity into a so-called biological performance diversity, i.e., to create chemical libraries that are not only diverse but also have a high pharmacological hit rate (Pavlinov et al., 2019). In this context it is worth stressing that i) any given antioxidant will manifest only a segment of its scavengome in a biological microenvironment under oxidative stress, and ii) the formed metabolites contain chemical information directly translated from the local conditions of oxidative stress, i.e., the types and amounts of ROS/RNS present. Considering the co-evolution of the biochemical machinery (i.e., potential drug targets) of animals and humans with their food rich in plant secondary metabolites, polyphenols, etc. (i.e., potential drugs), it seems reasonable to postulate that scavengome of a dietary antioxidant is an organic part of the signaling network related to oxidative stress. This notion suggests that a diversity-oriented chemical approach to explore the scavengome is a promising strategy towards biological performance diversity.

In the followings, we aim to provide a summary of what is known about the scavengome of a few selected abundant natural antioxidants, i.e., a stilbene (resveratrol), a pair of hydroxycinnamates (caffeic acid and methyl caffeate), and a flavonol (quercetin). We approach this subject by overviewing the chemical complexity of their metabolites prepared through their oxidative chemical transformations, in view of the biomimetic value of the oxidants. We also provide some characteristic examples on the bioactivity of several metabolites and evaluate their positioning in the drug-like chemical space.

2. BIOMIMETIC OXIDATIVE CHEMISTRY – EXPLORING THE SCAVENGOME

For modeling biological processes with an *in vitro* chemical approach, it is especially important to characterize and classify the models based on the translational distance from the studied human

physiological process. In the case of oxidative stress or antioxidant studies, model developments typically focus on mitochondrial, macrophage and neutrophil-related, and microsomal free radical formation processes (Bartesaghi & Radi, 2018). In this context, the classical approach basically distinguishes three main classes of biomimetic models (López-Alarcón & Denicola, 2013) that evaluate i) the scavenging activity towards stable free radicals, e.g., 2,2-Diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) and 2,2′-Azinobis-(3-ethylbenzothiazole-6-sulphonate radical cation ($ABTS^{\bullet+}$), ii) the reduction of metal ions, e.g., Ferric Reducing Antioxidant Power (FRAP) and Cupric ion-Reducing Antioxidant Capacity (CUPRAC) to evaluate the sample's capacity to reduce ferric or cupric ions in aqueous media, and iii) the oxidation of low-density lipoprotein (LDL). In relation with the scavengome concept, a comprehensive work is not limited to classical and directly biorelevant oxidative stress studies on the transformations of selected model compounds. In our novel approach to introduce potentially diverse oxidative chemical model systems in exploring a broad chemical space of oxidized antioxidant metabolites, we use a hierarchical classification system in our related studies, and this classification will also serve as an organizing principle to this chapter. This is based on biocompatibility or translational goodness in terms of bioequivalence, i.e., oxidative transformation of an antioxidant by any chemical system can be classified as a biorelevant (A), biomimetic (B), or biomimetic-related chemical (C) oxidation. Accordingly, we consider biorelevant (A) any *in vitro* chemical models that directly provide oxidative agents/free radicals present in the body, such as $^{\bullet}OH$: metalloporphyrin/H_2O_2 (Mazoir et al., 2020), Fe^{2+}/H_2O_2 (Miller et al., 2021), H_2O_2: Cu^{2+}/ascorbic acid (Shen et al., 2021), and $ONOO^-$ (Ferrer-Sueta et al., 2018), and chemical systems for which there is significant experimental evidence of their suitability for biological oxidative stress (DPPH, AAPH, AIBN AMVN) (Marano et al., 2021; Takatsuka et al., 2022) are also included in this group. We classify as biomimetic (B) the chemical systems in which the oxidative reaction medium contains an aqueous component in addition to a cosolvent (in some cases dissolved O_2 as a prooxidant) providing a medium with better suitability to the physiological system. The last class, i.e., chemical related to biomimetic (C), differs from class B only in the anhydrous organic solvent medium, which may still provide relevant models of oxidative processes within biological membranes. In some cases, we also classify oxidative conditions using biologically less relevant pH values into class C. In the model systems class B and C, three subgroups can be distinguished: (a) oxygen atom donors (H_2O_2, *t*-BuOOH, O_2 in alkaline media) (Costa et al., 2021; Wenz et al., 2019) (b) electron donors (e.g. $NaIO_4$, $K_3Fe(CN)_6$, Ru^{3+}, Cu^{2+}, Fe^{2+}/Fe^{3+}, Au^{3+}) (Galletti et al., 2018; Pradhan et al., 2020; Wu et al., 2021; Yang et al., 2016) (c) agents that mimic the nitrating capacity of peroxynitrite (eg. $NaNO_2$) (Ozyurt & Otles, 2020; Vossen & De Smet, 2015). Figure 1 shows our hierarchical classification of oxidative chemical model systems according to their applicability to explore chemical metabolite space within the scavengome concept.

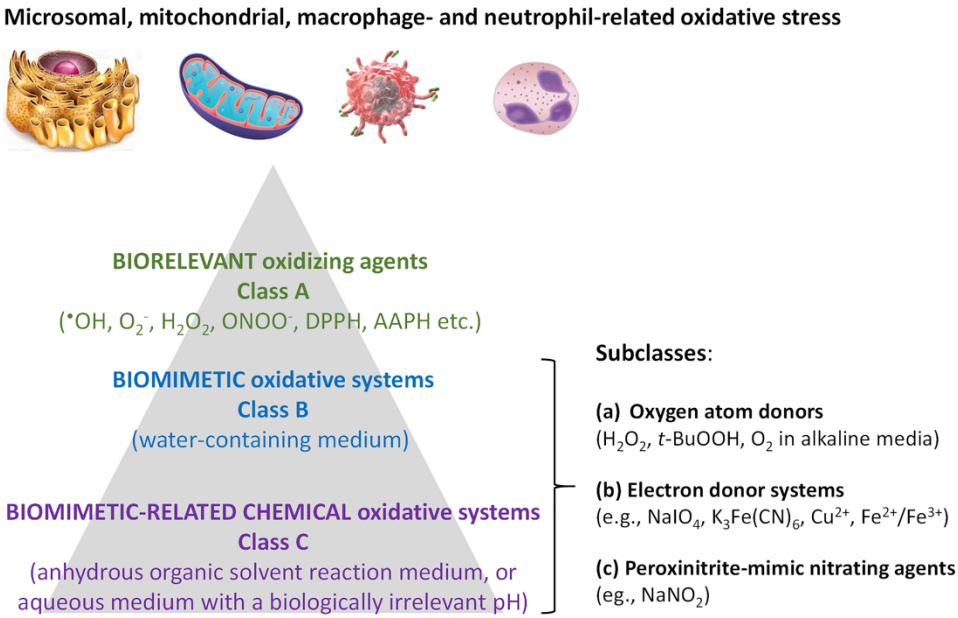


Figure 1. Hierarchical classification of oxidative chemical systems based on their bioequivalence value to model ROS/RNS scavenging reactions.

