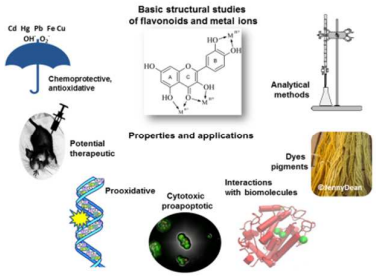
RSC Advances




ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Flavonoid metal complexes have a wide spectrum of activities as well as potential and actual applications.



Journal Name

RSCPublishing

ARTICLE

# Properties and applications of flavonoid metal complexes

Cite this: DOI: 10.1039/xoxxoooooox

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

www.rsc.org/

Maria M. Kasprzak,[a] Andrea Erxleben[c], Justyn Ochocki[a]

[a] Departement of Bioinorganic Chemistry, Medical University of Lodz, Ul. Muszynskiego 1, Lodz, Poland
[c] School of Chemistry, National University of Ireland, Galway, Ireland
*Corresponding author: maria.kasprzak@umed.lodz.pl*

Flavonoids are widely occurring polyphenol compounds of plant origin with multiple biological and chemical activities. Due to the presence of carbonyl and hydroxyl groups they can coordinate metal ions and form complexes. Metal complexes of flavonoids have many interesting properties: they are colored, often fluorescent, anti- or pro-oxidant, antimicrobial, antiproliferative and biologically active in many other ways. There are many papers covering specific aspects of activity of flavonoid metal complexes, *e.g*. their antioxidant properties, enzyme-mimicking behavior, therapeutic potential or use in chemical analysis. However, for a researcher interested in this theme, it would be useful to find an extensive review on more than one selected area. Our aim was to cover a wide spectrum of possible activities and potential applications of flavonoids coordinated to metal ions in order to give our readers a broad view on the topic of this class of compounds, their activity and potential applications. While a significant amount of information on the chemical properties and biological activity of flavonoid metal complexes can be found in the literature, an in-depth understanding of structure-property relationships is still lacking. In an attempt to address this issue, a comprehensive discussion of the available data is presented.

**Keywords:** flavonoids; metal ions; complexes

## 1. Introduction

Flavonoids, a group of compounds based on the 2-phenylchromen-4-one (2-phenyl-1-benzopyran-4-one) structure, occur in nature mostly as polyphenol compounds of plant origin (Table 1). Depending on the degree of oxidation of the γ-pyranone ring, they can be categorised into different subclasses, such as flavones, flavonols, and flavanones (Table 1). In nature flavonoids occur mostly as glycosides, as the sugar group enhances the water solubility of the hydrophobic flavonoid molecules. There are also many synthetic flavonoid-derived substances.[1-5] Most, if not all, flavonoids are colored substances (flavonoid from Latin *flavus* 'yellow') and thus, along with carotenoids, chlorophyll, etc., are the source of color in leaves, flowers and fruits of many plants. Natural flavonoids are widely believed to prevent many conditions, such as cancer or cardiovascular diseases.[6] Their beneficial properties are ascribed to their antioxidant activity which depends mostly on the number and position of hydroxyl moieties.[7] However, the wide variety of biological activities of flavonoids is not limited to antioxidant properties. They also act as enzyme inhibitors,[8] signaling molecules,[9,10] DNA intercalators[11,12] and chelators of metal ions.[13] Furthermore, they are not always beneficial for health.[8] For example, quercetin alone is carcinogenic in rats[14] and upon oxidation it may produce toxic metabolites and cause depletion of the intracellular thiol pool.[15]

Due to the presence of oxo and hydroxyl groups (and nitrogen donors in some synthetic compounds[5]), many flavonoids can coordinate and chelate various metal ions (Fig. 2).[16-27] However, reactions between flavonoids and metal ions are not always limited to simple coordination bond formation. Depending on the metal ion, redox reactions between the metal ions and the ligands may occur, as most flavonoids have good reducing capacity. This is particularly relevant for metal ions with oxidizing properties, *e.g.* $Fe^{III}$,[28] $Ru^{IV}$,[29] $Ru^{III}$,[30] $Au^{III}$,[31] or $Os^{VIII}$.[32] This review focuses on the ability of flavanones, flavones and flavonols, as well as their glycosides to form metal complexes, the various consequences of this process, and the properties and applications of such complexes. Isoflavones and flavanols (catechins), because of their distinct structure that affects the geometry of their chelating sites (Fig 1), will not be the subject of this paper.

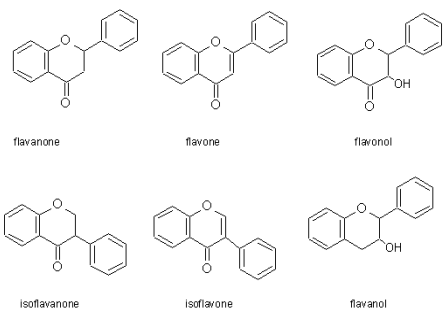
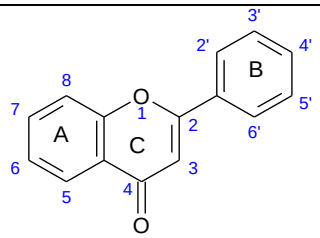



**Fig. 1** Structures of different classes of flavonoids.

**Table 1** Examples for natural flavonoids

| Basic structure of selected flavonoids, with carbon atoms numbered | |
|---|---|
|  | |
| **Flavanones** | |
| Naringenin | 5,7-dyhydroxyflavanone |
| Liquirtigenin | 7,4'-dihydroxyflavanone |
| Hesperetin | 5,7,5'-trihydroxyflavanone |
| Eriodictyol | 5,7,3',4'-tetrahydroxyflavanone |
| Homoeriodictyol | 5,7,4'-trihydroxy-3'-methohyflavanone |
| Pinostrobin | 5-hydroxy-7-methoxyflavanone |
| **Flavones** | |
| Chrysin | 5,7-dihydroxyflavone |
| Baicalein | 5,6,7-trihydroxyflavone |
| Luteolin | 5,7,3',4'-tetrahydroxyflavone |
| Apigenin | 5,7,4'-trihydroxyflavone |
| Scutellarein | 5,6,7,4'-tetrahydroxyflavone |
| Wogonin | 5,7-dihydroxy-8-methoxyflavone |
| Tangeritin | 5,6,7,8,4'-pentamethoxyflavone |
| Diosmetin | 5,7,3'-trihydroxy-4'-methoxyflavone |
| **Flavonols** | |
| Flavonol | 3-hydroxyflavone |
| Galangin | 3,5,7-trihydroxyflavone |
| Kaempferol | 3,5,7,4'-tetrahydroxyflavone |
| Morin | 3,5,7,2',4'-pentahydroxyflavone |
| Myricetin | 3,5,7,3',4',5'-hexahydroxyflavone |
| Quercetin | 3,5,7,3',4'-pentahydroxyflavone |
| Fisetin | 3,7,3',4'-tetrahydroxyflavone |
| Azaleatin | 3,7,3',4'-tetrahydroxy-5-methoxyflavone |

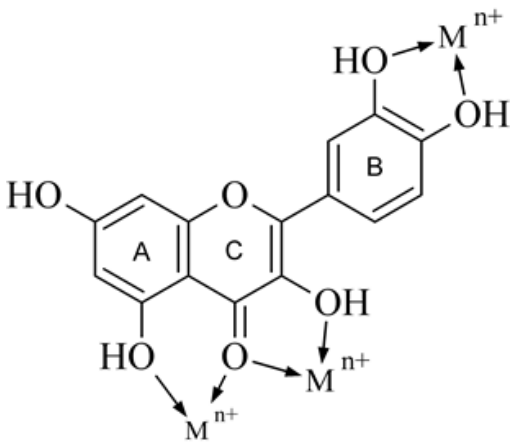



**Fig. 2** Possible chelating sites of quercetin. Flavonoids can coordinate metal ions in their neutral (as shown) or anionic form.

## 2. Metal ion chelation

### 2.1. Metal binding sites and complex stability

As shown in Fig. 1, flavonoids present more than one possible chelating site for metal ions. Metal coordination to the pentahydroxyflavone quercetin, the most common flavonoid, can occur *via* the 3-hydroxyl and 4-carbonyl group of the C ring (denoted as 3-4 site), the 4-carbonyl-5-hydroxyl site of the A and C rings (4-5 site) or *via* the catechol moiety of the B ring (3'-4' site). The preferred binding site depends on the flavonoid, the metal ion and on the pH value. The latter is relevant to the *in vivo* properties of flavonoids as dietary components or pharmaceuticals (*e.g*. strongly acidic conditions in the stomach *vs.* blood pH; acidic pH in lysosomes; pathological acidosis in tumors and inflamed tissue). Flavonoids as polyphenols are weak acids that undergo deprotonation which strongly affects the coordination of metal ions.[33] A large number of experimental and theoretical studies on the coordination properties of flavonoids have been published and often seemingly contradictory results have been obtained with regard to the binding site and metal/ligand stoichiometry[33-38] which, however, is due to the variation in experimental conditions in the different studies. It is generally agreed that in alkaline solution $Fe^{3+}$, $Cu^{2+}$ and $Al^{3+}$ ions have the strongest affinity for the ortho-dihydroxyl group of quercetin.[39-42] Bodini *et al*. reported that the catechol group remains the major chelation site for $Fe^{3+}$ in acidic solution,[43] whereas Dimitric Markovic *et al*. presented data that supported the opposite.[33] They reported the formation of a 1:2 $Fe^{3+}$ quercetin complex in acidic solution with coordination *via* the 3-4 or 4-5 site and Fe binding to the catechol group in a 1:1 metal/ligand ratio at higher pH. However, different solvent systems were used in the two studies. The formation of a 1:2 Fe fisetin complex involving coordination through the 3-4 site in acidic solution was also observed by Dimitric Markovic *et al*.[44] The reduction of $Fe^{3+}$ to $Fe^{2+}$ by fisetin at acidic pH was described, while at higher pH $Fe^{3+}$ and $Fe^{2+}$ complexes co-exist and coordination occurs through the ortho-dihydroxyl group with a 1:1 metal/ligand stoichiometry. Cornard and Merlin reported the formation of a 1:1 $Al^{3+}$ quercetin complex with chelation through the 3-4 or 4-5 site in acidic solution.[45] At alkaline pH, these authors observed binding of a second $Al^{3+}$ ion to the catechol moiety leading to a 2:1 metal/ligand stoichiometry. By contrast, Dimitric Markovic *et al*. reported the stepwise coordination of $Al^{3+}$ to the 3-4 and 3'-4' sites of fisetin also at pH 5 (albeit at a $Al^{3+}$ : ligand ratio different to that in Cornard and Merlin's study)[46] and the 2:1 $Al^{3+}$ complex of luteolin involving coordination to the 3-4 and 3'-4' sites was proposed by Rygula *et al*. to co-exist with the 1:1 complex in acidic solution.[38] On the other hand, the suggestion that the ortho-dihydroxyl group of the B ring is not a binding site for $Al^{3+}$ in strongly acidic medium is in line with a report that flavonoids that lack the 3- and 5-hydroxy groups, such as 3'4'-dihydroxyflavone do not react with $Al^{3+}$ at acidic pH.[47] Ahmedova *et al*. carried out a combined $^{1}H$, $^{13}C$ MAS NMR and DFT study on the Al quercetin complex and pointed out that the chelating site in the solid-state and in solution are likely to be different.[48] $Cr^{3+}$ ions were found to favor the deprotonated 4-5 site of quercetin[49] and luteolin,[50] while $Pb^{2+}$ ions preferentially bind to the 3'-4' site.[51,52] There are a large number of theoretical studies on the preferential coordination mode in metal flavonoid complexes in the literature that will be discussed in section **2.4**.

**Table 2** Stability constants of flavonoid complexes

| M | M:L | pH / medium[a] | logβ / logK | ref. |
|---|---|---|---|---|
| *quercetin* | | | | |
| $Al^{3+}$ | 1:1 | EtOH | - 5.79 [b] | 60 |
| $Al^{3+}$ | 1:2 | | 24.1 | 65 |
| $Ca^{2+}$ | 1:1 | | 2.91 | 61 |
| $Cd^{2+}$ | 1:1 | pH 4.4 | 6.35 | 62 |
| | | pH 7.4 | 6.89 | |
| $Co^{2+}$ | 1:1 | pH 5.0 | 4.87 | 63 |
| $Co^{2+}$ | 1:1 | [b] | 8.37 | 35 |
| $Cu^{2+}$ | 1:1 | [b] | 19.30 | 35 |
| $Fe^{3+}$ | 1:2 | pH 4 [c] | 9.56 | 33 |
| $Fe^{2+}$ | 1:2 | pH 4 [d] | 10.70 | 64 |
| $Fe^{3+}$ | 1:1 | pH 8 [c] | 5.50 | 33 |
| $Fe^{2+}$ | 1:1 | pH 8 [d] | 6.30 | 64 |
| $Fe^{3+}$ | 1:1 | [c] | 44.2 | 35 |
| $Fe^{2+}$ | 1:2 | | 13.3 | 65 |
| $Fe^{2+}$ | 1:1 | pH 7.2 | 6.65 [e] | 64 |
| $Fe^{2+}$ | 1:2 | pH 7.2 | 10.70 | 64 |
| $La^{3+}$ | 1:2 | $H_2O$/diox (1:1) | 13.77 | 66 |
| $Ni^{2+}$ | 1:1 | 5.0 | 5.57 | 63 |
| $Mg^{2+}$ | 1:1 | | 3.51 | 61 |
| $Ni^{2+}$ | 1:1 | [b] | 9.53 | 35 |
| $Pb^{2+}$ | 1:1 | MeOH | 4.87 | 52 |
| $Pb^{2+}$ | 1:2 | MeOH | 7.71 | 52 |
| $Pb^{2+}$ | 2:1 | MeOH | 8.23 | 52 |

| | | | | |
|---|---|---|---|---|
| $Pd^{2+}$ | 1:1 | pH 5.0 | 6.05 | 63 |
| *3-hydroxyflavone* | | | | |
| $Al^{3+}$ | 1:2 | MeOH | 12.3 | 67 |
| | | | 11.2 | 68 |
| $Al^{3+}$ | 1:1 | pH 6, MeOH/$H_2O$ | 5.17 | 69 |
| $Al^{3+}$ | 1:2 | pH 6, MeOH/$H_2O$ | 9.91 | 69 |
| $Cd^{2+}$ | 1:1 | pH 6.2 | 5.90 | 63 |
| $Co^{2+}$ | 1:1 | pH 6.2 | 10.87 | 63 |
| $Eu^{3+}$ | 1:2 | pH 6.0 | 13.47 | 63 |
| $Fe^{2+}$ | 1:2 | pH 7.2 | 11.30 | 64 |
| $Fe^{3+}$ | 1:1 | EtOH/$H_2O$ | 13.29 | 37 |
| $Ni^{2+}$ | 1:1 | pH 6.1 | 7.63 | 63 |
| $Pb^{2+}$ | 1:1 | pH 6.1 | 7.74 | 63 |
| $Pb^{2+}$ | 1:1 | MeOH | 4.97 | 67 |
| $Zn^{2+}$ | 1:1 | | 6.60 | 67 |
| $Zn^{2+}$ | 1:1 | pH 5.8 | 8.51 | 63 |
| *5-hydroxyflavone* | | | | |
| $Al^{3+}$ | 1:1 | MeOH | 6.5 | 67 |
| $Al^{3+}$ | 1:2 | pH 6, MeOH/$H_2O$ | 8.37 | 69 |
| $Pb^{2+}$ | 1:1 | MeOH | 4.51 | 67 |
| $Zn^{2+}$ | 1:1 | MeOH | 5.33 | 71 |
| *3'4'-dihydroxyflavone* | | | | |
| $Al^{3+}$ | 1:1 | MeOH | 6.7 | 67 |
| $Al^{3+}$ | 1:1 | pH 6, MeOH/$H_2O$ | 4.85 | 69 |
| $Al^{3+}$ | 1:2 | pH 6, MeOH/$H_2O$ | 9.24 | 69 |
| $Fe^{2+}$ | 1:2 | pH 7.2 | 11.08 [e] | 64 |
| $Fe^{3+}$ | 1:1 | EtOH/$H_2O$ | 20.87 | 37 |
| $Pb^{2+}$ | 1:1 | MeOH | 5.09 | 67 |
| $Pb^{2+}$ | 1:2 | MeOH | 8.82 | 67 |
| $Zn^{2+}$ | 1:1 | MeOH | 5.19 | 71 |
| *3,7-dihydroxyflavone* | | | | |
| $La^{3+}$ | 1:1 | diox/$H_2O$ | 7.27 | 66 |
| *5,7-dihydroxyflavone* | | | | |
| $Al^{3+}$ | 1:2 | MeOH | 11.67 | 72 |
| $Fe^{3+}$ | 1:1 | EtOH/$H_2O$ | 11.40 | 37 |
| *3,7,3'-trihydroxyflavone* | | | | |
| $Ga^{3+}$ | 1:2 | MeOH | 7.74 | 75 |
| $La^{3+}$ | 1:2 | diox/$H_2O$ | 14.36 | 66 |
| *baicalein* | | | | |
| $Fe^{3+}$ | 1:1 | pH 6.0 | 4.43 | 33 |
| $Fe^{3+}$ | 1:1 | pH 6.0 [d] | 6.47; 6.00 [g] | 73 |
| *morin* | | | | |
| $Cu^{2+}$ | 1:2 | pH 5.8 | 4.94 | 63 |
| $Fe^{3+}$ | 2:1 | EtOH/ $H_2O$ | 8.22 | 74 |
| $Zn^{2+}$ | 1:2 | pH 5.5 | 6.74 | 63 |
| $La^{3+}$ | 1:2 | diox/$H_2O$ | 12.24 | 66 |
| *3,3'-dihydroxyflavone* | | | | |
| $Ga^{3+}$ | 1:2 | MeOH | 8.46 | 75 |
| $La^{3+}$ | 1:1 | MeOH | 6.36 | 76 |
| *luteolin* | | | | |
| $Fe^{3+}$ | 1:1 | EtOH | 8.4 | 77 |
| *rutin* | | | | |
| $Co^{2+}$ | 1:1 | pH 5.0 | 6.04 | 63 |
| $Co^{2+}$ | 1:1 | [b] | 8.23 | 35 |
| $Cu^{2+}$ | 1:2 | pH 6.1 | 10.76 | 63 |
| $Cu^{2+}$ | 1:1 | [f] | 23.51 | 35 |
| $Fe^{2+}$ | 1:2 | pH 7.2 | 11.85 [e] | 64 |
| $Fe^{3+}$ | 1:1 | [c] | 44.1 | 35 |
| $Ni^{2+}$ | 1:2 | pH 6.2 | 8.95 | 63 |
| $Pb^{2+}$ | 1:2 | pH 4.5 | 13.81 | 63 |
| $Zn^{2+}$ | 1:1 | pH 6.2 | 4.68 | 63 |
| *fisetin* | | | | |
| $Fe^{3+}$ | 1:1 | pH 7.0 [c] | 4.78 | 44 |
| $Fe^{3+}$ | 1:1 | pH 9.0 [c] | 4.95 | 44 |
| $Fe^{3+}$ | 1:2 | pH 4.0 [c] | 9.85 | 44 |
| $Fe^{3+}$ | 1:1 | pH 7.0 [d] | 4.80 | 44 |
| $Fe^{3+}$ | 1:1 | pH 9.0 [d] | 4.79 | 44 |
| $Al^{3+}$ | 1:1 | pH 2.0 | 4.32 | 46 |
| $Al^{3+}$ | 1:1 | pH 5.0 | 5.05 | 46 |
| $Al^{3+}$ | 1:2 | pH 5.0 | 6.52 | 46 |
| $Al^{3+}$ | 1:1 | pH 8.0 | 7.13 | 46 |
| $Al^{3+}$ | 1:2 | pH 8.0 | 10.41 | 46 |
| *kaempferol* | | | | |
| $Fe^{3+}$ | 1:1 | pH 8.0 | 4.10 | 78 |
| $Fe^{3+}$ | 1:1 | pH 4.0 | 3.35 | 78 |

[a] aqeous solution unless stated otherwise. EtOH = ethanol; MeOH = methanol; diox = dioxane. [b] K = $[AlL][H^+]^3/[Al^{3+}][L]$; $[Cu(OH)L]/[Cu][L][OH]$; $[CoL]/[Co][L]$; $[NiL]/[Ni][L]$; $[Fe(OH)_2L]/[Fe][L][OH]^2$. [c] acetate buffer. [d] phosphate buffer. [4] average of two methods. [f] K = $[Cu(H_2L)]/[Cu][L]^2$. [g] stability constant calculated by different methods.