OXIDATIVE TRANSFORMATIONS OF SELECTED ANTIOXIDANTS

3.1 Resveratrol (I).

Resveratrol (*trans*-3,5,4′-trihydroxystilbene; compound **I**) is a natural polyphenol and phytoalexin, and probably the most popular dietary antioxidant due to its well-known presence in red wine. Other than that, it is also present in many common foods including, e.g., a variety of berries, tomato skin, peanuts, pistachios and cocoa (Dybkowska et al., 2018). Resveratrol was reported to have a myriad of beneficial bioactivities in health and disease (Baur & Sinclair, 2006), and its antioxidant properties are at the hallmark of these. Resveratrol can efficiently scavenge various types of ROS/RNS, resulting in radical intermediates stabilized by the delocalization of electrons between the two aromatic rings and the unsaturated methylene bridge joining them (Karlsson et al., 2000). This gives ample opportunities to C-C coupling at various regions of the resveratrol molecule. Interestingly, in a biological environment there is a high chance that the most likely reaction partner to such couplings would be another resveratrol molecule; this is because of the strong self-association of resveratrol fixed by strong π-π stacking in aqueous solution (Bonechi et al., 2008; Velu et al., 2013). This may be the reason why biorelevant / biomimetic oxidation of resveratrol very frequently

results in various dimers. The various oxidative reaction conditions applied are summarized in Table 1, and chemical structures of the metabolites obtained are presented in Figure 2.

Table 1. Oxidative transformations of resveratrol (**I**).

Reagents	Experimental conditions	Metabolites obtained	References
Biorelevant			
$NaNO_2$	0.1M phosphate buffer, pH 3.0, 37°C	**I/1–11**	Panzella et al., 2006
Peroxynitrite	0.1M, pH 7.4, phosphate-buffered ethanol solution	Unspecified dimers, nitro- and dinitro-derivatives	Holthoff et al., 2010
DPPH	MeOH	**I/1**, **I/12**	Wang et al., 1999
Biomimetic			
$K_3Fe(CN)_6$	pH 5.5 phosphate buffered CH_3CN	**I/1**	Sako et al., 2004
$FeCl_3$	EtOH(aq)	**I/1**, **I/12**, **I/16**	Shingai et al., 2011
Formic acid	Reflux	**I/13–15**	Li et al., 2003
Ruthenium chloride	MeOH(aq), 35°C	**I/16**, **I/17**	Yadav et al., 2019
Biomimetic-related chemical			
AgOAc, Ag_2O, Ag_2CO_3, $AgNO_3$, $Mn(OAc)_3$ CuOAc, $Cu(OAc)_2$	Dry MeOH, 50°C	**I/1**	Sako et al., 2004
$Tl(NO_3)_3$	MeOH, -50°C	**I/16**	Takaya et al., 2005
$Tl(NO_3)_3$	MeOH, -30°C	**I/16**	
$K_3[Fe(CN)_6]/K_2CO_3$	MeOH, 25°C	**I/1**, **I/16**, **I/18**	
$Ce(SO_4)_2$	MeOH, -50°C	**I/1**, **I/16**, **I/18**	
$FeCl_3$	Acetone, 25°C	**I/1**, **I/16**, **I/18**	
MnO_2	CH_2Cl_2, 25°C	**I/1**, **I/18**	
$K_3[Fe(CN)_6]$, sodium acetate	Aqueous acetone under reflux	**I/1**, **I/18–22**	Xie et al., 2015
AgOAc or $FeCl_3$	EtOH	**I/16**, **I/23–25**	El Khawand et al., 2020

The biomimetic oxidation of stilbenes may lead to various regioselective couplings depending on the oxidant, solvent, and substitution (Velu et al., 2008). Compound **I/1** was a major dimer formed by several biorelevant and biomimetic oxidants, and it was also a common oxidized product of resveratrol when it was reacted with various transition metals in less biomimetic experimental setups. An impressive, nearly quantitative (97%) yield of **I/1** was achieved when resveratrol was oxidized by AgOAc or Ag_2O (Sako et al., 2004). Similarly high (>90%) yields of **I/1** were obtained with $FeCl_3$ in acetone or MnO_2 in CH_2Cl_2 at 25°C, while ε-viniferin (**I/16**) became the major product with no detectable formation of **I/1** when resveratrol was oxidized by thallium(III) nitrate at –50°C (Takaya et al., 2005).

In a broader context of the scavengome, it is of interest that stressing grapevine leaves (var. Chasselas) by *Plasmopara viticola* (downy mildew) infection or UV-C irradiation also leads to the oxidative dimerization of resveratrol to form a mixture of products including the *E*/*Z* isomers of δ- and ε-viniferins (**I/1** and **I/16**–**17**) (Pezet et al., 2003). Both *P. viticola* infection and UV-C irradiation induces oxidative stress in grapevine leaves (Carvalho et al., 2015). While resveratrol dimers may certainly have been biosynthesized in the used experimental setup through various stress-activated enzymatic processes, the involvement of direct free radical scavenging may also not be excluded.

Oxidizing resveratrol by AgOAc in ethanol resulted in oxidative coupling to form **I/16** and three additional dimers, **I/23**–**I/25** (El Khawand et al., 2020). It may be worth stressing that, while the presence of ethanol as a solvent makes these conditions of less use to model pathophysiological ROS/RNS scavenging, such oxidative processes can naturally occur in wine that also contains transition metals, mainly iron and copper (Płotka-Wasylka et al., 2018).