Besides 1:1, 1:2 and 2:1 complexes other metal/ligand stoichiometries are in principle possible, including 1:3, 2:2, 2:3, and 3:1.[36,45,53-55] However, for steric reasons, complexes with more than two flavonoid ligands are usually restricted to rare-earth metal ions.[55-59] It should be noted that the simultaneous binding of two metal ions to the 3-4 and 4-5 site is unfavorable because of steric repulsion.

Table 2 gives an overview of the stability constants of flavonoid complexes. Because of the poor aqueous solubility of flavonoids, in many cases complex stabilities were determined in alcoholic solution or water/alcohol mixtures and often a direct comparison of the data is difficult. The polarity of the solvent and its ability to form hydrogen bonds has an impact on metal ion - ligand interactions. It has been shown, for example, that the degree of $Al^{3+}$ complexation by 3-hydroxyflavone, 5-hydroxyflavone and 3'4'-dihydroxyflavone in methanol decreases significantly in the presence of water.[69] Other factors that have an influence include the choice of buffer. Especially the effect of phosphate buffer on the degree of complex formation has been discussed in the literature.[44] Thus, care has to be taken, when extrapolating stability data to *in vivo* complexing properties. However, some general conclusions can be drawn: Most 1:1 flavonoid metal complexes have moderate ($5 < \log\beta < 10$) or high ($\log\beta > 10$) stabilities. Biologically relevant $Fe^{3+}$ as a hard Lewis acid has a high affinity for the hard oxygen donor atoms of flavonoids and forms highly stable

complexes, while the corresponding complexes of $Fe^{2+}$ as a borderline hard/soft Lewis acid have lower stabilities. It appears that, in general, a weakly acidic pH (around 5 - 6) allows stable complex formation. The presence of a catechol or trihydroxy group in the B ring is advantageous for Fe binding (*cf*. logβ for quercetin and 3',4'-dihydroxyflavone complexes *vs*. baicalein and kaempferol complexes). Complexes of 5-hydroxyflavone have lower stability constants than complexes of 3-hydroxyflavone owing to the presence of a strong O5-H···O4 hydrogen bond in 5-hydroxyflavone.[79] Extrapolated to quercetin, this may suggest that in acidic solution biologically relevant Fe ions should prefer the 3-4 site of quercetin over the 4-5 site. Mladenka *et al*. compared 26 flavonoids in order to elucidate the contribution of the different binding sites to the overall Fe binding activity.[80] They showed that the Fe chelating power decreases in the order bacailein (5,6,7-trihydroxyflavone) > 7-methoxybacailein > 5-hydroxyflavone and concluded that flavonoids with a 6,7-dihydroxyl group are the most potent Fe chelators due to the favorable deprotonation of the 7-hydroxyl group.

### 2.2. Structural properties

The number of structurally characterized metal flavonoid complexes is rather limited. So far, the X-ray structures of $[Cu^{I}(3HF)(PPh_3)_2]$,[81,82] $[Cu^{II}(3HF)_2]$,[83,84] $[Zn(MSA)(H_2O)_2]_n$, $Na_2[Zn(MSA)_2(H_2O)]$,[85] $[Fe^{II}(3HF)_2Cl(CH_3OH)]$[86] and $[Fe^{III}(4'OMeFla)_3]$[87] were reported (3HF = 3-hydroxyflavone; MSA = morin-5-sulfonic acid; 4'OMefla = 4'-methoxyflavonol). Structures of ternary flavonoid complexes containing additional mono-, di-, tri- or tetradentate *N*- or *N,O*-donor ligands are somewhat more abundant and include $Fe^{II}$,[88] $Fe^{III}$,[89,90] $Cu^{II}$,[91-98] $Co^{II}$,[88,91,99,100] $Mn^{II}$,[87,90,91] $Zn^{II}$,[91,101] and $Ni^{II}$ [88,91,102] flavonol and 4'-methoxyflavonol complexes of type $[M(fla)L]^{n+}$, $[M(fla)LX]^{n+}$ and $[M(fla)_2L_2]$ as well as the oxido-bridged $Fe^{III}$ complex $[Fe(6\text{-}Ph_2TPA)Fe(3HF))_2(\mu\text{-}O)](ClO_4)_2$ (6-$Ph_2$TPA = *N,N*-bis(6-phenyl-2-pyridyl)methyl)-*N*-((2-pyridyl)methylamine).[91] These complexes were mainly synthesized with the aim to model the enzyme-substrate complex of quercetin 2,3-dioxygenase, the enzyme that catalyzes the oxidative degradation of quercetin to the depside.

Except for square-planar $[Cu^{II}(3HF)_2]$[83,84] and distorted tetrahedral $[Cu^{I}(3HF)_2(PPh_3)_2]$[81,82] the metal centers in flavonoid complexes are five- or six-coordinate. The majority of ternary Cu complexes have either a distorted square-pyramidal or a distorted trigonal-bipyramidal coordination geometry. In square-pyramidal $[Cu(fla)L]^{n+}$ and $[Cu(fla)LX]$ complexes the O3 and O4 oxygens of the flavonoid occupy basal positions, while in trigonal-bipyramidal complexes the flavonoid ligand spans an apical and an equatorial site. In all structures a slight shortening of the C3-O3 bond and an elongation of the C4=O4 bond is observed with the C-O and C=O distances ranging from 1.30 to 1.36 Å and from 1.24 to 1.30 Å, respectively, as compared to 1.357(3) and 1.232(3) Å in free 3HF [103] (see figure in Table 1 for the numbering scheme). Speier, and co-workers discussed the structural changes in the pyranone ring of 3HF on metal coordination to the 3-4 site.[82,95] They attribute the observed increase of the C2-O1 and C9-C10 bond distances and the decrease of the C3-C4 bond length to the extended delocalization of the π system over the whole molecule. This should impact on the quercetinase mimetic properties, as the extended delocalization should make oxygenation less favorable. Sun *et al*., on the other hand, described a small elongation of the C2=C3 bond in [M(3HF)L] complexes with M = $Fe^{II}$, $Co^{II}$ and $Ni^{II}$ and pointed out that this may promote the bond cleavage during the reaction with $O_2$.[88,99] Moreover, it was proposed that the slight pyramidalization of C2 on metal coordination stabilizes the substrate radical formed during the oxygenation reaction (see below).

The Cu-O bond lengths are significantly shorter in the $Cu^{II}$ complex $[Cu(3HF)_2]$[83,84] than in $[Cu^{I}(3HF)(PPh_3)_2]$[81,82] demonstrating that the flavonolato ligand binds more strongly to $Cu^{II}$ than to $Cu^{I}$. Likewise, $[Fe^{II}(3HF)L]$ features longer Fe-O bonds (C=O 2.0297(16), C-O 2.1893(17) Å)[88] than $Fe^{III}$ complexes (1.912 - 1.968, 2.100 - 2.139 Å). It is noteworthy that the M-O3 and M-O4 bond distances are clearly different in all complexes ($\Delta d_{M\text{-}O=C/M\text{-}O\text{-}C}$ 0.145 - 0.184 Å for Fe and 0.089 - 0.264 Å for Cu). The relevance of this asymmetry to the monodentate substrate coordination assumed for quercetin 2,3-dioxygenase is mentioned in the literature.[91]

In the metal complexes the flavonoid ligand typically adopts a non-planar conformation with the B ring being twisted relative to the benzopyrane ring system. The O1-C2-C1'-C6' torsional angle $\phi$ is 40.51 ° in octahedral $[Mn(6\text{-}Ph_2TPA)(3HF)]^{+}$ and is significantly larger than that observed in the analogous Co and Ni complexes (-15.91 and -22.31 °, respectively).[91] The latter are more in line with the torsional angle range of -28.27 to 14.3 ° of all other X-ray structures. The flavonoid ligands in $[Co^{II}(L^{OMe})(3HF)]$ ($\phi$ = -1.329 °, $L^{OMe}$ = 2-{[bis (pyridin-2-ylmethyl)amino]methyl}-p-methoxy-benzoate),[99] $[Fe^{II}L(3HF)]$ ($\phi$ = -2.487 °, L = 2-{[bis(pyridin-2-ylmethyl)amino]methyl} benzoate),[88] $[Fe^{III}(3HF)Cl(CH_3OH)]$ ($\phi$ = 2.896 °)[86] and $[Cu(3HF)_2]$ ($\phi$ = -0.878 °)[84] are closest to planarity. In an interesting study by Sun *et al*. the X-ray structures of a series of $Co^{II}$ flavonolate complexes with differently substituted tripodal co-ligands revealed a linear relationship between the torsion angle and the Hammett constant.[99] Thus, it seems that the conformation of the flavonoid is - at least partly - determined by the electronic effect of metal coordination.

### 2.3. Optical properties

As flavonoids are colored compounds, they have specific absorption bands in the UV-VIS region and can be analyzed by means of spectrophotometric methods.[104] The UV-VIS spectra of most flavonoids (especially flavonols, due to the presence of the 3-OH moiety) display two main absorption bands: the benzoyl band in the 240-280 nm range and the cinnamoyl band in the 320-385 nm range (Fig. 3). The bands may be attributed

to HOMO-LUMO energy gaps, and the first absorption band has $\pi \rightarrow \pi^*$ character.[105] After coordinating metal ions, the UV spectra of flavonoids change, displaying a bathochromic (red) shift. Some authors suggest that the bathochromic shift is caused by a strong charge transfer from the flavanoid to the metallic center,[105] while others attribute the shift to a decrease in the HOMO-LUMO gap in the flavonoid molecule, rather than to an LMCT (Ligand to Metal Charge Transfer) transition.[105] As discussed below, theoretical calculations have been reported that support a decrease of the HOMO-LUMO gap in flavonoids (*e.g.* quercetin or luteolin) on complexation of metal ions.[44,101] The change in the energetic state may also affect the antioxidant activity of flavonoids which often increases with metal ion chelation (see below).[106]

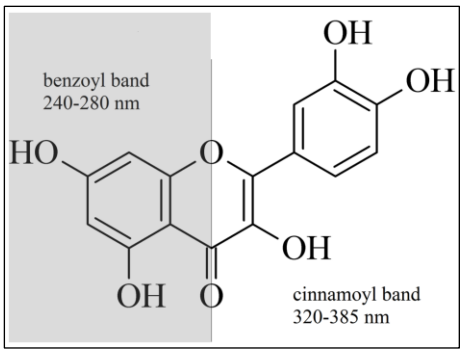



**Fig. 3** Structural moieties of quercetin (as an example of flavonoids) responsible for the main bands in its absorption spectrum.[171]

### 2.4. Computational studies

Flavonoids and their complexes have been studied in great detail by theoretical methods in order to understand the relationship between structure, properties and antioxidant activity. Much attention has been paid to Fe and Cu complexes due to the biological relevance of these metal ions and their major role in the production of reactive oxygen species (ROS) in living organisms.[44,51,78,105-107]

Ren *et al.* reported first-principle electronic structure calculations of Fe complexes with quercetin, luteolin, galangin, kaempferol and chrysin.[105] The calculated binding energies suggest that the most preferable chelating site is the 3-4 site of the C ring followed by the 4-5 site of the A and C rings and the catechol moiety of the B ring. Leopoldini *et al.* carried out a comprehensive DF-B3LYP study on the $Fe^{II}$-quercetin system considering complexes with both neutral and anionic quercetin as well as bare and hydrated $Fe^{2+}$.[51] In contrast to the results obtained by Ren *et al*. they found Fe binding to the 3-4 binding site of neutral quercetin to be 10.1 kcal $mol^{-1}$ less stable than binding to the 4-5 site. Likewise the global minimum for $Fe^{2+}$/anionic quercetin involves $Fe^{2+}$ coordination to the carbonyl group and the deprotonated 5-hydroxyl group. Leopoldini *et al.* argued that a six-membered chelate ring should introduce a greater stabilizing effect. However, the calculated energy separation between the 3-4 and 4-5 coordination modes of anionic quercetin is small ($\Delta E$ = 1.8 kcal $mol^{-1}$) and seems to be further reduced by water molecules in the coordination sphere of $Fe^{2+}$. When four water molecules were added to the coordination sphere, the stability order changed and the 3-4 bound $Fe^{2+}$ complex became the most stable one. The DF-B3LYP calculations indicated a low chelating ability of the catechol group of both neutral and anionic quercetin towards Fe.

Ren and co-workers calculated the binding energies for Fe quercetin complexes with different stoichiometries and found the highest binding energy for the 1:2 Fe quercetin complex (2.39 eV per quercetin for 1:2, 2.16 eV for 1:3 and 2.09 eV for 1:1 Fe:quercetin; coordination *via* the 3-4 site).[105] The binding energy of the 2:1 Fe quercetin complex (binding *via* the 3-4 and 3'-4' sites) was shown to be 0.6 eV lower than twice the binding energy of the 1:1 complex (binding *via* the 3-4 site). Binding energy calculations also showed that an orthogonal orientation of the two quercetin ligands to each other is favored over a co-planar arrangement in the 1:2 complex leading to a tetrahedral coordination geometry in the absence of additional water ligands.[105] When two water ligands are added to the 3-4 bound 1:2 complex, the global minimum is represented by two completely coplanar quercetin ligands, while the axial water molecules show a slight deviation from an ideal octahedron.

A detailed DFT investigation of the structural, electronic and optical properties of Cu flavonoid complexes by Lekka *et al.* demonstrated that $Cu^{2+}$ – like $Fe(H_2O)_2^{2+}$ – preferentially coordinates to the deprotonated 3-4 site of quercetin, luteolin and galangin and that this chelation site is energetically favored for complexes with 1:1, 1:2 and 1:3 metal/ligand stoichiometry.[106] As found for Fe, the highest binding energy for the 1:2 complex was obtained, when the two quercetin ligands were orthogonal to each other. A band decrease by 0.3 eV of quercetin upon complexation was calculated in line with the bathochromic shift observed in the UV spectra as already discussed. A similar result was reported for Fe quercetin[105] and Al apigenin.[108] The bathochromic shift in the spectra of $[Al(H_2O)_4(apigenin)]^{2+}$ and $[Al(H_2O)_2(apigenin)_2]^{+}$ (binding *via* the 4-5 site) was rationalized by the calculated decrease in the HOMO-LUMO gap from 4.11 eV in apigenin to 3.73 eV in $[Al(H_2O)_2(apigenin)_2]^{+}$ to 3.59 eV in $[Al(H_2O)_4(apigenin)]^{2+}$.[108]