Concerning the bioactivity of oxidized resveratrol metabolites, many of these compounds are widely recognized as phytoalexins, i.e., antibiotic compounds produced by plants in response to environmental stress stimuli (Jeandet, 2015). Other than that, a wide range of bioactivities of resveratrol dimers have been reported, including antiinflammatory, antioxidant, neuroprotective, and vascular protective activities. Related literature on ε-viniferin (**I/16**) has been most recently reviewed; this compound appears to exhibit particularly strong bioactivities against inflammatory and oxidative stress both *in vitro* and *in vivo* (Beaumont et al., 2022).

Unlike resveratrol and its open-ring dimer (**I/12**), δ- and ε-viniferins **I/1** and **I/16** have 5-LOX inhibitory activity (Shingai et al., 2011). Additional anti-inflammatory activities of some oxidized resveratrol metabolites were also reported in models where resveratrol was found less active or inactive. Compounds **I/1**, **I/23** and **I/24** reduced the NO production in LPS-induced RAW 264.7 macrophages with around twice the potency of resveratrol (IC_{50} values <10μM) (El Khawand et al., 2020). At 10 μM concentrations, compounds **I/13** and **I/14** inhibited leukotriene B_4 (LTB_4) biosynthesis, inhibited the adhesion of HL-60 cells to capillary endothelial cells, and acted as LTD_4 antagonists (Li et al., 2003).
Both δ- and ε-viniferins (**I/1** and **I/16**) exerted significant protective functions on vascular endothelial cells (Wu et al., 2020), and compound **I/1** was reported as a potent antioxidant against hemoglobin (Hb)-induced oxidative stress in erythrocytes, and to protect Hb from methemoglobin formation (Ficarra et al., 2016).

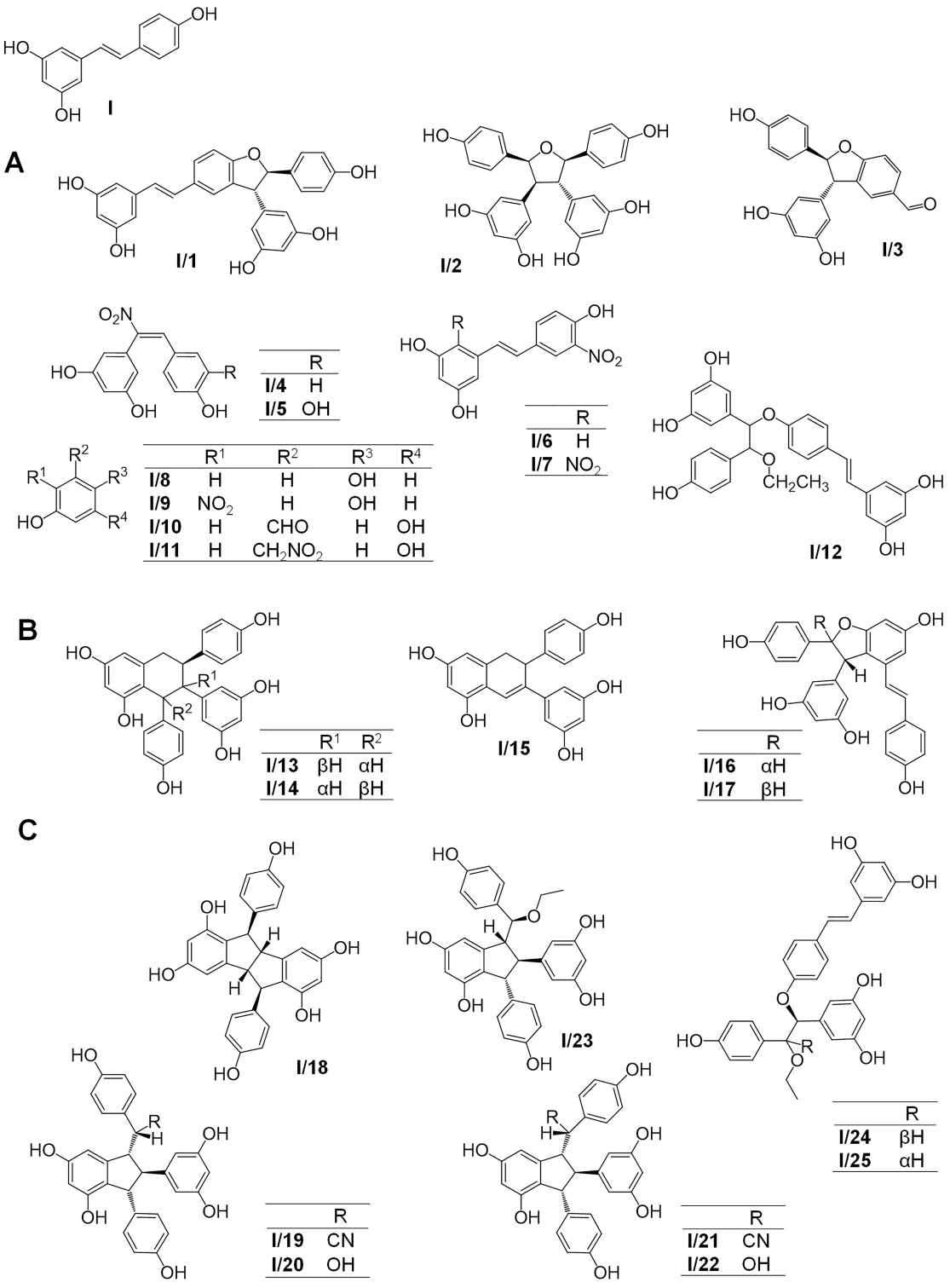


Figure 2. Oxidized resveratrol derivatives formed in reactions with biorelevant (**A**), biomimetic (**B**), or biomimetic-related chemical oxidants (**C**).

3.2. Caffeic acid (II) and methyl caffeate (III)

Caffeic acid is widely distributed in plants, and it is the most abundant hydroxycinnamic acid in many fruits and vegetables including, e.g., black chokeberries, lingonberries, dates, plums, olives, and potatoes, beverages, e.g., coffee, wines, and beers, seeds, like sunflower seeds, and a wide range of herbs and spices, like common sage, thyme, oregano, etc. It may occur in free form or glycosides, and it is valued for a wide range of beneficial bioactivities (Birková, 2020). While it is a very small and simple molecule, it is considered a highly valuable building block in the design of new bioactive compounds (Touaibia et al., 2011). Both caffeic acid (**II**) and its naturally occurring methyl ester (**III**) have been described as chain-breaking, radical scavenging antioxidants due to their hydrogen atom or electron donating capacity and to the ability to delocalize/stabilize the resulting phenoxyl radical (Teixeira et al., 2013). Caffeic acid is susceptible to autoxidation and it was reported to react with transition metals, glutathione and other thiols (cysteine, thioglycolic acid or thiocresol), and ascorbic acid (Cilliers & Singleton, 1990; Heleno et al., 2015). The behavior of compounds **II** and **III** under oxidative conditions have been studied in various experimental setups; these are summarized in Table 2, and chemical structures of the metabolites obtained are presented in Figure 3.

Table 2. Oxidative transformations of caffeic acid **(II)** and methyl caffeate **(III)**.

Reagents	**Experimental conditions**	**Metabolites obtained**	**References**
Biorelevant			
Iron-porphyrin Fe(TDCPPS)Cl, H_2O_2	MeOH(aq)	**II/1**–**4**	Šmejkalová & Piccolo, 2006 Šmejkalová et al., 2006
Peroxynitrite	50mM phosphate buffer, pH 7	**II/1**	Rice-Evans & Miller, 1996
Peroxynitrite	Continuous-flow, $ONOO^-$ prepared in situ, NaCl and glycine buffer present	**III/6**	Fási et al., 2020
Fenton oxidation	Aq. solution of Fe^{2+}/EDTA (1:1) and 3% H_2O_2	**II/5**, **II/6**	Antolovich et al., 2004
2,2′-Azobis(2,4-dimethylvaleronitrile) (AMVN)	CH_3CN, reaction in the presence and absence of ethyl linoleate, 37°C	**III/1**–**5**	Masuda et al., 2014
AAPH	CH_3CN:Water (2.5:1, v/v), 60°C	**III/6**, **III/7**	Fási et al., 2020
Biomimetic			
Oxygen	Bubbled through aqueous solution containing KOH	**II/2**, **II/3**, **II7**–**10**	Cilliers & Singleton, 1991
$NaIO_4$	Aqueous solution ranging from pH 2 to 7	**II/11**, **II/12**	Fulcrand et al., 1994

$NaIO_4$	Citric acid solution (pH 3.5), reaction in the absence and presence of L-cysteine, NaOH to adjust the pH	**II/1**, **II/13**	Bassil et al., 2005
$NaIO_4$	CH_3CN:Water (5:1, v/v)	**II/5**, **II/6**	Antolovich et al., 2004
$NaIO_4$	Water	**II/14**, **III/8**	Tazaki et al., 2003
$FeCl_3$	Caffeic acid in EtOH with the oxidant dissolved in water	**II/15**	Masuda et al., 2014

Unsurprisingly, the *o*-quinone derivative **II/1** was frequently observed as the primary intermediate during the oxidative coupling of caffeic acid (Antolovich et al., 2004; Tazaki et al., 2003), and thus it was reported as a common product in several oxidative transformations. As an interesting biomimetic element of the reaction setup, **II** was subjected to oxidation in the presence of L-cysteine (Bassil et al., 2005). The thiol group of cysteine residues plays a central role in redox signaling and stress response (Fra et al., 2013). Conjugation of caffeic acid with L-cysteine took place to yield **II/13** and this somewhat (by ca. 7%) enhanced the antiradical efficiency compared to that of caffeic acid (Bassil et al., 2005). More importantly, however, this also demonstrates the potential of reactive caffeic acid intermediates, particularly **II/1**, to modulate proteins' function through covalent coupling to accessible cysteine residues. Bubbling oxygen gas through the alkaline aqueous solution of caffeic acid led to the formation of compounds **II/7**–**10** (Cilliers & Singleton, 1991). Several related 1,4-benzodioxane-type neolignans were obtained when **II** was transformed by a non-specified crude mixture of pear enzymes, and this study revealed the enhanced COX-2 inhibitory activity of **II/7** (IC_{50}=7.5 μM) and **II/8** (IC_{50}=25.6 μM) as compared to the inactive parent compound **II** (Bae & Kim, 2012). Among the oxidized metabolites of caffeic acid derivatives, perhaps the most significant bioactivity changes were observed for compound **III/6**. This dihydrobenzofuran lignan was formed when methyl caffeate (**III**) was oxidized in biorelevant (Fási et al., 2020) or biomimetic (Pieters et al., 1999) experimental setups. Compound **III/6** was identified as a highly promising antitumor agent in various *in vitro* and *in vivo* experimental models. Its 2*R*,3*R* enantiomer (but not the inactive 2*S*,3*S*) acted as an anti-tubulin agent as potent (IC_{50}=6.0 μM) as combretastatin A-4 (Pieters et al., 1999), and exerted strong antiangiogenic activity apparently without interfering with fibroblast growth factor-2 (FGF-2) or vascular endothelial growth factor (VEGF) (Apers et al., 2002). Further, the racemate of **III/6** was found to suppress 4T1 tumor metastasis in mice at a dose of 100 μg/kg through increasing the interleukin-25 (IL-25) secretion of tumor-associated fibroblasts (Yin et al., 2016). In our previous work, we investigated the possible formation of **III/6** in HeLa, SiHa, MCF-7 and MDA-MB-231 cancer cells treated with **III** and incubated with or without *t*-BuOOH-induced oxidative stress. While no direct evidence was found for an *in-situ*

formation of **III/6**, the cytotoxic activity of **III** was altered by oxidative stress in a way that coincided with the cell line specificity of compound **III/6** (Fási et al., 2020).