With regard to the biological activity, redox properties and possible interactions of flavonoid complexes with biomolecules, understanding the effect of metal coordination on the geometric parameters of flavonoids is of utmost importance and several theoretical papers have addressed this question. In most cases the calculations predicted only small changes in the bond lengths at the chelating site, while C-O and C-C bond distances in rings not involved in metal binding are not affected.[9,44,47,68,71,72] Chelation through the C or A/C rings



generally results in a slight increase of the predicted C=O bond length and a slight decrease of the C-O bond length in line with the reported X-ray data described in section **2.2**. Likewise, geometry optimisations indicated a shortening of the C-O catechol bonds on metal coordination to the B ring. As for the more relevant question of the dihedral angle between the B ring and the benzopyrone moiety authors have come to different conclusions depending on the theoretical model used. DFT calculations at the B3LYP/6-31G*/LANL2DZ level indicated a C3-C2-C1'-C2' torsional angle $\phi$ of 179.9 °, when $Fe^{2+}$ coordinates to the 4-5 site of quercetin, comparable to the value for the free flavonoid calculated at similar level of theory.[51] Coordination to the 3-4 site, however, causes a significant twist ($\phi \sim 146$ °, same theoretical model). DFT calculations at the B3LYP/6-31(d,p) level also showed that the planar structure of the flavonoid is retained, when Pb coordinates two quercetin molecules *via* the catechol moiety.[52] Using the same basis set, Dimitric Markovic *et al.* obtained a dihedral angle of about 9 ° between the B and C rings in the catechol-bound 1:1 Fe quercetin complex, while the quercetin ligands in the optimized 1:2 complex involving Fe chelation through the 3-4 site adopts a planar conformation.[44] By contrast, the B3LYP/6-311+G(d,p) method indicated planarity of the ligand in both the catechol-bound 1:1 and the 3-4-bound 1:2 Fe fisetin complex.[44] However, the M06/6311+G(d,p) functional gave a 8.7 ° dihedral angle between the B and A/C rings in the case of the same 1:1 complex.[44] Both methods revealed a higher stability of the *trans* isomer of the 1:2 Fe fisetin complex compared to the *cis* isomer, albeit by only 0.25 kcal $mol^{-1}$. The same authors optimized the geometry of the 2:1 $Fe^{2+}$ morin complex $[Fe_2(CH_3OH)_2(morin)]$ (binding *via* the 3-4 and 4-5 sites) using the M052x functional and 6-311G(d,p) basis set and reported a C3-C2-C1'-C2' torsional angle of 23.90 °, significantly lower than the experimentally determined angle in free morin (43.4 °[109]). On coordination of $Fe^{3+}$ to kaempferol, a change from a non-planar (O1-C1-C1'-C2' = 7.8 °) to the planar conformation (O1-C1-C1'-C2' = 0 °) was predicted (M052x/6-311G(d,p).[78] Cornard and Merlin carried out AM1 semi-empirical calculations to reveal the effect of $Al^{3+}$ complexation on the structural parameters of quercetin and reported a dihedral angle of 8.8 ° on coordination of Al to the 3-4 site. On addition of a second metal to the 3'-4' site the quercetin ligand became essentially planar with a C1-C2-C1'-C6' torsional angle of 2.4 °.[45] The AM1 model also predicted a twist for the free flavonoid (dihedral angle of 26.7 °) which is in contrast to DFT results.[110] This discrepancy can be attributed to the fact that AM1 underestimates the stabilizing contribution of π electron delocalisation. It should be noted that the X-ray structure of quercetin monohydrate showed a torsional angle of -1.0 °.[111] Quantum semi-empirical AM1 methods also indicated a non-planar structure of 5,7-dihydroxyflavone, when coordinating $Al^{3+}$ *via* the 4-5 site in a 1:2 metal/ligand stoichiometry ($\phi$ = 26.8 ° *vs.* 27.5 ° in the free flavonoid).[72]

Flavones, like apigenin, that lack the 3-hydroxy group (and consequently C-H···O hydrogen bonding that enforces coplanarity between the B and A/C rings) should be slightly twisted and this is reproduced by DFT calculations for apigenin at the B3LYP/6-31G(d) level ($\phi$ = 16.4 °) or B3LYP/6-31G** level (15 °) and by the RHF/STO-3G model ($\phi$ = 16.5 °).[112] By contrast, the B3LYP/6-311++G* basis set gives a C3-C2-C1'-C2' torsional angle of 0.0 ° for apigenin.[113] Amat *et al.* investigated the Al apigenin system using the B3LYP/6-31G** basis set.[108] The optimized geometry of $[Al(H_2O)_4(apigenin)]^{2+}$ (binding *via* the 4-5 site) shows a dihedral angle $\phi$ between the B and A/C rings of 2.6 ° as compared to 15 ° in uncoordinated apigenin. However, in the case of the 2:1 complex $[Al(H_2O)_2(apigenin)_2]^+$ the decrease in the dihedral angle on metal coordination is less pronounced ($\phi$ = 9.6 °). Geometry optimization of the 1:1 and 1:2 luteolin complexes, $[Al(H_2O)_4(lu)]^{2-}$ and $[Al(H_2O)_2(lu)_2]^-$, with Al coordination to the catechol moiety showed a non-planar ligand in both cases. Geometry optimization of the tetrahedral $Al(3HF)_2$ complex showed an increase of the O1-C2-C1'-C2' angle from 0.2 in free 3HF to 5.6 °. Again, semi-empirical calcualtions gave different results. Using the AM1 method Boudet *et al.* reported $\phi$ 27.88 and 15.15 ° for free 3HF and $Al(3HF)_2$, respectively, *i.e.* a decrease by ~12 °.[114] Similar discrepancies between the two methods were described for the Al complex of 3',4'-dihydroxyflavone with AM1 calculations giving a more staggered conformation ($\phi$ = 30.1 °) than DFT ($\phi$ = 20.3 °).[47]

Despite the significant efforts that have been devoted to modelling the geometric parameters of flavonoid complexes,[44,51,71,78,105-114] a better insight into their structural properties is clearly desirable, in particular in view of the limited X-ray data available. The question whether the ligand is planar or not is not only relevant to possible interactions with biomolecules (*e.g.* intercalation into DNA, section **7**), but will also contribute to our understanding of the effect of metal chelation on the antioxidant properties (section **6**). Planarity is associated with a maximum of conjugation which in turn influences the redox potential.

Besides the planarity/non-planarity of the flavonoid ligand, the geometry of the chelate ring formed with the metal ion is of interest with regard to flavonoid-biomolecule interactions. In the optimized geometry of the 4-5 bound Fe quercetin complex the six-membered chelate ring is co-planar with the quercetin ligand.[51] Likewise, when Fe or Al bind to the 3-4 site of deprotonated quercetin or 3HF, geometry optimisation shows co-planarity of the five-membered ring and the ligand.[114]

Lapouge *et al.* have studied the structures of $Zn^{II}$ complexes of 3-hydroxyflavone, 5-hydroxyflavone and 3',4'-dihydroxyflavone (1:1 metal/ligand stoichiometry) using different basis sets.[71] Independent of the basis set, the five-membered chelate ring in the 3',4'-dihydroxyflavone complex was always found to form a dihedral angle of ~20 ° with the B ring, while in the case of 3-hydroxyflavone and 5-

hydroxyflavone the chelate rings were co-planar with the benzopyrone part. The dihedral angle between the B and A/C rings, however, varied significantly with the basis set. For example, depending on the basis set, values between 1.7 and 16.7 ° were obtained for the 3-hydroxyflavone complex.

Several authors have used time-dependent DFT (TDDFT) calculations to identify binding sites and complex stoichiometries by comparing the computed optical absorption spectra with the experimental ones.[44,60,106] Lekka *et al.* reported that the experimental absorption spectrum at physiological pH is best reproduced by the calculated spectrum of the 1:2 Cu quercetin complex with four-coordinate Cu.[106] Furia *et al.* reported a combined potentiometric, spectroscopic and computational study on the Al quercetin system.[60] Their DFT calculations indicated $[Al(quer^{-})(OH)_2(H_2O)_2]$ with Al coordinating to the 4-5 site to be the most stable complex. However, taking the $pK_a$ values of the 3- and 5-OH groups and of Al-bound water as well as the experimental and theoretical UV/Vis spectra into account, the authors suggested the co-existence of $[Al(quer^{2-}\text{-}4\text{-}5)(OH)(H_2O)_3]$ and $[Al(quer^{2-}\text{-}3\text{-}4)(OH)(H_2O)_3]$ in solution ($quer^{2-}$ = quercetin deprotonated at O3 and O5, *3-4* and *4-5* denotes the binding site). DFT calculations gave an energy difference between the two isomers of only 0.8 kcal $mol^{-1}$ and confirmed that interconversion is kinetically possible.

### 3. Applications in chemical analysis

The ability of flavonoids to coordinate metal ions is sometimes applied in the development of preparative[115] and analytical[116] methods, concerning those two chemical species. For example, a promising strategy developed to distinguish isomers of a flavonoid by tandem mass spectrometry relies on the complexation of the flavonoid with a transition metal ion.[117-119] Some flavonoid compounds, especially flavonols, display fluorescence after excitation with visible or UV light due to their molecular structure and electron configuration. The (more or less) flat, rigid rings with a system of conjugated double bonds and delocalized π electrons make the molecules highly sensitive colorimetric and fluorimetric reagents in analytical chemistry. Chelation of certain metal ions often affects or quenches the fluorescence in a specific way that can be measured and applied for the quantitative determination of a flavonoid or a metal ion. The most widely used fluorescent flavonoid is "flavonol" *i.e.* 3-hydroxyflavone. Its fluorescence is highly enhanced by coordination of *e.g.* $Al^{3+}$ ions.[120]

A comprehensive and excellent review on flavonoids as analytical reagents has been published recently.[121] Therefore only some selected examples for spectrophotometric and spectrofluorimetric applications are presented here to give the reader an insight into the potential of flavonoid complexes in analytical chemistry.

A range of colorimetric and fluorimetric methods have been developed that use different flavonoids to measure metal ions in water samples (*e.g.* $Zr^{4+}$,[50,122,123] $Al^{3+}$,[123,124] $Cr^{3+}$,[125] $W^{VI}$,[125] $Fe^{3+}$,[124] and $V^{V}$ [124]), food (*e.g.* $Sn^{4+}$ [46,126-128]), pharmaceuticals and cosmetics (*e.g.* $Au^{III}$,[18,20] $Ru^{IV}$,[18] $Fe^{3+}$ [125]) and metallurgical products (*e.g.* $Ga^{3+}$,[129] $In^{3+}$,[129] $Cr^{3+}$,[130] $W^{VI}$,[130] $Rh^{3+}$,[131] and $Ir^{3+}$ [131]). Detection limits range from 0.1 to $10^3$ μg/L. Flavonoids, on the other hand, can be determined using metal ions as analytical reagents. Examples include $MoO_4^{2-}$, $Sb^{3+}$, $Bi^{3+}$ and $Al^{3+}$ to determine flavonoids in plant extracts,[116,132] $Al^{3+}$ and $Tb^{3+}$ for the fluorimetric determination of flavonoids in orange juices,[133] and $Cu^{2+}$ for the determination of quercetin in pharmaceuticals.[134] $F^{-}$ anions can replace the flavonoid ligand in ternary $Al^{3+}$ and $Zr^{4+}$ flavonoid complexes causing a change in fluorescence and this can be exploited for the quantification of $F^{-}$.[135,136] Pyrophosphate has been shown to quench the fluorescence of 3-hydroxyflavonolate-diphenyltin(IV)chloride with good selectivity and high sensitivity, suggesting potential applications for the determination of pyrophosphate in biological samples.[137] Cao *et al.* proposed to use the increase in fluorescence of the Al kaempferol complex in the presence of DNA and Ag nanoparticles for fluorescence staining in gel electrophoresis patterns.[138]

### 4. Photochemical and electrochemical applications

As mentioned above, flavonoids, especially flavonols, are photoactive compounds, *i.e.* they display luminescence.[139] So do their metal complexes.[140] From the structural and computational studies described in **2.2** and **2.4** it is clear that chelation of metal ions often enhances the luminescence of flavonols by affecting the system of delocalized π orbitals. These phenomena are the basis for the use of flavonoid complexes in photochemical applications. Especially 3-hydroxyflavone is an interesting photoactive compound. Itself, it is a fluorescent compound and easily undergoes photodegradation[141] or tautomerization, depending on the surrounding environment.[142] The emission spectrum of 3-hydroxyflavone depends on the polarity of the solvent, pH,[143] temperature[144] and the presence of water molecules.[145] When excited with UV light, 3-hydroxyflavone emits deep-violet light and a yellow-green light; the latter is assigned to its mono-solvated tautomeric form that is formed by an excited-state proton transfer (ESPT) process.[141] Complexation of a metal ion by 3-hydroxyflavone which usually involves deprotonation of the 3-hydroxyl group erases the ESPT effect. Even if metal coordination is not accompanied by deprotonation, complexed 3-hydroxyflavone may not exhibit the ESPT effect due to geometric hindrance. Theoretical calculations showed, for example, that $Mg^{2+}$ in acetonitrile preferentially binds to the carbonyl group of 3-hydroxyflavone leading to a rotation of the H atom of the hydroxyl group away from the carbonyl group.[146]

Coordination of $Al^{3+}$ to 3-hydroxyflavone leads to an increase in fluorescence and improved photostability of the complex.[141,147] This phenomenon may be applied to study the interaction of $Al^{3+}$ ions with soil organic matter (flavonol as a model of a soil organic compound)[41] and to produce flavonoid-based photoactive materials,



such as wavelength-shifting systems.[147] Several Be and other metal complexes of hydroxyflavones have been investigated with regard to potential applications in organic light-emitting diodes (OLED).[148] The V complex of 3-hydroxyflavone, $VO_2$(3HF), was tested as an interface in a Schottky diode.[149]

**5. Applications as pigments and dyes**

Flavonoids, as natural chromophores, and their metal complexes have been used as natural dyes and pigments since ancient times. The sources of flavonoids were plants, *e.g.*: *Reseda luteola* (weld containing luteolin and apigenin), *Maclura tinctoria* (old fustic containing morin and maclurin), *Genista tinctoria* (dyer's broom containing genistin and luteolin).[150] Adding certain metal salts (*e.g.* alum - aluminum potassium disulphate) to the extracts of the plants resulted in a change in color and improved pigment stability on account of the bathochromic shift in the absorption spectra of flavonoids following metal coordination (see **2.3**).[8] Weld lake, due to its intense yellow color, was used as a pigment for decorating medieval manuscripts and in easel paintings. Preparing the lake included precipitating the plant extract with alum and purifying and drying the precipitate.[108] Dyeing textiles demands a different lake preparation, but still flavonoid metal complexes play the main role in the process. When applied to fibers like wool, flavonoid plant extracts are prone to be washed out easily and the use of metal ions as mordants is crucial to make the dyestuffs more water-proof.[151] $Al^{3+}$, $Fe^{3+}$, $Cu^{2+}$ and other metal ions bind to carbonyl, sulfhydryl or amino groups of keratin (wool, silk), thus linking the dye molecules to the wool fiber. The choice of metal salt as mordant influences not only the color (Fig. 4) and its stability, but also the textile properties and degradation rate. The use of ferric mordant with flavonoid dyestuffs causes the greatest loss in strength and extensibility in wool fibers, dyed and irradiated with solar-like fluorescent light up to 2500 h. The effect is less pronounced when using cupric mordant, and Al mordant does not reduce the strength and leads only to a minor loss in extensibility of the dyed wool fibers.[152] Transition metal ions like $Fe^{2+}$ and $Cu^{2+}$ can catalyze the production of free radicals (see section **6**) which can cause oxidative damage of the keratin fibers. Certain metal ions increase the dye's photosensitivity and susceptibility to bleaching,[153] due to the increased reactivity of such complexes. Some mordant metal ions, *e.g.* $Fe^{3+}$, $Cu^{2+}$, $Sn^{IV}$ undergo UV light-induced redox reactions, which can enhance the phototendering of woolen fibers and subsequent damage. Thus, metal-mordanted flavonoid dyestuffs are susceptible to fading or photodegradation. Generally, flavonol-based dyes (*e.g.* quercetin, kaempferol) are more prone to light-induced fading than flavone-based dyes (luteolin and apigenin), whose colors darken with time.[151] The greater photodegradation of flavonol-based dyes can be confirmed by the presence of the degradation products, mainly hydrobenzoic acids, that arise from the oxidation of the C2-C3 bond and C2-C3 and C3-C4 bond breaking.[150] UV irradiation may activate the process, and metal ions can probably catalyse it. The significant resistance of flavones to photodegradation indicates the role of the 3-hydroxyl group in this process. The C2-C3 bond has a greater electron density in flavonols than in flavones, what makes its reaction with singlet oxygen one to two orders of magnitude faster.[150] Quercetin and kaempferol alone, in concentrated solutions, can effectively dissipate the excitation energy without degradation due to an intramolecular dual proton transfer involving the 5-hydroxy-4-keto and 3-hydroxy-4-keto groups. When the flavonols are complexed with $Al^{3+}$, this dual proton transfer is impossible due to the presence of coordination bonds[150] so that degradation takes place. However, there are also findings showing that $Al^{3+}$ coordination blocks - rather than increases - the photodegradation of 3-hydroxyflavone in 50% methanolic solution, when irradiated with a xenon lamp for up to 200 min. at a wavelength adjusted to the maximal absorption peak of the complex (~400 nm).[141] This contradiction may probably be explained by the wavelength of the irradiating light, with shorter wavelength light (UV range), but not longer (400 nm range) causing the degradation.[147,150,151] This has implications for the storage of precious pieces of art to prevent their damage.

Understanding the nature, composition and degradation of metal-complex based pigments is an important topic in arts conservation and this prompted some of the theoretical work aimed at finding relationships between structural and optical properties of flavonoid complexes described in section **2.4**. Luteolin and apigenin are the main chromophores in weld and there is combined evidence from experimental spectroscopic data and DFT calculations that for both flavonoids the preferred chelation site is the 4-keto-5-hydroxy group.


**Fig.4** Examples of natural dyer's plants and dyed fibers. a) *Serratula tinctoria*, (sawwort) plant containing luteolin and 3-methylquercetin[151]; b) Samples of wool dyed with *S. tinctoria* extract with different mordants: 1-3 alum, 4-5 alum + subsequently ferrous sulphate, 6-7 alum + subsequently copper sulphate (courtesy of Jenny Dean, Jenny Dean's Wild Colour); c) Wood chips from *Maclura tinctoria* (old fustic) containing morin and maclurin[150]; d) Samples of wool dyed with *M. tinctoria* extract with different mordants: 1 – alum, 2 – ferrous sulphate, 3 - stannous chloride (courtesy of Cynthia Slatcoff, Brush Creek Wool Works).

Apigenin forms a 2:1 complex with $Al^{3+}$, when the metal ion concentration is low ($8 \cdot 10^{-5}$ mol/L) and a 1:1 complex at higher $Al^{3+}$ concentration ($10^{-3}$ mol/L).[108,154] Luteolin probably forms three different complexes with $Al^{3+}$ at different concentrations of the metal ion: a 2:1 complex at low $Al^{3+}$ concentration ($8 \cdot 10^{-6}$ mol/L), a 1:1 complex at higher concentration ($5 \cdot 10^{-5}$ mol/L), and a 1:2 complex which is in equilibrium with the 1:1 complex at ~$10^{-3}$ mol/L concentration. In the latter the second $Al^{3+}$ binds to the 3'-4'-dihydroxyl moiety.[108] On the basis of the computed spectra of the $Al^{3+}$ luteolin complexes and DFT calculations Amat *et al*. proposed that the co-precipitate of luteolin and alumina contains the 1:1 complex with $Al^{3+}$ binding to the 4-5 site and that this complex is the main contributor to the color of weld lake.[108]

Metal complexes of flavonoid dyes are also important in the chemical analysis of pieces of art. On the one hand the use of poorly water soluble $Al^{3+}$ complexes can make such an analysis difficult.[155] On the other hand, coordination of $Al^{3+}$ or $ZrO^{2+}$ ions to flavonoid dyes in the laboratory lowers the detection threshold of many historical natural dyes.[156] This is particularly important, because samples from such pieces of art must be as small as possible.

**6. Redox properties of flavonoids and their metal complexes**

There is no doubt that interactions with endogenous redox active metal ions play a major role in the biological behavior and medicinal applications of flavonoids. Several groups have studied the ability of flavonoids to reduce $Fe^{3+}$ and $Cu^{2+}$.[33,157,158] Pekal and co-workers have shown that binding of $Cu^{2+}$ to the 3-4 site of quercetin leads to the rapid reduction of $Cu^{2+}$ to $Cu^{+}$ accompanied by the oxidation of the flavonoid to benzoquinone-type products.[158] Addition of EDTA to remove $Cu^{+}$ did not restore the original quercetin spectrum indicating that the oxidation reaction is irreversible. Ryan and Hynes reported that the initial formation of a 2:1 $Fe^{3+}$ quercetin complex is followed by an electron transfer step leading to the decomposition of the complex.[157] On the other hand, binding of $Fe^{2+}$ to polyphenol ligands was shown to promote its oxidation to $Fe^{3+}$ which is consistent with the greater stability of $Fe^{3+}$ complexes compared to $Fe^{2+}$ complexes (see **2.1**).[159,160] Several studies have established that flavonoids have a higher reducing capacity for $Cu^{2+}$ than for $Fe^{3+}$, in line with the standard redox potentials of $Cu^{II}/Cu^{I}$ (+0.15 V) and $Fe^{III}/Fe^{II}$ (+0.77 V).[36,42,161] It is noteworthy that only the flavones myricetin and quercetin seem to have a high reducing capacity for $Fe^{3+}$.[31,37,59] The importance of the C2=C3 double bond conjugated with the 4-oxo group and the presence of both the 3-hydroxyl group and the 3'-4' dihydroxyl site for $Fe^{3+}$ reducing activity has been pointed out.[3]6 Likewise, the 3-hydroxy group in quercetin and kaempferol appears to enhance the oxidation rate of these flavonoids in the presence of $Cu^{2+}$, as luteolin and rutin that lack the 3-hydroxy group do not undergo oxidation in the presence of $Cu^{2+}$ as easily.[41]

**6.1. Antioxidant activity**

A large portion of oxidative stress in all living organisms is related to the chemistry of metal ions. In particular Fe and Cu are involved in the generation of reactive hydroxyl radicals through the Fenton reaction:

$$M^{n+} + H_2O_2 \rightarrow M^{(n+1)+} + OH\cdot + OH^-$$

$$M^{(n+1)+} + H_2O_2 \rightarrow M^{n+} + HOO\cdot + H^+$$

Coordination of the metal ions to a ligand can affect the kinetics as well as the thermodynamics of the reaction. Thus, flavonoids are considered to be antioxidants not only due to direct radical scavenging, but also due to their chelating properties, in particular for Fe ions.[162,163] Melidou *et al*. studied the ability of a range of flavonoids to protect cellular DNA from $H_2O_2$-induced single-strand breaks and found no correlation between scavenging ability and prevention of DNA damage.[164] Rather, the ability to protect DNA seemed to correlate with the ability to chelate intracellular redox active Fe ions: Flavonoids with an ortho-dihydroxyl moiety in either ring A or ring B displayed strong DNA-protecting capacity and the presence of a 3-hydroxyl group in combination with a 4-carbonyl group enhanced the DNA protective ability. The importance of the catechol group is also evident from work by Cheng and Breen who demonstrated that luteolin and quercetin suppress the generation of hydroxyl radicals by the $Fe^{2+}$-ATP complex more efficiently than baicalein and naringenin.[165] However, baicalein has a trihydroxy group on the A ring and Cheng and Breen suggested that B ring substituents had a more pronounced effect on the antioxidant properties. This hypothesis was supported by two other studies, although conflicting observations have also been described.[166,167] Perron *et al*. carried out a comprehensive study on the prevention of DNA damage by a series of polyphenol compounds that confirmed the correlation between Fe-binding ability and antioxidant properties, thus supporting the idea that chelation of intracellular Fe is the cause for the protection of DNA against $H_2O_2$-mediated damage.[168] While there is a bulk of evidence supporting the hypothesis that metal chelation is the origin of antioxidant activity, there are also studies showing that chelation of Fe is independent from the scavenging activity of flavonoids[114] or may even contribute to pro-oxidant activity (see **6.2**). The main problem in establishing structure-antioxidant activity relationships is the fact that the antioxidant activity properties depend on the radical-scavenging rate constants, the stability of the resulting flavonoid radical and the redox potential. These data are typically determined under different conditions and for environments distinct from biological systems.[164,169]

A vast amount of research shows that flavonoids act as protective agents in cell cultures, plants and animals in case of metal ion poisoning or oxidative stress caused by metal ions.[170] This is relevant to essential microelement overload (Fe, Zn or Cu) as well as to xenobiotic metal exposure, *e.g*. Cd, Pb, Ni, Hg or Pt compounds (see **6.3**). Undoubtedly, the protective activity must (at least partially) be based on the ability to form complexes with those ions. The consequences of complexation are twofold: firstly, chelated ions have limited possibility to



interact with vital bio-macromolecules and are possibly more easily excreted and secondly, chelation can prevent the metal-catalysed Fenton reaction leading to free radicals. Bearing the latter in mind, one may raise the question, whether the complexes themselves are antioxidants or not. The answer is yes, they often are. There is a significant body of experimental data that reveal that most flavonoid complexes are even more effective radical scavengers than free flavonoids. Examples include the $Fe^{2+}$, $Fe^{3+}$, $Cu^{2+}$ and $Zn^{2+}$ complexes of rutin, epicatechin and dihydroquercetin (Table 3). Complexes with a 1:1 stoichiometry seem to be better antioxidants and cytoprotectants than 2:1 (metal:flavonoid) complexes.[171] The pronounced antioxidant effect of some flavonoid complexes is observed in cell cultures[172] as well as in cell free models or non-aqueous milieu.[27,173,174] Metal coordination changes the redox potential of a ligand and thus affects the antioxidant activity of the flavonoid. Pekal *et al*. reported an increase of the radical scavenging activity of quercetin upon Cu binding which is in line with the lower redox potential measured for Cu quercetin compared to free quercetin.[158] The more facile oxidation of quercetin in the coordination sphere of Cu was attributed to a destabilization of the flavonoid structure on metal binding. The mechanism of quercetin oxidation, however, is not well understood and may vary with the oxidizing agent and reaction medium.[171,175]

However, the observation that metal complexes have enhanced antioxidant properties compared to the parent flavonoid is not a rule (Table 4). A recent study by Yang *et al*., for example, revealed that the $Fe^{3+}$ complex of luteolin displays a lower scavenging activity in the DPPH assay than luteolin on its own in agreement with its higher reduction potential determined by cyclovoltammetry.[77] A possible cause of the often superior chemo-protective properties of flavonoid complexes is the increase in electron-donating ability of the flavonoid molecule upon complexation[7] and/or the presence of additional effective superoxide dismutating metal centers.[176-178] Flavonoids can exert antioxidant activity through two mechanisms, the hydrogen transfer (1) and the single electron transfer (2) mechanisms. Both generate less reactive flavonoid aroxyl radicals:

$$ArOH + R^{\cdot} \rightarrow ArO^{\cdot} + RH \qquad (1)$$

$$ArOH + R^{\cdot} \rightarrow ArOH^{\cdot +} + R^{-} \rightarrow ArO^{\cdot} + RH \qquad (2)$$

Chen *et al*. carried out DFT calculations to rationalize the higher radical scavenging activity of the $Cr^{III}$ quercetin complex compared to quercetin.[7] The gas-phase bond dissociation enthalpy of the 4'-OH group calculated at B3LYP/6-311++G(2d,2p)/B3LYP/LANL2DZ level is 0.61 kcal $mol^{-1}$ lower for the quercetin complex (assuming chelation *via* the 4-5 site) than for quercetin. The ionization potential of quercetin decreases from 165.98 kcal $mol^{-1}$ to 149.57 kcal $mol^{-1}$ upon complexation and the HOMO of the complex (-0.20657 Hatree) has a higher energy than that of quercetin (-0.21118 Hatree). Thus, $Cr^{III}$ coordination seems to lead to a stronger H atom transfer and electron-donating ability with the latter one being more important. Furthermore, spin density analysis showed that the spin density of the $Cr^{III}$ quercetin complex radical is distributed over the whole molecule, while it concentrates on the A and B rings in the case of the quercetin radical, indicating that the quercetin complex radical is more stable.

Furthermore, flavonoid complexes with Fe, Cu and other metals such as Pd and Pt were shown to have superior superoxide-dismutase activity.[64,179,180] Furuno *et al*.,[181] de Souza *et al*.,[34] Afanas'ev *et al*.[182] and Kostyuk *et al*.[178] reported the reaction of $Fe^{2+}$ flavonoid complexes with $O_2^-$ to $H_2O_2$ and a semiquinone-radical. Bodini and co-workers described the disproportionation of protonated $O_2^-$ to $O_2$ and $H_2O_2$ in the presence of the $Fe^{2+}$ quercetin complex in an aprotic solvent. No $Fe^{2+}$ could be detected by cyclovoltammery measurements after the reaction as $H_2O_2$ oxidizes the $Fe^{2+}$/quercetin to the $Fe^{3+}$ complex (an oxidation potential lower than that of free quercetin was determined). $H_2O_2$ is consumed and the production of hydroxyl radicals by the $Fe^{II}$-mediated Fenton reaction is precluded.[43] $Cu^{2+}$ complexes are significantly more effective in scavenging $O_2^-$ than $Fe^{2+}$ or $Fe^{3+}$ complexes.[178] The $Cu^{2+}$ complexes of rutin and taxifolin, for example, were found to be eight and six times more effective cytoprotectants against superoxide-induced oxidative injury of phagocytic cells than rutin or taxifolin alone.[183]

**Table 3** Complexes with higher antioxidant activity than the parent flavonoid.

| Metal ion | Flavonoid | Antioxidant assay [a] | Ref |
|---|---|---|---|
| $La^{3+}$, $Nd^{3+}$, $Eu^{3+}$, $Gd^{3+}$, $Tb^{3+}$, $Dy^{3+}$, $Tm^{3+}$, $Y^{3+}$ | quercetin | NBT | 184 |
| $Mn^{2+}$, $Co^{2+}$, $Ni^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Pb^{2+}$ | quercetin | NBT | 185 |
| $Fe^{2+}$, $Fe^{3+}$, $Cu^{2+}$, $Zn^{2+}$ | rutin, dihydroquercetin | NBT | 183 |
| $Fe^{2+}$, $Fe^{3+}$, $Cu^{2+}$ | taxifolin, catechin, quercetin, rutin, fisetin, luteolin, kaempferol | superoxide scavengers (%) in xanthine/ hypoxanthine system | 26 |
| $Fe^{2+}$ , $Fe^{3+}$, $Cu^{2+}$ | rutin, taxifolin, epicatechin, luteolin | superoxide-mediated reduction of nitroblue tetrazolium by photochemically reduced riboflavin | 178 |
| $Cr^{3+}$ | quercetin | DPPH | 7 |
| $Cu^{2+}$ | quercetin | DPPH | 173 |
| $Co^{2+}$ | quercetin | DPPH | 174 |
| $Cu^{2+}$ | morin | DPPH | 27 |
| $Zr^{4+}$, $MoO_4^{2-}$ | morin | DPPH | 186 |
| $Mg^{2+}$, $Ca^{2+}$ | morin | DPPH | 187 |
| $Ce^{4+}$ | naringenin, chrysin, daidzein | DPPH | 28 |
| $Fe^{2+}$, $Fe^{3+}$, $Cu^{2+}$ | rutin | inhibition of cyt. C reduction by xanthine | 172 |

| | | | |
|---|---|---|---|
| $Cu^{2+}$ | naringenin-2-hydroxy benzoyl hydrazone | NBT reduction, safranin bleaching | 188 |
| $Cu^{2+}$, $Ni^{2+}$, $Zn^{2+}$ | 1,2-di(4'-imino-naringenin)ethane | NBT reduction, safranin bleaching | 189 |
| $Cu^{2+}$ | naringin | DPPH | 190 |
| $VO^{2+}$ | hesperidin | SOD-like activity assay | 176 |
| $Fe^{3+}$ | pinostrobin oxime | DPPH, ABTS | 191 |
| $VO^{2+}$ | chrysin | ABTS, deoxyribose degradation by hydroxyl radical, measured with the thiobarbituric acid method | 192 |

[a] DPPH = (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging assay; FRAP = ferric reducing antioxidant power; ABTS = 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt radical redeuction assay; NBT = nitro blue tetrazolium reduction by superoxide assay; safranin bleaching = hydroxyl radical scavenging assay; SOD – superoxide dismutase

**Table 4** Complexes with similar or lower antioxidant activity than the parent flavonoid

| **Metal ion** | **Flavonoid** | **Antioxidant assay [a]** | **Ref.** |
|---|---|---|---|
| $Fe^{2+}$ | rutin, fisetin | superoxide scavengers (%) in xanthine/ hypoxanthine system | 26 |
| $Sn^{2+}$ | quercetin | DPPH, ABTS and FRAP | 193 |
| $Fe^{3+}$ | quercetin, baicalein | DPPH | 74 |
| $Ni^{2+}$, $Zn^{2+}$ | naringenin-2-hydroxy benzoyl hydrazone | superoxide radical scavenging assay (NBT reduction) hydroxyl radical scavenging assay (safranin bleaching) | 189 |
| $Eu^{3+}$, $Y^{3+}$ | naringenin-2-hydroxy benzoyl hydrazone | hydroxyl radical scavenging assay (safranin bleaching) | 184 |
| $Fe^{3+}$ | quercetin, morin | DPPH, EPR spectroscopy (DPPH and tempone radical) | 33 |
| $VO^{2+}$ | hesperidin | DPPH, ABTS | 176 |
| $VO^{2+}$ | chrysin | DPPH, NBT | 192 |
| $Cu^{2+}$ | pinostrobin oxime | DPPH, ABTS | 191 |
| $Fe^{3+}$ | luteolin | DPPH | 77 |
| $Cd^{2+}$ | quercetin | DPPH, ABTS | 62 |

[a] DPPH = (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging assay; FRAP = ferric reducing antioxidant power; ABTS =2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt radical reduction assay; EPR - electron paramagnetic resonance; NBT = nitro blue tetrazolium reduction by superoxide assay; safranin bleaching = hydroxyl radical scavenging assay

### 6.2. Pro-oxidant activity

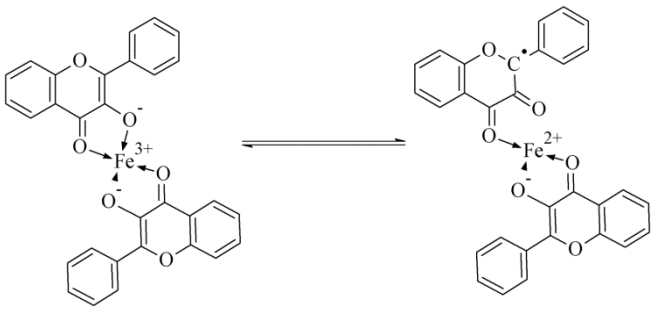


**Fig. 5** Possible formation of $Fe^{2+}$ and a radical *via* the redox reaction between flavonolate and $Fe^{3+}$. [86]

Flavonoids alone,[194] in the presence of metal ions[195] or flavonoid metal complexes may also have pro-oxidant properties.[32] The pro-oxidant activity has been attributed to the ability of flavonoids such as *e.g.* quercetin, amentoflavone or myricetin to reduce $Cu^{2+}$ and $Fe^{3+}$ (Fig. 5), thus generating $Fe^{2+}$ and $Cu^{+}$ as catalysts for the production of hydroxyl radicals through the Fenton reaction.[88,196-198] Flavonols containing a catechol moiety in ring B can undergo autoxidation in the presence of redox active transition metal ions and dioxygen and generate ROS, semiquinone-type radicals and quinone metabolites (Fig. 6). As metal ions localize near the negatively charged sugar phosphate backbone of DNA *via* electrostatic attraction or bind covalently to DNA nucleobases, these radicals are formed in close proximity of DNA. There is evidence that metal complexes of flavonoids cause oxidative damage of DNA and induce apoptosis.[88,199,200] Especially the $Cu^{2+}$ complex of quercetin promotes the formation of 8-oxoguanine, resulting in extensive oxidative DNA damage.[197201,202]

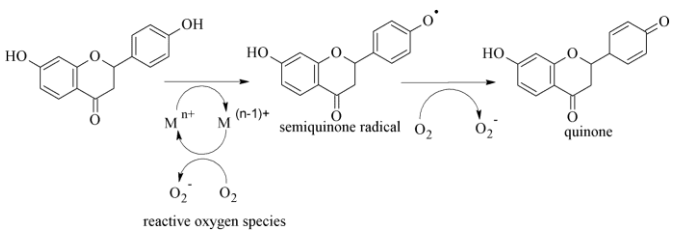



**Fig. 6** Reaction of flavonoids in the presence of redox active metal ions leading to reactive oxygen species.[203]

The pro-oxidant *vs.* antioxidant activity of flavonoids appears to depend on the concentration,[80,198,203] metal ions to flavonoid ratio,[156] the surrounding milieu (solvent, pH) and the presence of other metal ions, dioxygen, antioxidants, and chelators.[28,198] For example, myricetin can enhance the repair of Fe-induced DNA damage in rat hepatocytes cultured *in vitro*,[204] while it can act as a pro-oxidant and cause DNA damage and lipid peroxidation in isolated rat liver nuclei. The latter effect is enhanced in the presence of $Fe^{3+}$ or $Cu^{2+}$.[200] Flavonoids differ in their pro-oxidant properties.[199] Under



the same conditions kaempferol and luteolin, for example, produce significantly less oxidative DNA damage in the presence of $Cu^{2+}$ ions than quercetin does.[205] Also, no pro-oxidant activity was observed for catechin or taxifolin in the presence of $Cu^{2+}$ ions.[206] In general, pro-oxidant activity seems to prevail mainly at low flavonoid concentrations. Thus, the role of flavonoids as chemopreventive agents due to their antioxidant properties is not unequivocally proven and depends on many factors. This should be considered before advising the intake of flavonoids as antioxidant diet supplements to reduce the risk of cancer. Pro-oxidant and antioxidant properties of flavonoids and their metal complexes probably occur in parallel and highly depend on other factors, such as competing chelators that can prevent binding of $Fe^{3+}$ or $Cu^{2+}$ to the flavonoid, the surrounding environment and the target biomolecule. For example, the $Fe^{II}$-promoted generation of OH· radicals from $H_2O_2$ is stimulated by flavonoids in the presence of EDTA, but not in the presence of ATP or citrate.[207] The latter is in line with UV/Vis spectroscopic studies that showed that quercetin can effectively compete with ATP and citrate for $Fe^{2+}$ under physiologically relevant conditions.[64] While both pro-oxidant and antioxidant effects have been observed for the metal-catalyzed oxidation of proteins and DNA, antioxidant activity seems to dominate in the case of Fe- or Cu-catalyzed peroxidation of lipids.[22,163,205,208-212] Tsai *et al*. even associate pro-oxidant activity with antioxidant activity of flavonoids from propolis. They suggest that the antioxidant protection of treated cells may occur by means of exposing of the cells to moderate oxidative stress and an adaptation process, after which the exposed cells become more resistant to further oxidative stress.[213] The antiproliferative activity of flavonoids may be partly due to their pro-oxidant properties (see section **7**).