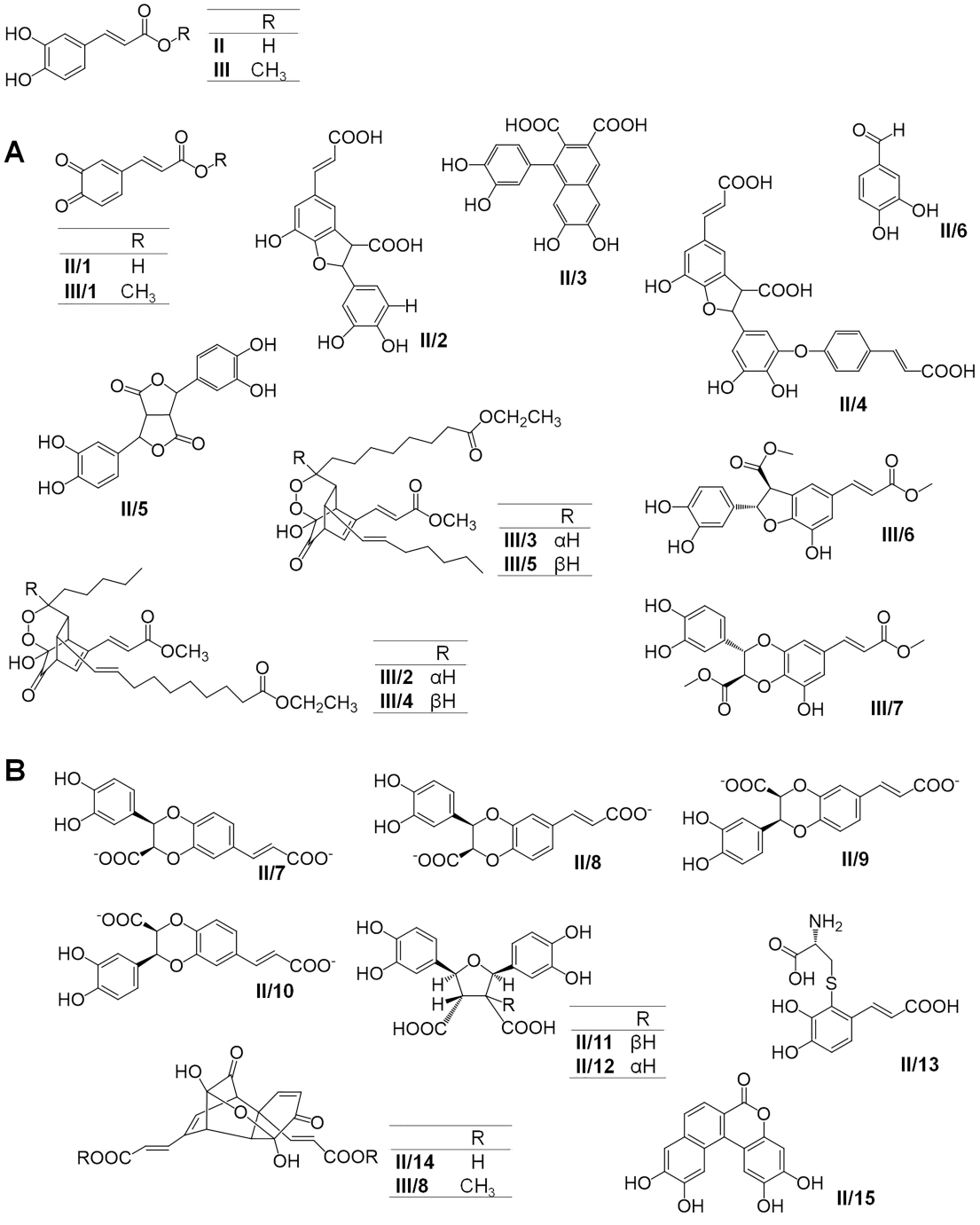


Figure 3. Oxidized caffeic acid or methyl caffeate derivatives formed in reactions with biorelevant (**A**) or biomimetic (**B**) oxidants.

3.3 Quercetin (IV)

Quercetin is one of the most common dietary flavonols due to its widespread occurrence in many common fruits and vegetables, including apples, blueberries, cherries, onions, lettuce, Chinese cabbage, etc., as well as in popular beverages like black tea and red wine (Dabeek & Marra, 2019). The free radical scavenging properties of quercetin have been thoroughly studied; it is well known to possess ROS/RNS scavenging properties due to i) the *o*-dihydroxy (catechol) structure in its B ring; ii) the 2,3-double bond conjugated with the 4-keto function; and iii) the additional presence of both 3- and 5-OH groups (Buchner et al., 2006; Makris & Rossiter, 2002-02; Rice-Evans & Miller, 1996). The oxidation of quercetin has been studied in many experimental models including enzymatic, electrochemical, chemical, and microbial systems, and a wide spectrum of products were identified, arising from dimerization, skeleton alteration and/or decomposition (Zhou & Sadik, 2008). Herein, we discuss the chemical models; experimental setups are summarized in Table 3, and structures of the metabolites are shown in Figure 4.