### 6.3 In vitro and in vivo chemo-protective activity

The ability to chelate metal ions along with their antioxidant properties make flavonoids agents that may attenuate the effects of metal ion toxicity. Essential metal ions (*e.g*. $Cu^{2+}$, $Fe^{3+}$) in excess and xenobiotic metals (*e.g*. $Pb^{2+}$, $Hg^{2+}$, $Cd^{2+}$, $Al^{3+}$) are toxic for all living organisms. The mechanism of their toxicity is based on direct binding to vital biomolecules, affecting transcriptional and signaling cellular pathways besides causing oxidative stress.[170]

There are many studies investigating the preventive effects of flavonoids in metal ion exposure, both *in vivo* and *in vitro*. Quercetin as an effective chelator of $Fe^{2+}$ was shown to modulate the transport of the metal species. The Fe quercetin complex is transported *via* glucose transporters (GLUT1). However, the effect is inhibited at quercetin concentrations above 10 μM or by other GLUT inhibitors. This may suggest a potential way of chemotherapy of Fe-overload diseases.[214] Primary rat hepatocytes *in vitro*, when treated with ferric nitriloacetate - Fe(NTA) - undergo DNA damage and lipid peroxidation. Addition of myricetin was shown to prevent lipid peroxidation and accumulation of oxidation products in DNA. What is more interesting are findings that myricetin stimulates excision repair enzymes for DNA and induces the expression of polymerase β in a dose-dependent manner.[204] Thus, myricetin not only protects cells against Fe overload, but also enhances the activity of cellular repair enzymes.

Supplementing the diet of Fe-overfed mice with 1% baicalin or quercetin attenuated the effects of Fe toxicity in the liver. The addition of the flavonoids inhibited lipid and protein oxidation in the liver and decreased the Fe content in the liver and hepatic collagen. It also increased the serum non-heme Fe level, but not the serum ferritin level. Animals fed with flavonoid-supplemented food excreted more Fe in feces in comparison to those given only Fe-enriched food.[215] Baicalin as well as quercetin also protected the liver of rats overfed with Fe, improved hepatic biochemical markers and inhibited tyrosine nitration in the liver[24] and in other tissues.[216] Naringin, quercetin and myricetin stimulated the activity of cellular protective selenoenzymes (thioredoxin reductase and glutathione peroxidase) in the liver of rats that had been overfed with $Fe^{3+}$ in the diet.[217] Pretreatment with antioxidants, *e.g.* quercetin, before giving ferric nitriloacetate to rats suppressed lipid peroxidation, but not other markers of Fe nephrotoxicity (glutathione level, catalase activity, glutathione peroxidase) and Fe concentration in rat kidneys.[218]

Flavonoids can affect the cellular expression of metallothionein (MT, antioxidant peptide rich in sulfhydryl groups) in human intestinal Caco2 cells. For example, genistein and biochaninA increase the expression of MT, while quercetin decreases it.[219] However, quercetin as well as genistein increase the $Cu^{2+}$-induced MT production (though genistein does not react with $Cu^{2+}$ ions chemically). On the other hand, quercetin reduces the Zn-stimulated expression of MT and has no effect on the Cd-stimulated expression of MT.[220] The data above indicate that the mechanism of interactions between flavonoids, metal ions and self-protective cellular mechanisms is complex and is beyond the simple chelation of free metal ions.

Quercetin moderately decreases the oxidative damage of DNA in human gastric mucosa cells exposed to micromolar concentration of $NiCl_2$. However, it does not affect the rate of repair of DNA, when added after exposure of $Ni^{2+}$.[221] This is quite different from the observations by Abalea *et al*.,[204] who found that myricetin enhanced DNA repair after Fe-induced damage, but similar to that of Barcelos *et al*., who found that quercetin protects HepG2 cells against Hg-induced oxidative DNA damage only when added before or simultaneously with Hg compounds, but not when added after Hg exposure.[23] $Co^{2+}$ exposure of cultured cells may be considered as *in vitro* cellular model of hypoxia. The flavonoid glycoside scutellarin can protect rat pheochromocytoma cells PC12 against apoptosis induced by $CoCl_2$ treatment. The flavonoid affects radical production in the cells, as well as the biochemical apoptosis markers caspase 3, Bcl-$X_L$ expression and p38 MAPK phosphorylation.[222] Naringin partly protects human lymphocytes against Cd-induced damage. It decreases total chromosomal aberrations caused by Cd exposure, but does not affect sister chromatid exchange.[223]

Quercetin and catechin were shown to attenuate some toxic effects of Pb and Cd salts administered to rats. The flavonoids increased the pathologically lowered Mg levels in tissues and increased the pool of

free branched chain amino acids, also affected by the toxic metal salts.[224] $Cd^{2+}$ ions act as nephrotoxins and hepatotoxins. Quercetin can mitigate the nephrotoxicity of $Cd^{2+}$ in rats, while not affecting its renal content. This suggests mainly oxidative damage caused in kidneys by $Cd^{2+}$ and antioxidative protection by quercetin.[225-227] However, in the case of the hepatic toxicity of Cd, quercetin improved the oxidative status of the rat tissues, but did not prevent Cd-caused liver damage. This indicates that Cd does act as hepatic toxin not only by means of oxidative damage, but also in other ways.[228] Naringenin can also attenuate the nephrotoxicity of $Cd^{2+}$ exposure.[229] Similarly, naringenin can attenuate nephrotoxicity of cisplatin in rats, but does not affect its content in kidneys.[225] This is important, because the unaffected bioavailability of the antitumor drug cisplatin is crucial for the therapeutic action of the drug.[230] Like hesperetin[231] and isoquercitrin[232] naringenin seems to ameliorate the damaging effects of Cd on liver function in rats and to restore the antioxidant defense mechanisms in the liver.[233] Additionally, quercetin can attenuate Cd-induced damage of male murine germ cells,[234] rat testes[235] and rat heart tissue, along with improved dyslipidemia markers.[236]

$Ni^{2+}$, besides $Cd^{2+}$, is also a toxic metal ion that causes inflammation and oxidative stress. Ni-induced kidney toxicity, oxidative stress in rats as well as Ni-induced liver damage can be alleviated by administration of naringin.[237,238] With regard to other toxic metal ions, quercetin relieves nerve functional disturbances (impaired synaptic plasticity) caused by $Pb^{2+}$ in rat brains[239] and protects against methylmercury-induced damage in rats. It improves the glutathione and glutathione peroxidase status and decreases DNA damage in liver and leukocytes of methylmercury-exposed rats.[233]

Flavonoid accumulation in plants may be attributed to a protection mechanism against metal ion toxicity. Flavonoid accumulation in *Populus sp*. (poplar) was observed as a response to Cd stress.[238] Elevated accumulation of the flavonoid taxifolin and other phenolic compounds is also observed in the Al-tolerant maize variety Cateto when compared with Al-sensitive maize. This may be, besides increased Al exclusion, the mechanism of resistance of Cateto maize to high Al content in soil.[241]

Quercetin, as well as its derivatives, may act as reductive detoxifiers of $Cr^{VI}$ species in soil. This method is probably even more effective than bioremediation.[242] Flavonoid complexes of *e.g*. $Cu^{2+}$, $Ca^{2+}$, $Mg^{2+}$, $Al^{3+}$ can preserve wood and wooden objects against oxidation, putrefaction and microorganisms. Such complexes are often hardly soluble in water and have antioxidant and antimicrobial properties. Domratcheva suggests that for these reasons flavonoids may be used as preservatives of wooden pieces of art and buildings.[243]

## 7. Applications in medicine

This section and the following one give a brief summary of the pharmaceutical activities of flavonoid complexes and their reaction behavior towards biomolecules. For a more detailed overview, the interested reader is referred to a comprehensive recent review focusing on the significance of flavonoid complexes as potential therapeutic agents.[244]

### 7.1. Cytostatic activity

Flavonoid metal complexes often display antiproliferative activity against cancerous cell lines.[55,245] Rare earth metal complexes of quercetin, for example, were shown to affect DNA transcription and to inhibit tumor cell growth by binding to DNA.[55,246] Often the activity of the complex is superior to the activity of the flavonoid alone.[55] Several compounds are highly cytotoxic at concentrations similar to those of the potent metallodrug cisplatin.[5,19,32,247] A number of flavonoid complexes display promising selectivity towards cancerous cells over normal cells. The vanadyl complexes of quercetin or chrysin, for example, exhibit selectivity towards tumoral osteogenic cell lines (as does free quercetin).[192,248] It was reported that the $VO^{2+}$ quercetin complex can also promote osteoblast differentiation which is a crucial stage in bone physiology.[248] The $Pt^{II}$ complex of 3-aminoflavone is cytotoxic and also more selective than cisplatin towards cancer cells *vs*. normal lymphocytes.[249,250] $Ru^{II}$ complexes with flavanone-derived ligands are toxic toward the cisplatin-sensitive and cisplatin-resistant bladder cancer cell lines EJ and EJcisR.[19] However, they are less toxic to normal human lymphocytes, and are more selective than cisplatin toward cancer cells *in vitro*. Selectivity between cancer and healthy cells is a crucial feature of potential antitumor agents and the antiproliferative properties of flavonoid-metal complexes make the compounds good candidates for becoming cytostatic antineoplastic drugs.

One of the causes of the cytotoxicity of flavonoid complexes is their ability to cause oxidative damage to DNA by generating ROS (see above). This feature is strongly associated with the induction of strand breaks which directly impairs cell proliferation and may induce apoptosis.[32] Especially the $Cu^{II}$ complex of quercetin was shown to oxidatively damage DNA.[177,201,202] Several authors have described $Fe^{III}$-flavonoid and $Fe^{III}$-polyphenol complex-mediated DNA strand scissions *in vitro*.[86,208,251,252] However, it should be noted that despite the rich redox chemistry of V, the cytotoxic $VO^{2+}$ complex of chrysin described above does not generate ROS, at least not those that can be detected using the dihydrorhodamine oxidation assay.[192] Some complexes of quercetin with divalent metal ions such as $Mn^{2+}$ or $Zn^{2+}$ can cleave DNA *via* a hydrolytic mechanism, producing single and double strand breaks.[253,254] Mixed-ligand complexes with flavonoids and planar diimine co-ligands such as phenanthroline and bipyridine have also been studied as DNA cleavage and antitumor agents.[255]

Many, if not most, flavonoid complexes bind to DNA more strongly than free flavonoids. They usually intercalate into DNA strands, whereas flavonoids alone bind to DNA in other ways or intercalate into DNA to a lesser extent. In contrast to quercetin that interacts with DNA electrostatically, the La complex of quercetin intercalates into DNA.[256] The La chrysin[257] and Eu quercetin[258] complexes behave in a similar way. The Zn,[259] Cu,[260] and Bi[261] morin complexes bind to DNA mainly by intercalation, whereas free morin interacts with DNA *via* a non-intercalating mode.[262] The higher intercalative binding affinity of the complexes can be attributed to the greater planarity of the coordinated flavonoid (see **2.2** and **2.4**) and additional electrostatic interactions between the metal cation and



the negatively charged phosphate backbone. It is worthy of note that due to its high intercalative binding affinity, the use of the $Cu^{II}$ complex of luteolin in electrochemical DNA biosensors has been proposed.[263] Other examples for DNA intercalators are the Cu complexes of quercetin[264] and naringin[265] and the Ni quercetin complex.[266] The intercalated complexes may react with nucleobases and thus cause oxidative damage or hydrolytic cleavage.[267] The cytotoxic Zn and Ni quercetin complexes preferably intercalate into GC-rich regions of the DNA double helix. At the same time they cause a decrease in the level of the anti-apoptotic proteins survivin and Bcl. It was proposed that the complexes may bind to the GC-rich survivin core promoter, down-regulating the expression of the protein.[268]

Some flavonoids, *e.g*. luteolin, may act as topoisomerase inhibitors or poisons (see section **8.3**).[269] Topoisomerase poisons or inhibitors are cytotoxic, because they impair DNA relaxation and therefore cause cellular death. η-Arene $Ru^{II}$ complexes can inhibit or poison topoisomerase [270] and Kurzwernhart *et al*. reported a series of $Ru^{II}$ arene flavonoid complexes that display cytotoxic properties.[271,272] The compounds bind covalently to DNA, and additionally have the ability to interact with human topoisomerase II–α.[179, 180] Tabassum *et al*. designed heterobimetallic quercetin $Cu^{II}$-$Sn^{IV}_2$ and $Zn^{II}$-$Sn^{IV}_2$ complexes that bind to DNA *via* electrostatic interactions between $Sn^{IV}$ and phosphate and covalent binding of the $M^{II}$ metal to a nucleobase nitrogen.[273] The $Cu^{II}$-$Sn^{IV}_2$ complex is a potent topoisomerase inhibitor. In addition it exhibits superoxide dismutase and nuclease activity. Such complexes are good candidates to be multi-targeted anticancer agents, with a broader spectrum of activity than classical one-targeted agents.

In summary, the cytotoxic and pro-apoptotic activity of flavonoid complexes is based on various mechanism, including ROS generation, DNA intercalation and damage, interaction with topoisomerases and affecting apoptosis-related proteins.

### 7.2. Antimicrobial and antiviral activity

Many metal compounds are known for their antibacterial activity[274,275] as are some flavonoids.[276] Therefore metal complexes of flavonoids often display antibacterial properties, depending on the metal, ligand and bacteria strain. The antibacterial activity of quercetin complexes depends strongly on the metal ion used. The most active complex against the four tested bacterial strains *S. aureus, B. cereus, P. aeruginosa, E. coli* and *K. pneumoniae* is the $Hg^{2+}$ quercetin complex.[29] Complexes containing $Mn^{2+}$, $Co^{2+}$ and $Cd^{2+}$ have lower antibacterial activity. This is not surprising, because it is widely known that Hg and its compounds have strong bactericidal properties.[277] Complexes of $Mg^{2+}$ and $Ca^{2+}$ with morin have remarkable antibacterial properties against *Micrococcus flavus* and *Staphylococcus aureus*.[187] Complexes of $Cu^{2+}$, $Ni^{2+}$, $Co^{2+}$, $Zn^{2+}$, $Fe^{3+}$, $Cr^{3+}$, $Cd^{2+}$, and $Mn^{2+}$ with the natural product 5-hydroxy-7,4′-dimethoxyflavone were tested with regard to their antibacterial activity and appeared to be more active than the ligand alone.[278] The $Cu^{2+}$ and $Al^{3+}$ complexes of baicalin have MIC (Minimum Inhibitory Concentration) values in the range of $10^{-4}$ – $10^{-3}$ mol/L with the Cu complex being more active against several bacterial strains than $Al^{3+}$ and the free ligand.[279] Similarly, the $Cu^{2+}$ complex of naringin exhibits increased antimicrobial activity compared to the flavonoid on its own, although the activity is moderate.[190] $Ru^{II}$ polypyridyl complexes containing 3-hydroxyflavones as co-ligand also show antibacterial properties.[280,281] Kopacz *et al*. reported that the La, Gd and Lu complexes of morin are highly active against *Eschericia coli, Klebsiella pneumoniae* and *Staphylococcus aureus* which was attributed to their antioxidant properties.[282]

The antiviral activity of flavonoid complexes remains equivocal. The $Ru^{II}$ complex of 3-hydroxyflavone derivatives and the $Fe^{III}$ complex of 3-hydroxy-4'-benzyloxy-flavone have some anti-HIV properties *in vitro*.[281,283] On the other hand, the $Cu^{II}$ and $Fe^{III}$ complexes of pinostrobin oxime have negligible anti-HIV activity *in vitro* and almost no selectivity towards virus *vs*. uninfected cells.[191] Lee and coworkers studied a series of derivatives of the flavonoid galangin representing structural analogs of aryl diketo acids, known inhibitors of hepatitis C virus (HCV) NS5B polymerase. Such compounds have some anti-HCV activity *in vitro*, depending on the presence of specific functional groups. Although no metal complexes were studied, the antiviral mechanism of the galangin derivatives is believed to be partly due to the ability to chelate metal ions.[284] To date, there is no strong evidence supporting the relevance of flavonoid complexes as potential antiviral drugs.

### 7.3. Anti-inflammatory activity

The inflammation process involves many factors to occur. It engages many signal molecules, *e.g*. interleukins, histamine, serotonin, and is often accompanied by elevated oxidative stress. Clinical manifestations of the inflammatory process usually include swelling, redness of the affected tissue, capillary vessel permeability, increased temperature, pain or itching. Flavonoids are often known to possess anti-inflammatory activity. Several flavonoid complexes were synthesized and tested towards their anti-inflammatory properties in animal models or *in vitro*.