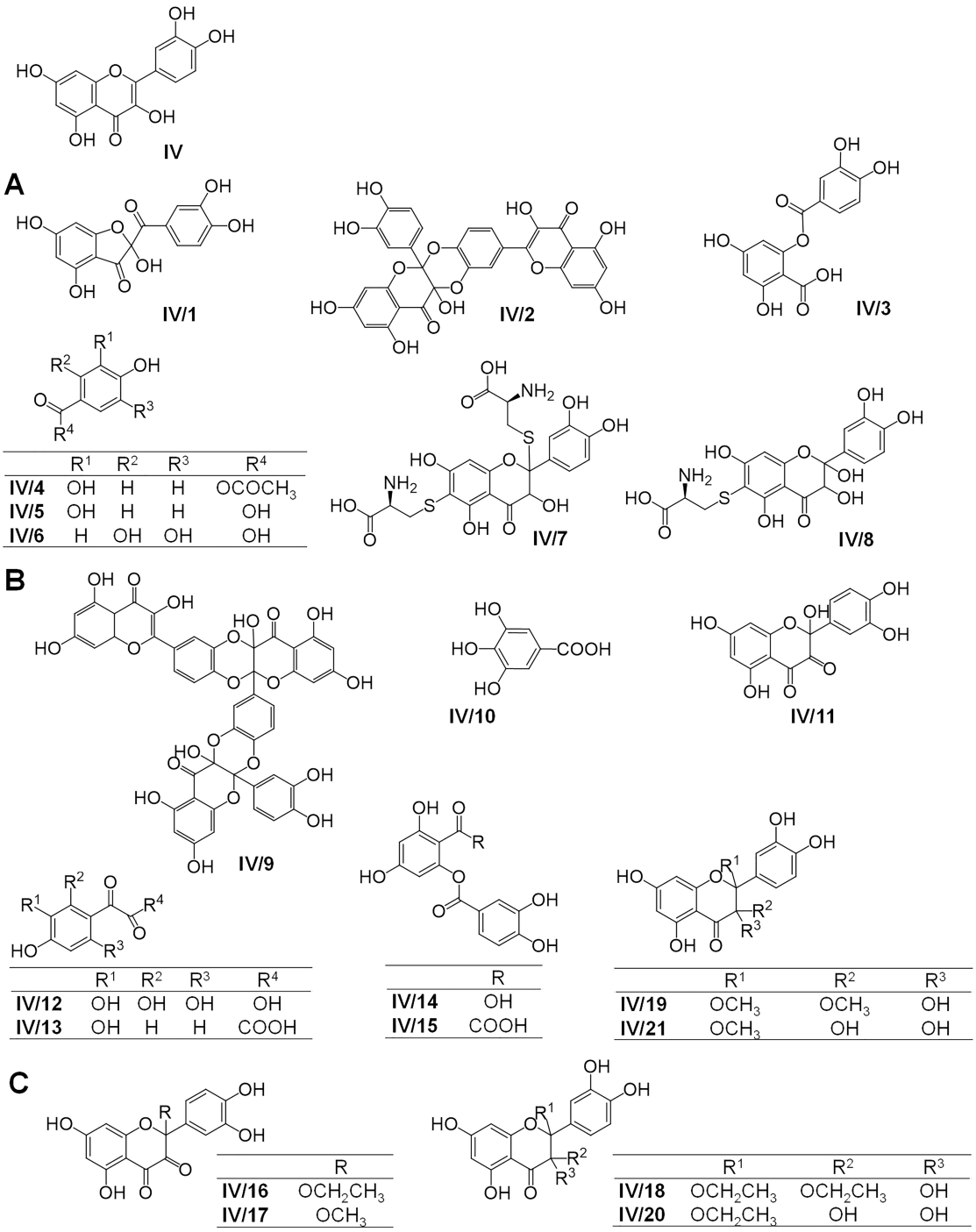
Table 3. Oxidative transformations of quercetin (**IV**).

Reagents	**Experimental conditions**	**Metabolites obtained**	**References**
Biorelevant			
Cu^{2+}	Phosphate-citrate buffer solution (pH 7.5), reaction in the presence of L-ascorbic acid	**IV/1**	Bobolaki et al., 2018
Cu^{2+}	Phosphate-citrate buffer solution (pH 7.5), reaction in the presence and absence of L-cysteine	**IV/1**, **IV/5**–**8**	Photiades et al., 2020
DPPH	MeOH	**IV/2**	Furusawa et al., 2003
Biomimetic			
2,2′-azobis-isobutyronitrile (AIBN)	CH_3CN, 60°C	**IV/1**–**4**	Krishnamachari et al., 2002
$NaNO_2$	KCl-HCl solution (pH 1-3)	**IV/1**	Takahama et al., 2003
$K_3[Fe(CN)_6]$	Aqueous phosphate buffer (0.05 M, pH 7.4)	**IV/2**, **IV/9**	Chervyakovsky et al., 2008
t-BuOOH	*t*-BuOH–water (2:1 v/v), reaction in NaOH, neutralized with sodium phosphate buffer (pH 7.4)	**IV/2**	Mallepu et al., 2018
$K_3[Fe(CN)_6]$, $NaHCO_3$	70% CH_3CN	**IV/6**, other unspecified products	Makris & Rossiter, 2002
$CuSO_4/H_2O_2$ $NaIO_4$	70% aq. CH_3CN	**IV/6**, other unspecified products	Makris & Rossiter, 2002-02

Fe^{3+}, Fe^{2+} and Cu^{2+}	70/30 (v/v) mixtures of aqueous acetic acid and different organic modifiers; (CH_3CN, CH_3OH and EtOH)	**IV/1**	Jungbluth et al., 2000
$CuSO_4$	(CH_3CN/water: 8/2, v/v), pH adjusted from 4.5 to 2 at the end of the reaction	**IV/1**	Gülşen et al., 2007
$K_3[Fe(CN)_6]$, Na_2CO_3	CH_3CN	**IV1**, **IV/2**	Gülşen et al., 2007
Air	NaOH (pH 12)	**IV/1**, **IV/5**, **IV/6**, **IV/11**–**15**	Fuentes et al., 2017
$[AuCl_4]^-$	0.1M $HClO_4$ aqueous solution (1:1, v:v)	**IV/11**, **IV/19**, **IV/21**	Bondžić et al., 2013
Biomimetic-related chemical			
$CuC1_2$	MeOH and EtOH	**IV/16**–**21**	Utaka & Takeda, 1985
Ru + Ag + NaOH $PdCl_2$ K_2OsCl_6 Solid Pt and Au in aqua regia.	MeOH	**IV/1**	Balcerzak et al., 2004
$PbCl_2$	MeOH	**IV/1**	Cornard et al., 2005