The $Mg^{2+}$ complex of rutin displays activity against serotonin-induced rat paw edema that is comparable to that of rutin alone, but is ineffective against histamine-induced paw edema.[285] Complexes of rare earth metal ions such as $La^{3+}$, $Ho^{3+}$, $Yb^{3+}$, $Lu^{3+}$, and $Y^{3+}$ with luteolin do have an inhibitory effect on xylene-induced ear edema in mice (at 100 mg/kg concentration) which is comparable to that of luteolin alone and that of the control drug dexamethasone (5 mg/kg), but the effect is weaker than that of luteolin alone in the carrageenan-induced paw edema model.[286] In contrast to this, the coordination of transition metal ions ($Fe^{2+}$, $Fe^{3+}$, $Cu^{2+}$, $Zn^{2+}$) to rutin and dihydroquercetin enhances the anti-inflammatory properties of the flavonoids in the *in vitro* model of asbestos-induced cell injury.[96] The increase in the protective properties is probably due to the better antioxidant capacity of the complexes compared to the ligands, and the absorption of the complexes to the chrysotile asbestos fibers. The authors proposed that the results provide a potential tool to treat inflammation induced by asbestos fiber inhalation. In a study by Korkina *et al*. it was found that when administered orally, the Fe and Cu complexes of rutin prevented lung inflammation and fibrosis in rats exposed to asbestos or bleomycin.[172] The naringin $Cu^{II}$ complex,

in which the metal ion is coordinated *via* the 4-carbonyl-5-hydroxyl group of the flavonoid, has a stronger anti-inflammatory activity (at 10 and 100 mg/kg concentrations) than naringin and is comparable to the control drug indomethacine (10 mg/kg).[190] In summary, the anti-inflammatory effect of flavonoid-metal complexes depends on the flavonoid and metal ion used, and is probably correlated to the antioxidant capacity of the complex.

### 7.4. Applications in non-cancer diseases

Flavonoids form stable complexes with the $VO^{2+}$ species due to the presence of carbonyl and hydroxyl oxygen atoms.[287] V compounds are known to act as insulin-mimics, lowering blood glucose levels, but they also have significant toxicity.[288] Data obtained from animal models with alloxan-induced diabetes indicate that $VO^{2+}$ flavonoid complexes (*e.g*. the $VO^{2+}$ complex of kaempferol-3-neohesperidoside) may act as antidiabetic agents.[289] Flavonoids such as quercetin - besides enhancing the glycaemia-lowering activity of V - also attenuate its toxicity by decreasing the oxidative stress in streptozotocin-induced diabetic mice.[288] Another metal ion which plays a significant role in the control of glycaemia is $Zn^{2+}$.[290] However, in excess it is toxic, as are all other essential metal ions. The 3-hydroxyflavone complex of $Zn^{2+}$ has a low toxicity and significant antidiabetic activity in streptozotocin-induced diabetic rats.[291]

The $Cu^{2+}$ complex of rutin acts as protective (anticonvulsant) agent in rats with experimentally induced epilepsy. The authors of the study associate this anticonvulsant activity with the antioxidative properties of the $Cu^{2+}$ complex.[25]

The $Al^{3+}$ complex of hesperidin and the $Cu^{2+}$ complex of rutin can prolong the activated partial thromboplastin time (aPTT) *in vitro* and might have potential anticoagulant activity.[292]

Metal ions are sometimes associated with amyloid-β aggregation in Alzheimer's disease (AD) and a few metal-chelating flavonoids have been reported to inhibit the formation of amyloid fibrils.[293] He and coworkers studied the influence of metal-chelating flavonoids on amyloid aggregation *in vitro* and found that although certain flavonoids could remove $Cu^{2+}$ and $Zn^{2+}$ from metallo-Aβ, they did not suppress metal-mediated Aβ aggregation.[294] On the other hand, Tay *et al*. presented kinetic data that suggested that quercetin and myricetin bind but do not remove Cu from CuAβ and that binding *via* the hydroxy-keto group inhibits CuAβ-mediated redox reactions.[295] By contrast, catechin and epicatechin bind *via* the catechol group and this binding mode results in the two flavanols being easily oxidized by CuAβ.[295] In another study, Liu and coworkers studied the protective activity of luteolin against Cu-mediated toxicity in the β-amyloid (Aβ) precursor protein in Swedish Mutation Overexpressing SH-SY5Y cells.[296] They established that apart from reducing the intracellular ROS generation and anti-apoptotic activity in SH-SY5Y cells treated with Cu salt, luteolin down-regulated the expression of Aβ Precursor Protein and lowered the secretion of $Aβ_{1-42}$ subunits.[296] Another flavonoid, myricetin was effective against metal-associated Aβ over metal-free Aβ species. It was also able to regulate Aβ aggregation induced by metal ions and alleviate its neurotoxicity *in vitro*.[297] The impact of flavonoids on the amyloid protein associated with Alzheimer's disease is promising, but needs more profound research. It is worth mentioning that Tc and Re complexes of flavone derivatives may be used for SPECT imaging of β-amyloid plaques in AD-affected brains *in vivo*.[298,299]

## 8. Interaction with biomolecules other than DNA

Flavonoids are metabolized in living organisms and may affect the metabolism of other compounds. Consequently, it is important to study the interaction of flavonoid complexes with biomolecules, such as proteins, lipids and enzymes.

### 8.1. Interaction with lipids

Flavonoid molecules are often hydrophobic, and thus can interact with lipid bilayers. They have a tendency to accumulate in biological membranes where they can interfere with the function of receptors and signal transducers by modulating the lipid phase behavior and protecting the membrane lipids against oxidation. Metal coordination often affects the mode flavonoid lipid interactions. While the 1:1 complex of Fe and quercetin is less lipophilic than quercetin, the lipophilicity increases and rises above that of the free flavonoid, when two or three quercetin bind to Fe. In the 1:2 and 1:3 complex the most polar sites of the flavonoid are arranged inwards. Addition of $Fe^{2+}$ to liposome and flavonoid suspensions resulted in liposome aggregation and formation of giant vesicles, apparently due to the formation of flavonoid Fe complexes. The adhesion of liposomes is provided by an Fe link between flavonoid molecules integrated in adjacent bilayers.[300] The Cu complex of chrysin can stabilize or fluidize the lipid membrane of liposomes, depending on the concentration. Selvaraj *et al*. reported that at low concentrations (20 μM), the chrysin Cu complex exhibited twice the protection against hypotonic stress-induced membrane disruption when compared to chrysin. However, this stabilizing effect gradually decreased and the extent of protection became comparable to chrysin at higher concentrations. This biphasic behavior of the chrysin Cu complex could be further explored for therapeutic applications.[301]

Flavonoid Cu complexes can enhance the cross-link formation in collagen and elastin maturation. Lysyl oxidase seems to interfere with flavonoid Cu complexes to regulate the oxidative deamination of lysine ε-amino groups.[302,303]

### 8.2. Interaction with serum albumin

The absorption, bioavailability and activity of a compound or drug can be affected by protein binding. Serum albumin (SA), the main transport protein in blood, acts as a carrier for many endogeneous and exogeneous molecules or metal ions. SA drug interactions play a major role in drug efficacy. Flavonoids bind to bovine (BSA) or human (HSA) serum albumin *in vitro*, with the affinity being dependent on the molecule.[304] Metal ions present in the surrounding medium affect the binding affinity of flavonoids to SA.[305-308] While



several studies have investigated the structure-activity relationships for flavonoid-SA binding,[309-314] interactions between SA and flavonoid metal complexes are much less well explored. An increase[315] as well as a decrease[161,316,317] in affinity upon complexation was observed. This may be dependent on whether all the coordination sites are saturated. Peng *et al*. reported that $Cu^{2+}$ coordination to the 3'4'-site of quercetin, taxifolin and catechin reduced the binding constant for BSA by 1 to 5 orders of magnitude depending on the structure and concentration of the flavonoid.[318] A decrease of the binding constant by $10^5$ may have a significant impact on the transport and thus the biological effect of the flavonoid. These findings may occur to be important in investigating the behavior of flavonoids *in vivo*, under physiological or pathological conditions.

**8.3. Interaction with enzymes**

Flavonoids and their metal complexes can inhibit various enzymes by forming chelate complexes with metal ions in the enzyme's active site, or by binding of the already formed complexes to enzymes that do not contain metal ions. The inhibition of tyrosinase by several flavonoids involves the former mode of interaction. Tyrosinases are catechol oxidases of the type-3 Cu enzyme family (with two Cu ions in the active site) that oxidise o-diphenols to the corresponding quinones. Tyrosinase activity is crucial for pigmentation in plants, fungi and animals. Consequently, tyrosinase inhibitors can have improtant applications in medicine, cosmetology and agriculture with regard to preventing hyperpigmentation and browning.[319] A series of 25 flavonoids were studied with regards to their inhibitory activity towards mushroom tyrosinase, leading to the establishment of several structure-activity relationships.[319] For inhibitory properties the presence of catechol groups on the ether side of rings A and B of the flavone is critical. The most active compound of the studied series was 7,8,3',4,'-tetrahydroxyflavone ($IC_{50}$= 0.07 μM), and the least active was 5,4'-dihydroxyflavone ($IC_{50}$= 393 μM). Hydroxyl moieties on the carbonyl side of the A and B rings are associated with lower inhibitory activity, as they probably hamper the access of the molecule to the active site. Generally, flavonols are less active than flavones. The activity correlates positively with the flavonoid's ability to chelate Cu ions. Molecular docking studies showed two ways of placing 7,8,3',4,'-tetrahydroxyflavone in the active pocket of tyrosinase, with the catechol groups of either ring A or ring B chelating the dicopper center.[319] Other flavonoids, namely hesperetin,[320] morin[321] and rutin[322] can also competitively inhibit mushroom tyrosinase, with different $IC_{50}$ values. Again molecular docking studies showed coordination to the Cu ions in the active site. One can estimate from these studies that small Cu chelating molecules, with easy access to the active site are better inhibitors than larger ones. Kojic acid, a well established tyrosinase inhibitor, has an $IC_{50}$ value of 22.3±8 μM,[320] compared to 3.49 μM for kaempferol[319] and 81.3±12.1 μM (or 99 μM, depending of the study) for morin.[319,321] Larger molecules are less active. Hesperetin and rutin, for example, have $IC_{50}$ values of 11.25±1.73 mM[320] and 6.8±0.3 mM[322], respectively.

Flavonoids can also interact with Zn ions in Zn enzymes, such as angiotensin-converting enzyme (ACE), which plays a role in increasing blood pressure.[323] Molecular docking studies suggest that quercetin binds to the Zn ion through the 3-OH group, while epicatechin coordinates through the 7-OH group. The $IC_{50}$ values against ACE are 0.415 mM (quercetin) and 1.381 mM (epicatechin)[323]. For the quercetin glycoside rutin no coordination is predicted. Another Zn enzyme, histone deacetylase, can be inhibited by kaempferol, as suggested by molecular docking and *in vitro* studies. The flavonol therefore causes hyperacetylation of the H3 histone complex in cancer cell lines.[324]

Kaempferol as well as myricetin at micromolar concentrations also inhibit other metalloenzymes – bacterial allantoinase and dihydroorotase.[325] However, molecular docking calculations suggest that metal ion binding is probably not involved in these cases.

Enzyme inhibitors, such as HIV integrase inhibitors, play a role as antiviral drugs. HIV integrase (IN) is crucial for the integration of the viral genetic material into the host DNA. The enzyme contains two divalent metal ions ($Mg^{2+}$),[326] and already established integrase inhibitors have a two-ions-chelating moiety as a pharmacophore.[326,327] In a study of flavonoids as IN inhibitors, Li *et al*. designed various modified natural flavonoids. They included a hydrophobic aromatic group and showed that the inhibitory activity was optimal, when the hydrophobic group was placed on C7. The presence of a catechol moiety was found to be beneficial for the activity of flavonoids with a β-ketoenol group, supporting the metal-chelation mechanism of IN inhibition by flavonoids. The study showed that such modified flavonoids are potent novel HIV-1 integrase inhibitors.[327]

As mentioned above, already formed flavonoid metal complexes can inhibit enzymes that do not have any metal ions in their active sites. For example, Cu complexes of quercetin and rutin inhibit ribonuclease A in a non-competitive manner.[328] However, the exact molecular mechanism of the interaction is not clear.

The ability of some flavonoid complexes to inhibit topoisomerase has already been discussed in section **7.1**. Molecular docking studies show that the heterobimetallic complexes $[Cu(quercetin)_2(H_2O)_6Sn_2Cl_4]$ and $[Zn(quercetin)_2(H_2O)_6Sn_2Cl_4]$ bind to DNA in the major groove and form hydrogen bonds with neighbouring amino acids of the enzyme, thus inhibiting the topo-I-DNA complex.[273] The $IC_{50}$ value for the inhibitory activity of the complex $[Cu(quercetin)_2(H_2O)_6\text{-}Sn_2Cl_4]$ is 30 μM. The complex shows SOD mimic activity in the xanthine/xanthine oxidase assay (see **7.1**), but it also inhibits the natural enzyme, probably by inserting itself in the SOD active-site channel. The cationic core of $Sn^{IV}$, surrounded by Cl atoms, can coordinate to the active site Cu and a nearby guanidyl cation of Arg141 and positively charged ammonia of Lys134.

**9. Applications in biomimetic studies**

Metal complexes of flavonoids are widely employed to mimic and investigate the mechanisms of metalloenzymes that use flavonoids as

substrates. Certain flavonolate complexes of $Cu^{2+}$, $Cu^{+}$ or other metal ions, *e.g.* $Ce^{4+}$ may act as model compounds in biomimetic studies.[329] There is a body of evidence that many Cu flavonolate complexes have quercetinase (quercetin 2,3-dioxygenase) activity (Fig. 7). Quercetinases are catabolic enzymes of fungal or bacterial origin which oxidatively degrade quercetin into a phenolic carboxylic acid ester and carbon monoxide.[330,331] Some complexes of 3-hydroxyflavonolate with $Zn^{2+}$, $Cd^{2+}$ and $Hg^{2+}$ exposed to UV light under aerobic conditions give oxidative carbon–carbon bond cleavage and CO release.[332] Biomimetic catalysis is a well-established approach to investigate metalloenzymes and enzymatic mechanisms and the interested reader is referred to two excellent review papers on this topic.[333,334]

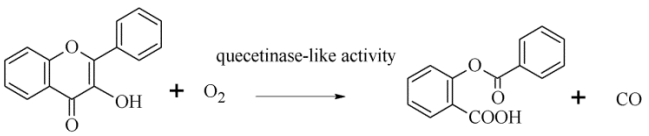



**Fig. 7** Quercetinase-like reaction, mimicked by many Cu flavonolate complexes. [329]

### 10. Conclusions

Flavones, flavanones and flavonols react with various metal ions. Depending on the properties of the flavonoid and the ion, the reaction may include formation of coordination bonds and/or a redox process. The formation of flavonoid metal complexes is confirmed in solution but the complexes are often also isolated and purified. The location and the number of coordinating or chelating sites in flavonoid molecules depends on the location and the number of electron-donating groups, usually carbonyl and hydroxyl moieties. Coordination of a metal ion to a flavonoid molecule affects its properties, *e.g.* color, fluorescence, stability, and oxidation state. Due to these phenomena, the formation of such complexes has been widely utilized in analytical chemistry (spectrophotometric and spectrofluorimetric methods), photochemistry and textile dyeing. Complexes of flavonoids and metal ions also display a wide spectrum of chemical and biological activity *in vitro* and *in vivo*. They are usually antioxidants, often more potent than the parent flavonoids, but they may be pro-oxidants as well. In biological systems, such complexes may act as chemo-protective agents, but sometimes they may enhance the oxidative damage of biomolecules. Some complexes of flavonoids with Cu or Fe gain superoxide-dismutating activity and specific flavonolate complexes can mimic enzymatic oxygenase activity. This indicates a complicated dependence between the ligand, the metal ion and the surrounding environment, and interactions of all those factors result in specific properties of a given complex. The redox and structural properties of flavonoid complexes allow the compounds to interact with many vital biological targets including DNA, proteins and lipids. They also affect the expression and activity of many regulatory molecules. Therefore, the complexes are biologically active: they have antimicrobial, antiproliferative and potential therapeutic properties. Chelation of metal ions is probably one of the reasons for the chemoprotective properties of flavonoids. Reactions of flavonoids with metal ions have been widely researched. The main scientific pursuit now is the development of applications of the many promising, chemically and biologically active flavonoid metal compounds.

## Acknowledgements

Authors acknowledge the financial support from the Polish National Science Centre (grant no. 6740/B/P01/2011/40; reg. no. N N405 674040) and the statute fund from Medical University of Lodz (503/3-016-02/503-31-001, 502-03/3-016-02/502-34-003).
We also acknowledge the courtesy of Jenny Dean (http://www.jennydean.co.uk/), the author of the pictures a) and b) from Fig.4, and Cynthia Slatcoff, (http://www.brushcreekwoolworks.com/), the author of the pictures c) and d) from Fig.4.

## Abbreviations

| | |
|---|---|
| AD | Alzheimer's disease |
| apig | apigenin |
| BSA | bovine serum albumin |
| diox | dioxane |
| EtOH | ethanol |
| fla | flavonol |
| 3HF | 3-hydroxyflavone |
| HAS | human serum albumin |
| MeOH | methanol |
| MIC | Minimum Inhibitory Concentration |
| MSA | morin-5-sulfonic acid |
| 4'OMefla | 4'-methoxyflavonol |
| 6-$Ph_2$TPA | *N*,*N*-bis(6-phenyl-2-pyridyl)methyl)-N-((2-pyridyl)methylamine |
| ROS | reactive oxygen spcies |
| SA | serum albumin |

## References

1. J. Cui and S. Li, Shaoshun, *Mini-Rev. Med. Chem*., 2013, 13, 1357-1368 and refs therein.
2. H. P. Kim and H. Park, *Nat. Prod. Sci*., 2010, 16, 59-67 and refs therein.
3. O. Burmistrova, M. T. Marrero, S. Estevez, I. Welsch, I. Brouard, J. Quintana and F. Estevez, *Eur. J. Med. Chem*., 2014, 84, 30-41.
4. S. Rubio, F. Leon, J. Quintana, S. Cutler and F. Estevez, *Eur. J. Med. Chem*., 2012, 55, 284-296.
5. E. Zyner, J. Graczyk and J. Ochocki, *Pharmazie*, 1999, **54**, 945-946.
6. B. H. Havsteen, *Pharmacol. Ther*., 2002, **96**, 67-202 and refs therein.
7. W. Chen, S. Sun, W. Cao, Y. Liang and J. Song, *J. Mol. Struct.*, 2009, **918**, 194-197.
8. G. Galati and P. J. O'Brien, *Free Radic. Biol. Med.*, 2004, **37**, 287-303.
9. R. J. Williams, J. P. E. Spencer and C. Rice-Evans, *Free Radic. Biol. Med.*, 2004, **36**, 838-849.
10. M. Lopez-Lazaro, *Mini-Rev. Med. Chem*., 2009, **9**, 31-59.
11. G. Rusak, I. Piantanida, L. Masic, K. Kapuralin, K. Durgo and N. Kopjar, *Chem.- Biol. Interact.*, 2010, **188**, 181-189.