When studying the effect of L-ascorbic acid (Bobolaki et al., 2018) and L-cysteine (Photiades et al., 2020) on the Cu^{2+}-induced oxidation of quercetin (**IV**) under several experimental conditions (pH and temperature), three oxidized products (**IV/5**, **IV/6** and **IV/1**) were tentatively identified by LC-DAD-MS. The product profile was pH-dependent, and when the oxidation was performed in presence of L-cysteine (Photiades et al., 2020), two tentatively assigned quercetin/L-cysteine adducts (**IV/7** and IV/**8**) were also observed. It was concluded that both ascorbyl and cysteinyl radicals may take an active part in the oxidation of quercetin. In view of the scavengome concept, this is an interesting example for the chemical environment significantly affecting the product profile connected to ROS/RNS scavenging by a small-molecule antioxidant. The dimer **IV/2** was observed as a main oxidation product in a photooxidation experiment, in which quercetin was oxidized by in situ formed *tert*-butoxyl radicals (*t*-BuO$^\bullet$) in a solvent mixture of *t*-BuOH water (2:1 v/v) containing *t*-BuOOH (Mallepu et al., 2018). Since the excess *t*-BuOH supposedly scavenged the $^\bullet$OH radicals also formed from the photolysis, results of this experiment suggest that such a dimerization of quercetin may also take place when it reacts with alkoxyl radicals. This dimer **IV/2** is also naturally present in the brownish scale of onion, and it was reported to exhibit stronger anti-platelet activity than quercetin (**IV**), and to rigidify liposomal and surface membrane bilayers (Furusawa et al., 2003). The membrane activity of **IV/2** may also be the reason for its increased anti-proliferative activity as compared to that of **IV**, based on its enhanced capacity to altering essential physicochemical properties

of tumor cells needed for their proliferation, metastasis and malignancy (Furusawa et al., 2006). Interestingly, several oxidized products of quercetin act as more potent antioxidants than quercetin itself (Vásquez-Espinal et al., 2019). In particular, compound **IV/1** was shown to exhibit improved antioxidant characteristics compared to **IV** (Gülşen et al., 2007). Further, it is of particular interest that a whole mixture of autoxidized quercetin metabolites was found to exert a ca. 20-fold stronger cytoprotective activity than quercetin on Hs68 and Caco2 cells exposed to indomethacin, and a metabolite, identified by ESI-MS/MS as **IV/1**, was 200-times stronger cytoprotective agent than its parent compound (Fuentes et al., 2017). These results strongly support the notion that the oxidized metabolites of quercetin are of potential value for drug discovery.



	R^1	R^2	R^3	R^4
IV/4	OH	H	H	$OCOCH_3$
IV/5	OH	H	H	OH
IV/6	H	OH	OH	OH

	R^1	R^2	R^3	R^4
IV/12	OH	OH	OH	OH
IV/13	OH	H	H	COOH

	R
IV/14	OH
IV/15	COOH

	R^1	R^2	R^3
IV/19	OCH_3	OCH_3	OH
IV/21	OCH_3	OH	OH

	R
IV/16	OCH_2CH_3
IV/17	OCH_3

	R^1	R^2	R^3
IV/18	OCH_2CH_3	OCH_2CH_3	OH
IV/20	OCH_2CH_3	OH	OH

Figure 4. Oxidized quercetin derivatives formed in reactions with biorelevant (**A**), biomimetic (**B**) or biomimetic-related chemical oxidants (**C**).

3. DRUG DISCOVERY VALUE OF THE CHEMICAL METABOLITE SPACE OF I–IV

To place the selected four antioxidants and their oxidized derivatives in the field of medicinal chemistry, we studied them by a popular method in cheminformatics, the t-distributed stochastic neighbor embedding (t-SNE) (Van der Maaten & Hinton, 2008) for dimensional reduction and visualization. The t-SNE defines a probabilistic distribution of the data points in the high dimensional space, in our case in the space of extended-connectivity fingerprints (ECFP4) (Rogers & Hahn, 2010), then defines a similar probability distribution in the low dimensional space. In the last step it minimizes the Kullback-Leibler divergence between the two distributions and provides a 2D projection of the high-dimensional space. In this projection we can state that objects similar in the high dimensional space fall close to each other in the low dimensional space with high probability. Figure 5 shows the t-SNE plot of the distribution of compounds **I**–**IV** and their metabolites over the chemical space of approved drugs (Wishart et al., 2017) that have a molar mass below 700 Da, a total of 2,378 compounds. Groups I to IV contain parent compounds and derivatives of resveratrol (**I**), caffeic acid (**II**), methyl-caffeate (**III**), and quercetin (**IV**). Parent compounds are also highlighted separately in Figure 5 for an easier identification.