12. A. H. Hegde, S. N. Prashanth and J. Seetharamappa, *J. Pharm. Biomed. Anal.*, 2012, **63**, 40-46.
13. I. B. Afanasev, A. I. Dorozhko, A. V. Brodskii, V. A. Kostyuk and A. I. Potapovitch, *Biochem. Pharmacol.*, 1989, **38**, 1763-1769.
14. A. M. Pamukcu, S. Yalciner, J. F. Hatcher and G. T. Bryan, *Cancer Res.*, 1980, **40**, 3468-3472.
15. A. W. Boots, H. Li, R. P. F. Schins, R. Duffin, J. W. M. Heemskerk, A. Bast and G. R. M. M. Haenen, *Toxicol. Appl. Pharmacol*., 2007, **222**, 89-96.
16. I. Esparza, I. Salinas, C. Santamaria, J. M. Garcia-Mina and J. M. Fernandez, *Anal. Chim. Acta*, 2005, **543**, 267–274.
17. S. A. B. E. van Acker, G. P. van Balen, D. J. van den Berg, A. Bast and W. J. F. van der Vijgh, *Biochem. Pharmacol*., 1998, **56**, 935-943.
18. G. R. Barcelos, J. P. Friedmann Angeli, J. M. Serpeloni, D. Grotto, B. A. Rocha, J. K. Bastos, S. Knasmueller and F. Barbosa Jr, *Mut. Res.*, 2011, **726**, 109-115.
19. Y. Zhang, Y. Huang, X. Deng, Y. Xu, Z. Gao and H. Li, *Eur. J. Pharmacol.*, 2012, **680**, 95-101.
20. V. V. Tsaryuk, A. I. Potapovich and V. A. Kostyuk, *Bull. Exp. Biol. Med.*, 2002, **133**, 334-335.
21. M. Y. Moridani, J. Pourahmad, H. Bui, A. Siraki and P. J. O'Brien, *Free Radic. Biol. Med.*, 2003, **34**, 243-253.
22. Q. K. Panhwar, S. Memon and M. I. Bhanger, *J. Mol. Struct.*, 2010, **967**, 47–53.
23. M. Jabbari and F. Gharib, *J. Mol. Liq.*, 2012, **168**, 36-41.
24. A. Bravo and J. R. Anacona, *Trans. Met. Chem.*, 2001, **26**, 20-23.
25. D. Nowak, A. Kuzniar and M. Kopacz, *Structural Chemistry*, 2010, **21**, 323-330.
26. R. Prajapati, S. K. Dubey, R. Gaur, R. K. Koiri, B. K. Maurya, S. K. Trigun and L. Mishra, *Polyhedron*, 2010, **29**, 1055-1061.
27. M. M. Kasprzak, L. Szmigiero, E. Zyner and J. Ochocki, *J. Inorg. Biochem.*, 2011, **105**, 518-524.
28. M. Balcerzak, A. Tyburska and E. Swiecicka-Fuchsel, *Acta Pharm*., 2008, **58**, 327-334.
29. M. Balcerzak, M. Kopacz, A. Kosiorek, E. Swiecicka and S. Kus, *Anal. Sci.*, 2004, **20**, 1333-1337.
30. J. Ochocki, M. Kasprzak, L. Checinska, A. Erxleben, E. Zyner, L. Szmigiero, A. Garza-Ortiz and J. Reedijk, *Dalton Trans*., 2010, **39**, 9711-9718.
31. M. Balcerzak, A. Kosiorek and E. Swiecicka, *J. Anal. Chem.*, 2006, **61**, 119-123.
32. A. Kosiorek-Rupinska, E. Swiecicka-Fuchsel and M. Balcerzak, *Anal. Lett.*, 2006, **39**, 589-602.
33. J. M. Dimitric Markovic, Z. S. Markovic, T. P. Brdaric, V. M. Pavelkic and M. B. Jadranin, *Food Chemistry*, 2011, **129**, 1567-1577.
34. R. F. V. de Souza, E. M. Sussuchi and W. F. De Giovanni, *Synth. React. Inorg. Met.-Org. Chem*. 2003, **33**, 1125-1144.
35. G. M. Escandar and L. F. Sala, *Can. J. Chem*., 1991, **69**, 1994-2001.
36. M. T. Fernandez, L. Mira, M. H. Florencio and K. R. Jennings, *J. Inorg. Biochem*. 2002, **92**, 105-111.
37. M. D. Engelmann, R. Hutcheson and I. F. Cheng, *J. Agric. Food Chem*., 2005, **53**, 2953-2960.
38. A. Rygula, T. P. Wrobel, J. Szklarzewicz and M. Baranska, *Vibr. Spectrosc*., 2013, **64**, 21-26.
39. A. Torreggiani, A. Trinchero, M. Tamba and P. Taddei, *J. Raman Spectrosc*., 2005, **36**, 380-388.
40. A. Torreggiani, M. Tamba, A. Trinchero and S. Bonora, *J. Mol. Struct*., 2005, 7**44-747**, 759-766.
41. J. E. Brown, H. Khodr, R. C. Hider, C. A. Rice-Evans, *Biochem. J*., 1998, **330**, 1173-1178.
42. L. Mira, M. T. Fernandez, M. Santos, R. Rocha, M. H. Florencio and K. R. Jennings, *Free Radical Res*., 2002, **36**, 1199-1208.
43. M. E. Bodini, G. Copia, R. Tapia, F. Leighton and L. Herrera, *Polyhedron*, 1999, **18**, 2233-2239.
44. J. M. Dimitric Markovic, Z. S. Marlovic, T. P. Brdaric and N. D. Filipovic, *Dalton Trans*., 2011, **40**, 4560-4571.
45. J. P. Cornard and J. C. Merlin, *J. Inorg. Biochem*., 2002, **92**, 19-27.
46. J. M. Dimitric Markovic, Z. S. Markovic, D. S. Veselinovic and J. B. Krstic, *J. Inorg. Biochem*., 2009, **103**, 723-730.
47. J. P. Cornard, A. C. Boudet and J. C. Merlin, *Spectrochim. Acta A*, 2001, **57**, 591-602.
48. A. Ahmedova, K. Paradowska and I. Wawer, *J. Inorg. Biochem*., 2012, **110**, 27-35.
49. L.-G. Gao, H. Wang, X.-L. Song and W. Cao, *J. Mol. Struct.*, 2013, **1034**, 386-391.
50. S. Sun, W. Chen, W. Cao, F. Zhang, J. Song and C. Tian, *J. Mol. Struct.*, 2008, **860**, 40-44.
51. M. Leopoldini, N. Russo, S. Chiodo and M. Toscano, *J. Agric. Food Chem*., 2006, **54**, 6343-6351.
52. J. P. Cornard, L. Dangleterre and C. Lapouge, *J. Phys. Chem. A*, 2005, **109**, 10044-10051.
53. R. F. V. De Souza and W. F. De Giovanni, *Spectrochim. Acta Part A*, 2005, **61**, 1985-1990.
54. E. Woznicka, M. Kopacz, M. Umbreit and J. Klos, *J. Inorg. Biochem*., 2007, **101**, 774-782.
55. J. Zhou, L. F. Wang, J. Y. Wang and N. Tang, *J. Inorg. Biochem*., 2001, **83**, 41-48.
56. A. A. Ansari, *J. Coord. Chem*., 2008, **61**, 3869-3878.
57. A. A. Ansari, *Main Group Chem*., 2008, **7**, 43-56.
58. A. A. Ansari, *Main Group Chem*., 2008, ***7***, 15-30.
59. J. Pusz, E. Woznicka, S. Wolowiec and M. H. Umbreit, *J. Therm. Anal. Calor*. 2009, **97**, 987-992.
60. E. Furia, T. Marino and N. Russo, *Dalton Trans*., 2014, **43**, 7269-7274.
61. G. Erdogan, R. Karadag, *Rev. Anal. Chem*., 2005, **24**, 9-23.
62. R. Ravichandran, M. Rajendran and D. Devapiram, *Food Chem*., 2014, **146**, 472-478.
63. D. Malesev and V. Kuntic, *J. Serb. Chem. Soc*., 2007, **72**, 921-939.
64. M. Guo, C. Perez, Y. Wei, E. Rapoza, G. Su and F. Bou-Abdallah, *Dalton Trans*., 2007, 4951-4961.
65. G. Erdogan, R. Karadag and E. Dolen, *Rev. Anal. Chem*., 2005, **24**, 247-261.
66. G. V. Ferrari, N. B. Pappano, N. B. Debattista and M. P. Montana, *J. Chem. Eng. Data*, 2008, **53**, 1241-1245.
67. L. Dangleterre and J.-P. Cornard, *Polyhedron*, 2005, **24**, 1593-1598.
68. Y. A. Davila, M. J. Sancho, M. C. Almandoz and S. E. Blanco, *Spectrochim. Acta Part* A, 2012, **95**, 1-7.
69. L. Dangleterre, J. P. Cornard and C. Lapouge, *Polyhedron*, 2008, **27**, 1581-1590.
70. J. P. Cornard, L. Dangleterre and C. Lapouge, *Chem. Phys. Lett*., 2006, **419**, 304-308.
71. C. Lapouge, L. Dangleterre and J. P. Cornard, *J. Phys. Chem. A*, 2006, **110**, 12494-12500.
72. G. T. Castro and S. E. Blanco, *Spectrochim. Acta Part A*, 2004, **60**, 2235-2241.
73. C. A. Perez, Y. Wei and M. Guo, *J. Inorg. Biochem*., 2009, **103**, 326-332.
74. J. M. Dimitric Markovic, Z. S. Markovic, I. A. Pasti, T. P. Brdaric, A. Popovic-Bijelic and M. Mojovic, *Dalton Trans*., 2012, **41**, 7295-7303.
75. G. V. Ferrari, N. B. Pappano, M. P. Montana, N. A. Garcia and N. B. Debattista, *J. Chem. Eng. Data*, 2010, **55**, 3080-3083.
76. G. V. Ferrari, M. P. Montana, F. C. D. Dimarco, N. B. Debattista, N. B. Pappano, W. A. Massad and N. A. Garcia, *J. Photochem. Photobiol. B*, 2013, **124**, 42-49.
77. A. H. Yang, X. Y. Shi, X. Li, F. F. Li, Q. Q. Zhang, S. X. Jiang, J. Z. Cui and H. L. Gao, *RSC Adv*., 2014, **4**, 25227-25233.
78. J. M. Dimitric Markovic, D. Amic, B. Lucic and Z. S. Markovic, *Monatsh. Chem*., 2014, **145**, 557-563.
79. M. Shoja, *Acta Cryst.*, 1990, **C46**, 517-519.
80. P. Mladenka, K. Macakova, T. Filipsky, L. Zatloukalova, L. Jahodar, P. Bovicelli, I. Proietti Silvestri, R. Hrdina and L. Saso, *J. Inorg. Biochem*., 2011, **105**, 693-701.
81. G. Speier, V. Fulop and L. Parkanyi, *J. Chem. Soc., Chem. Commun*., 1990, 512-513.
82. E. Balogh-Hergovich, J. Kaizer, G. Speier, V. Fulop and L. Parkanyi, *Inorg. Chem*., 1999, **38**, 3787-3795.
83. E. Balogh-Hergovich, G. Speier and G. Argay, *J. Chem. Soc., Chem. Commun*., 1991, 551-552.
84. E. Balogh-Hergovich, J. Kaizer, G. Speier, G. Argay and L. Parkanyi, *J. Chem. Soc., Dalton Trans*., 1999, 3847-3854.
85. E. Pieniazek, J. Kalembkiewicz, M. Dranka, E. Woznicka, *J. Inorg. Biochem*., 2014, **141**, 180-187.
86. F. B. A. El Amrani, L. Perello, J. A. Real, M. Gonzalez-Alvarez, G. Alzuet, J. Borras, S. Garcia-Granda and J. Montejo-Bernado, *J. Inorg. Biochem*., 2006, **100**, 1208-1218.

87. J. Kaizer, G. Barath, J. Pap, G. Speier, M. Giorgi and M. Reglier, *Chem. Commun*., 2007, 5235-5237.
88. Y.-J. Sun, Q.-Q. Huang, T. Tano and S. Itoh, *Inorg. Chem*., 2013, **52**, 10936-10948.
89. G. Barath, J. Kaizer, G. Speier, L. Parkanyi, E. Kuzmann and A. Vertes, *Chem. Commun*., 2009, 3630-3632.
90. A. Matuz, M. Giorgi, G. Speier and J. Kaizer, *Polyhedron*, 2013, **63**, 41-49.
91. K. Grubel, K. Rudzka, A. M. Arif, K. L. Klotz, J. A. Halfen and L. M. Berreau, *Inorg. Chem*., 2010, **49**, 82-96.
92. E. Balogh-Hergovich, J. Kaizer, G. Speier, G. Huttner and A. Jacobi, *Inorg. Chem*., 2000, **39**, 4224-4229.
93. E. Balogh-Hergovich, J. Kaizer, G. Speier, G. Huttner and L. Zsolnai, *Inorg. Chim. Acta*, 2000, **304**, 72-77.
94. I. Lippai, G. Speier, G. Huttner and L. Zsolani, *Acta Cryst*., 1997, **C53**, 1547-1549.
95. J. Kaizer, J. Pap, G. Speier and L. Parkanyi, *Eur. J. Inorg. Chem*., 2004, 2253-2259.
96. I. Lippai and G. Speier, *J. Mol. Catal. A*, 1998, **130**, 139-148.
97. I. Lippai, G. Speier, G. Huttner and L. Zsolnai, *Chem. Commun*., 1997, 741-742.
98. N. Okabe, E. Yamamoto and M. Yasunori, *Acta Cryst.*, 2003, **E59**, m715-m716.
99. Y.-J. Sun, Q.-Q. Huang and J.-J. Zhang, *Inorg. Chem*., 2014, **53**, 2932-2942.
100. W. Hiller, A. Nishinaga and A. Rieker, *Z. Naturforsch*., 1992, **47b**, 1185-1188.
101. E. Balogh-Hergovich, J. Kaizer, G. Speier, G. Huttner and P. Rutsch, *Acta Cryst*., 1999, **C55**, 557-558.
102. Y. Farina, B. M. Yamin, H.-K. Fun, B.-C. Yip and S.-G. Teoh, *Acta Cryst*., 1995, **C51**, 1537-1540.
103. M. C. Etter, Z. Urbanczyk-Lipkowska, S. Baer and P. F. Barbara, *J. Mol. Struct*., 1986, **144**, 155-167.
104. L. Jurd and T. A. Geissman, *Org. Chem.*, 1956, **21**, 1395-1401.
105. J. Ren, S. Meng, C. E. Lekka and E. Kaxiras, *J. Phys. Chem. B*, 2008, **112**, 1845-1850.
106. C. E. Lekka, J. Ren, S. Meng and E. Kaxiras, *J. Phys. Chem. B*, 2009, **113**, 6478-6483.
107. S. A. Payan-Gomez, N. Flores-Holguin, A. Perez-Hernandez, M. Pinon-Miramontes and D. Glossman-Mitnik, *J. Mol. Model*., 2011, **17**, 979-985.
108. A. Amat, C. Clementi, C. Miliani, A. Romani, A. Sgamellotti and S. Fantacci, *Phys. Chem. Chem. Phys*., 2010, **12**, 6672-6684.
109. V. Cody and J. R. Luft, *J. Mol. Struct*., 1994, **317**, 89-97.
110. T. Z. Todorova, M. G. Traykov, A. V. Tadjer and Z. A. Velkov, *Comput. Theor. Chem*., 2013, **1017**, 85–90.
111. S. Domagata, P. Munshi, M. Ahmed, B. Guilot and C. Jelsch, *Acta Cryst*., 2011, **B67**, 63-78.
112. S. A. van Acker, M. J. De Groot, D. J. van den Berg, M. N. Tromp, G. Doone-Op den Kelder and W. J. van derVijgh, *Chem. Res. Toxicol*., 1996, **9**, 1305-1312.
113. M. Leopoldini, I. Prieto Pitarch, N. Russo and M. Toscano, *J. Phys. Chem. A*, 2004, **108**, 92-96.
114. A.-C. Boudet, J.-P. Cornard and J.-C. Merlin, *Spectrochim. Acta A*, 2000, **56**, 829-839.
115. J. Zhang, L. Yue, K. Hayat, S. Q. Xia, X. M. Zhang, B. M. Ding, J. M. Tong and Z. X. Chen, *Sep. Purif. Technol.*, 2010, **71**, 273-278.
116. V. T. Papoti, S. Xystouris, G. Papagianni and M. Z. Tsimidou, *Ital. J. Food Sci.*, 2011, **23**, 252-259.
117. J. Zhang, J. Wang and J. S. Brodbelt, *J. Mass Spectrom*., 2005, **40**, 350-363.
118. R. March and J. Brodbelt, *J. Mass Spectrom*. 2008, **43**, 1581-1617.
119. O. Ilboudo, I. Tapsoba, Y. L. Bonzi-Coulibaly and P. Gerbaux, *Eur. J. Mass Spectrom.*, 2012, **18**, 465-473.
120. A. Sanzmedel, J. I. G. Alonso and E. B. Gonzalez, *Anal. Chem*., 1985, **57**, 1681-1687.
121. K. Pyrzynska and A. Pekal, *Crit. Rev. Anal. Chem.*, 2011, **41**, 335-345.
122. R. S. Chauhan and L. R. Kakkar, *Chem. Anal. (Warsaw)*, 1994, **39**, 571-576.
123. E. A. Saad, L. H. Khalil, M. T. M. Zaki and A. A. A. El-Ella, *Microchim. Acta*, 2002, **140**, 87-91.
124. R. Ghavami, A. Najafi and B. Hemmateenejad, *Spectrochim. Acta Part A*, 2008, **70,** 824–834.
125. A. A. Y. El-Sayed, E. A. Saad, B. M. M. Ibrahime and M. T. M. Zaki, *Microchim. Acta*, 2000, **135**, 19–27.
126. R. S. Kirk and W. Pocklington, Analyst, 1969, 94, 71-74.1-766.
127. B. Fitak and B. Pilkowska, *Roczniki Panstwowego Zakladu Higieny*, 1973, **24**, 331-336.
128. B. Fitak and T. Zaklika, *Roczniki Panstwowego Zakladu Higieny*, 1973, **24**, 627-633.
129. B. S. Garg and R. P. Singh, *Talanta*, 1971, **18**.
130. M. de la Guardia, A. Morales-Rubio, Comprehensive Analytical Chemistry, D. Barcelo (ed), 2003, 41, 1115-1171.126. H. M. Mykhalyna, T. Y. Vrublevs'ka, M. B. Urys and Y. Y. Lutsyshyn, *Mater. Sci.*, 2011, **47**, 412-416.
131. H. M. Mykhalyna, T. Y. Vrublevs'ka, M. B. Urys and Y. Y. Lutsyshyn, *Mater. Sci*., 2011, **47**, 412-416.
132. P. Viswanathan, V. Sriram and G. Yogeeswaran, *J. Agric. Food Chem.*, 2000, **48**, 2802-2806.
133. A. Andreu-Navarro, J. Manuel Fernandez-Romero and A. Gomez-Hens, *J. Sep. Sci.*, 2010, **33**, 509–515.
134. D. A. Kostic, G. Z. Miletic, S. S. Mitic, I. D. Rasic and V. V. Zivanovic, *Chem. Pap.*, 2007, **61**, 73-76.
135. S. Sathish, G. Narayan, N. Rao and C. Janardhana, *J. Fluoresc.*, 2007, **17**, 1-5.
136. J. S. Wu, F. Wang, W. M. Liu, P. F. Wang, S. K. Wu, X. H. Wu and X. H. Zhang, *Sens. Actuators, B*, 2007, **125**, 447-452.
137. R. Villamil-Ramos, V. Barba and A. K. Yatsimirsky, *Analyst*, 2012, **137**, 5229-5236.
138. Y. Cao, X. Wu and M. Wang, *Talanta*, 2011, **84**, 1188-1194.
139. T. Hayashi, S. Kawai and T. Ohno, *Chem. Pharm. Bull.*, 1971, **19**, 792-797.
140. H. Kunkely and A. Vogler, *Chem. Phys. Lett.*, 2001, **338**, 29-32.
141. S. Protti, A. Mezzetti, C. Lapouge and J.-P. Cornard, *Photochem. Photobiol. Sci.*, 2008, **7**, 109-119.
142. S. Tommasini, M. L. Calabro, P. Donato, D. Raneri, G. Guglielmo, P. Ficarra and R. Ficarra, *J. Pharm. Biomed. Anal.*, 2004, **35**, 389-397.
143. L. N. Ma, J. Y. Nan, F. Wu, W. Tian, W. J. Chen and G. L. Zhang, *Spectrosc. Spectr. Anal.*, 2009, **29**, 994-998.
144. S. Oncul and A. P. Demchenko, *Spectrochim. Acta, Part A*, 2006, **65**, 179-183.
145. B. Dick, *Ber. Bunsen-Ges. Phys. Chem.*, 1987, **91**, 1205-1209.
146. V. N. Agieienko, Y. V. Kolesnik and O. N. Kalugin, *J. Chem. Phys*., 2014, **140**, 194501-194514.
147. S. Protti, K. Raulin, O. Cristini, C. Kinowski, S. Turrell and A. Mezzetti, *J. Mol. Struct.*, 2011, **993**, 485-490.
148. Y. Hamada, T. Sano, H. Fujii, Y. Nishio, H. Takahashi and K. Shibata, *Appl. Phys. Lett.*, 1997, **71**, 3338.
149. K. S. Karimov, M. Mahroof-Tahir, M. Saleem and Z. Ahmad, *Physica B-Condensed Matter*, 2011, **406**, 3011-3017.
150. M. P. Colombini, A. Andreotti, C. Baraldi, I. Degano and J. J. Lucejko, *Microchem. J*., 2007, **85**, 174-182.
151. G. J. Smith, S. J. Thomsen, K. R. Markham, C. Andary and D. Cardon, *J. Photochem. Photobiol, A*, 2000, **136**, 87-91.
152. G. J. Smith, I. J. Miller and V. Daniels, *J. Photochem. Photobiol, A*, 2005, **169**, 147-152.
153. G. Erdogan, R. Karadag and A. Eler, *Main Group Met. Chem.*, 2010, **33**, 283-299.
154. G. Favaro, C. Clementi, A. Romani and V. Vickackaite, *J. Fluoresc.*, 2007, **17**, 707-714.
155. M. Leona, J. Stenger and E. Ferloni, *J. Raman Spectrosc.*, 2006, **37**, 981-992.
156. I. Surowiec, W. Nowik and M. Trojanowicz, *Microchim. Acta*, 2008, **162**, 393-404.
157. O. Ryan and M. J. Hynes, *J. Inorg. Biochem.*, 2008, **102**, 127-136.
158. A. Pekal, M. Biesaga and K. Pyrzynska, *Biometals*, 2011, **24**, 41-49.
159. T. Kawataba, V. Schepkin, N. Haramaki, R. S. Phadke and L. Packer, *Biochem. Pharmacol*., 1996, **51**, 1569-1577.
160. H. Kipton, J. Powell and M. C. Taylor, *Austr. J. Chem*., 1982, **35**, 739-756.