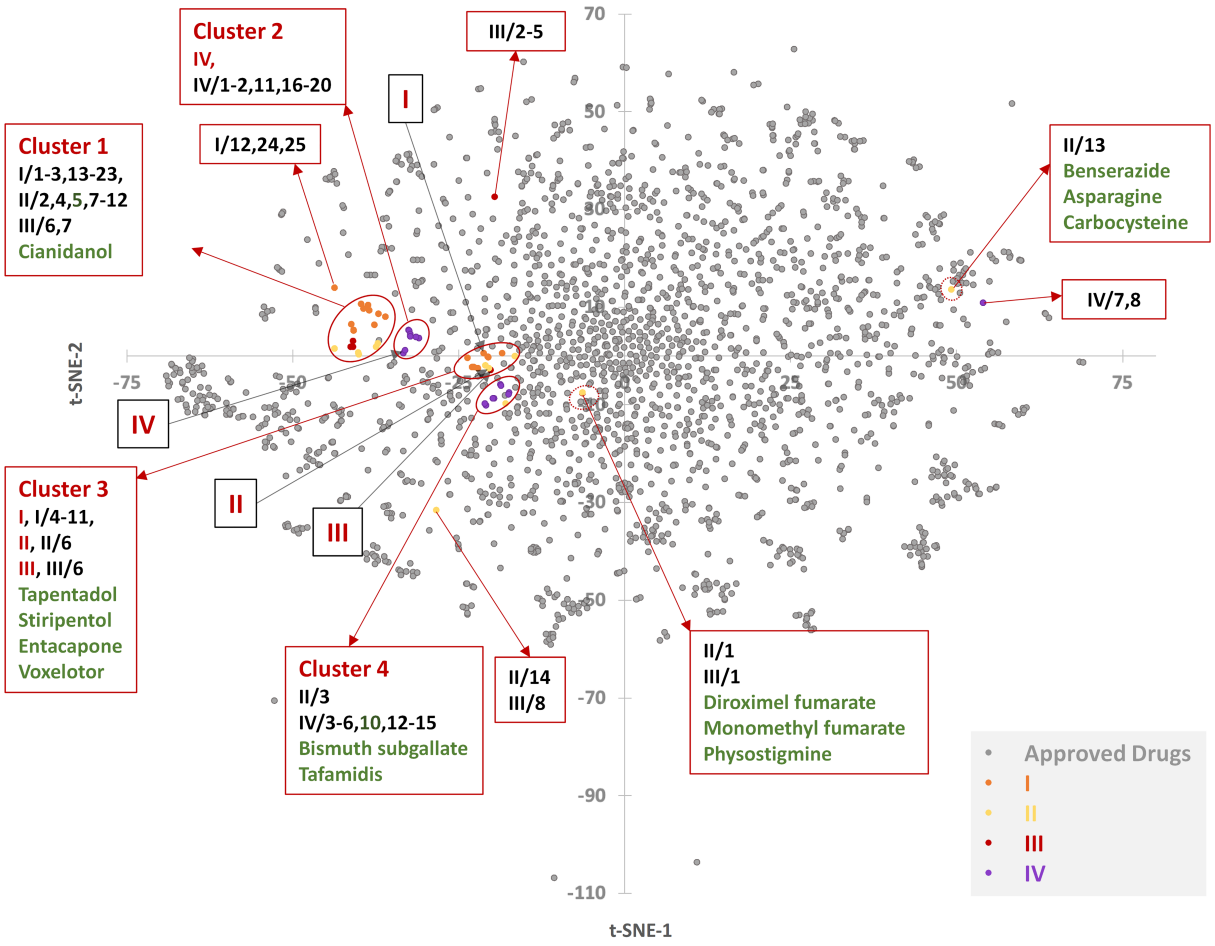


Figure 5. t-SNE plot of compounds **I**–**IV**, their oxidized derivatives, and approved drugs with a molecular mass below 700 Da. A total of 2,378 approved drugs are plotted that were retrieved from the DrugBank database. Each sphere represents a compound, and color codes are assigned to approved drugs (grey), and compound groups **I**, **II**, **III**, and **IV** (orange, yellow, red, and purple, respectively).

The investigated antioxidant compounds and their derivatives could be classified into four clusters and six additional small groups or singletons based on their structural similarity on the t-SNE map. Cluster groups that are located far from the origin of the t-SNE plot and in a space underrepresented by approved drugs can be considered unique, and thus to represent a new medicinal chemistry space. A good example for this is Cluster 1, which included only one known drug, Cyanidanol ((+)–catechin). This part of the t-SNE space is overall underrepresented. Another structurally valuable group is Cluster 2, which is close to Cluster 1 and contains only quercetin (**IV**) and its derivatives without any approved drugs. Clusters 3 and 4 are already much closer to the origin of the t-SNE plot, so that several structurally similar drugs could be included in them. It should be noted that Cluster 3 contains three of the parent compounds (**I**–**III**) and the

active substances Tapentadol (opioid analgesic) (Tzschentke et al., 2006), Stiripentol (anticonvulsant) (Quilichini et al., 2006), Entacapone (reversible inhibitor of COMT) (Chong & Mersfelder, 2000), and Voxelotor (hemoglobin oxygen-affinity modulator) (Hutchaleelaha et al., 2019); this predicts a variety of expectable pharmacological effects for compounds placed within this cluster. In terms of structural exclusivity, it is important to highlight the caffeic acid derivatives **II/14** and **III/8**, which are also located in the under-represented part of the t-SNE space. Their chemical exclusivity is presumably explained by the presence of a rigid heterocycle formed by oxidative cyclization and the relatively high number of oxygen atoms. Similarly, methyl-caffeate derivatives (**III/2**–**5**) and quercetin derivatives (**IV/7**–**8**) were identified as small groups providing new chemical entities. However, the cysteine-coupled derivative of caffeic acid (**II/13**) was identified as a chemically represented independent singleton in a cluster with Benserazide (DOPA decarboxylase inhibitor) (Shen et al., 2003), Asparagine, and Carbocysteine (mucolytic) (Zheng et al., 2008). Furthermore, the quinone derivatives of **II** and **III** (**II/1**, **III/1**) were identified as small groups, and their cluster group includes Diroximel fumarate (immunosuppressant) (Kourakis et al., 2020), Monomethyl fumarate (alters the NFE2L2 transcription factor) (Kourakis et al., 2020), and Physostigmine (reversible cholinesterase inhibitor) (Arens et al., 2018). Overall, the t-SNE map shows that although three of the four antioxidant parent compounds are in a common cluster (Cluster 1: **I**–**III**), their derivatives are scattered on the t-SNE map and distributed in a diverse way in the drug-like chemical space. Based on their appearance in several underrepresented areas of the t-SNE map and on the identified drugs in their cluster groups, a variety of pharmacological effects are expected from derivatives of compounds **I**–**IV** formed by biomimetic oxidation.

4. SUMMARY

The scavengome concept focuses on the drug discovery potential of metabolites that may be formed when small-molecule antioxidants scavenge reactive oxygen or nitrogen species. Naturally, any attempt to systematically explore such metabolites' chemical space requires an extensive use of biomimetic oxidative chemistry from a diversity-oriented perspective.

The examples discussed above demonstrate that antioxidants' transformation by a variety of biorelevant, biomimetic, or biomimetic-related chemical oxidants yields various product patterns and therefore significantly expands chemical space. At the same time, the high diversity of such product patterns is accompanied by a considerable overlap between products that are achievable through different chemical approaches, i.e., several less biomimetic oxidative conditions lead to the formation of products that are common with those formed in fully biorelevant models. This confers a plasticity to possible yield optimization initiatives for the selective preparation of any potential lead compounds identified from the scavengome.

The very high increase in the chemical complexity of oxidized metabolites as compared with that of their parent antioxidants (Figures 2–4), and their spreading in the drug-like chemical space (Figure 5) strongly suggest a great drug discovery value for such compounds. Accordingly, it may be postulated that a diversity-oriented exploration of the scavengome of antioxidants is a valid strategy to achieve an increase in biological performance diversity, and that using the scavengome concept as a guiding principle may serve as a novel strategy for antioxidant-inspired drug discovery.

ACKNOWLEDGEMENTS

The authors acknowledge support from the National Research, Development, and Innovation Office (NKFIH), Hungary (K-134704), and project no. TKP2021-EGA-32, implemented with the support provided by the Ministry of Innovation and Technology of Hungary from the NKFIH, financed under the TKP2021-EGA funding scheme.

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