161. H. El Hajji, E. Nkhili, V. Tomao and O. Dangles, *Free Radical Res*., 2006, **40**, 303-320.
162. I. Morel, G. Lescoat, P. Cogrel, O. Sergent, N. Pasdeloup, P. Brissot, P. Cillard and J. Cillard, *Biochem. Pharmacol.*, 1993, **45**, 13-19.
163. P. Sestili, A. Guidarelli, M. Dacha and O. Cantoni, *Free Radic. Biol. Med.*, 1998, **25**, 196-200.
164. M. Melidou, K. Riganakos and D. Galaris, *Free Radic. Biol. Med.*, 2005, **39**, 1591-1600.
165. I. F. Cheng, K. Breen, *Biometals*, 2000, **13**, 77-83.
166. A. Arora, M. G. Nair, G. M. Strasburg, *Free Radic. Biol. Med*., 1998, **24**, 1355-1363.
167. S. V. Jovanovic, S. Steenken, Y. Hara and M. G. Simic, *J. Chem. Soc. Perkin Trans*. 2, 1996, 2497-2504.
168. N. R. Perron, J. N. Hodges, M. Jenkins and J. L. Brumaghim, *Inorg. Chem.*, 2008, **47**, 6153-6161.
169. N. R. Perron, J. L. Brumaghim, *Cell. Biochem. Biophys*., 2009, **53**, 75-100.
170. K. Jomova and M. Valko, *Toxicology*, 2011, **283**, 65-87.
171. J. Joseph and K. Nagashri, *Appl. Biochem. Biotechnol.*, 2012, **167**, 1446-1458.
172. L. G. Korkina, E. A. Ostrachovich, G. A. Ibragimova and I. B. Afanas'ev, *Proceedings of the International Symposium on Trace Elements in Man and Animals*, 2000, 119-122.
173. S. B. Bukhari, S. Memon, M. Mahroof-Tahir and M. I. Bhanger, *Spectrochim. Acta, Part A*, 2009, **71**, 1901–1906.
174. S. B. Bukhari, S. Memon, M. M. Tahir and M. I. Bhanger, *J. Mol. Struct.*, 2008, **892**, 39-46.
175. M. F. Jimenez and F. Garcia-Carmona, *J. Agric. Food Chem*., 1999, **47**, 56-60.
176. S. B. Etcheverry, E. G. Ferrer, L. Naso, J. Rivadeneira, V. Salinas and P. A. M. Williams, *J. Biol. Inorg. Chem.*, 2008, **13**, 345-447.
177. M. Gonzalez-Alvarez, G. Alzuet, J. L. Garcia-Gimenez, B. Macias and J. Borras, *Z. Anorg. Allg. Chem.*, 2005, **631**, 2181-2187.
178. V. A. Kostyuk, A. I. Potapovich, E. N. Strigunova, T. V. Kostyuk and I. B. Afanas'ev, *Arch. Biochem. Biophys*., 2004, **428**, 204-208.
179. V. A. Kostyuk, A. I. Potapovich, T. V. Kostyuk and M. G. Cherian, *Cell. Mol. Biol*., 2007, **53**, 62-69.
180. H. Tang, X. Wang, S. Yang and L. Wang, *Rare Met*., 2004, **23**, 38-42.
181. K. Furuno, T. Akasako and N. Sugihara, *Biol. Pharm. Bull*., 2002, **25**, 19-23.
182. I. B. Afanas'ev, E. A. Ostrakhovitch, E. V. Mikhal'chik, G. A. Ibragimova and L. G. Korkina, *Biochem. Pharmacol*., 2001, **61**, 677-684.
183. V. A. Kostyuk, A. I. Potapovich, E. N. Vladykovskaya, L. G. Korkina and I. B. Afanas'ev, *Arch. Biochem. Biophys*., 2001, **385**, 129-137.
184. T.-R. Li, Z.-Y. Yang, B.-D. Wang and D.-D. Qin, *Eur. J. Med. Chem.*, 2008, **43**, 1688-1695.
185. J. Zhou, L. F. Wang, J. Y. Wang and N. Tang, *Transition Met. Chem.*, 2001, **26**, 57-63.
186. Q. K. Panhwar and S. Memon, *J. Coord. Chem.*, 2012, **65**, 1130–1143.
187. Q. K. Panhwar and S. Memon, *J. Coord. Chem.*, 2011, **64**, 2117-2129.
188. T.-R. Li, Z.-Y. Yang and B.-D. Wang, *Chem. Pharm. Bull.*, 2007, **55**, 26-28.
189. Y. Li, Z.-y. Yang and T.-R. Li, *Chem. Pharm. Bull.*, 2008, **56**, 1528-1534.
190. R. M. S. Pereira, N. E. D. Andrades, N. Paulino, A. C. H. F. Sawaya, M. N. Eberlin, M. C. Marcucci, G. M. Favero, E. M. Novak and S. P. Bydlowski, *Molecules*, 2007, **12**, 1352-1366.
191. A. A. Mashentseva, T. S. Seytembetov, S. M. Adekenov, B. I. Tuleuov, O. P. Loiko and A. I. Khalitova, *Russ. J. Gen. Chem.*, 2011, **81**, 96-101.
192. L. Naso, E. Gloria Ferrer, L. Lezama, T. Rojo, S. Beatriz Etcheverry and P. Williams, *J. Biol. Inorg. Chem.*, 2010, **15**, 889-902.
193. G. Dehghan and Z. Khoshkam, *Food Chemistry*, 2012, **131**, 101–104.
194. P. Filipe, J. Haigle, J. N. Silva, J. Freitas, A. Fernandes, J. C. Maziere, C. Maziere, R. Santus and P. Morliere, *Eur. J. Biochem.*, 2004, **271**, 1991-1999.
195. N. Sugihara, T. Arakawa, M. Ohnishi and K. Furuno, *Free Radic. Biol. Med*., 1999, **27**, 1313-1323.
196. Q. Uddin, A. Malik, S. Azam, N. Hadi, A. S. Azmi, N. Parveen, N. U. Khan and S. M. Hadi, *Toxicol. in Vitro*, 2004, **18**, 435-440.
197. J. Tan, B. Wang and L. Zhu, *J. Biol. Inorg. Chem.*, 2009, **14**, 727-739.
198. K. Macakova, P. Mladenka, T. Filipsky, M. Riha, L. Jahodar, F. Trejtnar, P. Bovicelli, I. P. Silvestri, R. Hrdina and L. Saso, *Food Chem.*, 2012, **135**, 2584-2592.
199. M. S. Ahmad, F. Fazal, A. Rahman, S. M. Hadi and J. H. Parish, *Carcinogenesis*, 1992, **13**, 605-608.
200. S. C. Sahu and G. C. Gray, *Cancer Lett.*, 1993, **70**, 73-79.
201. A. M. Oliveira-Brett and V. C. Diculescu, *Bioelectrochemistry*, 2004, **64**, 133-141.
202. A. M. Oliveira-Brett and V. C. Diculescu, *Bioelectrochemistry*, 2004, **64**, 143-150.
203. S. Long, Y. F. Tian, Z. Cao, J. L. He and D. M. Luo, *Sens. Actuators, B*, 2012, **166**, 223-230.
204. V. Abalea, J. Cillard, M. P. Dubos, O. Sergent, P. Cillard and I. Morel, *Free Radic. Biol. Med.*, 1999, **26**, 1457-1466.
205. N. Yamashita, H. Tanemura and S. Kawanishi, *Mutat. Res*., 1999, **425**, 107-115.
206. S. Teixeira, C. Siquet, C. Alves, I. Boal, M. P. Marques, F. Borges, J. L. F. C. Lima and S. Reis, *Free Radic. Biol. Med*., 2005, **39**, 1099-1108.
207. A. Puppo, *Phytochemistry*, 1992, **31**, 85-88.
208. J. F. Moran, R. V. Klucas, R. J. Grayer, J. Abian and M. Becana, *Free Radic. Biol. Med*., 1997, **22**, 861-870.
209. M. J. Laughton, B. Halliwell, P. J. Evans and J. R. S. Hoult, *Biochem. Pharmacol*., 1989, **38**, 2859-2865.
210. Y. Hanasaki, S. Ogawa, S. Fukui, *Free Radic. Biol. Med*., 1994, **16**, 845-850.
211. G. Cao, E. Sofic, R. L. Prior, *Free Radic. Biol. Med*., 1997, **22**, 749-760.
212. M. Ypshino, K. Murakami, *Anal. Biochem*., 1998, **257**, 40-44.
213. Y.-C. Tsai, Y.-H. Wang, C.-C. Liou, Y.-C. Lin, H. Huang and Y.-C. Liu, *Chem. Res. Toxicol.*, 2012, **25**, 191–196.
214. E. Vlachodimitropoulou, P. A. Sharp and R. J. Naftalin, *Free Radic. Biol. Med.*, 2011, **50**, 934-944.
215. Y. Zhang, H. L. Li, Y. L. Zhao and Z. H. Gao, *Eur. J. Pharmacol.*, 2006, **535**, 263–269.
216. Y. Zhang, Z. Gao, J. Liu and Z. Xu, *Nat. Prod. Res.*, 2011, **25**, 1150-1160.
217. A. Hodkova, P. Cerna, D. Kotyzova and V. Eybl, *Hemoglobin*, 2010, **34**, 278-283.
218. V. Eybl, D. Kotyzova, P. Cerna and J. Koutensky, *Hum. Exp. Toxicol.*, 2008, **27**, 347-353.
219. S. M. Kuo, P. S. Leavitt and C. P. Lin, *Biol. Trace Elem. Res.*, 1998, **62**, 135-153.
220. S. M. Kuo, C. T. Huang, P. Blum and C. S. Chang, *Biol. Trace Elem. Res.*, 2001, **84**, 1-10.
221. J. Blasiak, M. Arabski, T. Pertynski, E. Malecka-Panas, K. Wozniak and J. Drzewoski, *Cell Biol. Toxicol*., 2002, **18**, 279-288.
222. L.-X. Wang, J.-P. Zeng, X.-B. Wei, F.-W. Wang, Z.-P. Liu and X.-M. Zhang, *Chin. J. Physiol*., 2007, **50**, 301-307.
223. D. Yilmaz, N. C. Aydemir, O. Vatan, E. Tuzun and R. Bilaloglu, *Toxicol. Ind. Health*, 2012, **28**, 114-121.
224. K. Pasternak, M. Szpetnar and A. Boguszewska, *Magnesium Res*., 2004, **17**, 72-78.
225. A. I. Morales, C. Vicente-Sanchez, J. M. Santiago Sandoval, J. Egido, P. Mayoral, M. A. Arevalo, M. Fernandez-Tagarro, J. M. Lopez-Novoa and F. Perez-Barriocanal, *Food Chem. Toxicol.*, 2006, **44**, 2092-2100.
226. S. Milton Prabu, K. Shagirtha and J. Renugadevi, *Eur. Rev. Med. Pharmacol. Sci.*, 2010, **14**, 903-914.
227. J. Renugadevi and S. M. Prabu, *Exp. Toxicol. Pathol.*, 2010, **62**, 471-481.
228. C. Vicente-Sanchez, J. Edigo, P. D. Sanchez-Gonzalez, F. Perez-Barriocanal, J. M. Lopez-Novoa and A. I. Morales, *Food Chem. Toxicol.*, 2008, **46**, 2279-2287.

229. J. Renugadevi and S. M. Prabu, *Toxicology*, 2009, **256**, 128-134.
230. O. A. Badary, S. Abdel-Maksoud, W. A. Ahmed and G. H. Owieda, *Life Sci.*, 2005, **76**, 2125-2135.
231. L. Pari and K. Shagirtha, *Exp. Toxicol. Pathol.*, 2012, **64** 513-520.
232. R. Li, C. Yuan, C. Dong, S. Shuang and M. M. F. Choi, *Naunyn Schmiedebergs Arch. Pharmacol.*, 2011, **383**, 437-445.
233. G. R. Mazzaron Barcelos, D. Grotto, J. M. Serpeloni, J. P. Friedmann Angeli, B. A. Rocha, V. C. de Oliveira Souza, J. T. Vicentini, T. Emanuelli, J. K. Bastos, L. M. Greggi Antunes, S. Knasmueller and F. Barbosa, Jr., *Arch. Toxicol.*, 2011, **85**, 1151-1157.
234. T. Bu, Y. Mi, W. Zeng and C. Zhang, *Anat. Rec.*, 2011, **294**, 520-526.
235. E. O. Farombi, I. A. Adedara, S. A. Akinrinde, O. O. Ojo and A. S. Eboh, *Andrologia*, 2012, **44**, 273-284.
236. S. Milton Prabu, M. Muthumani and K. Shagirtha, *Eur. Rev. Med. Pharmacol. Sci.*, 2013, **17**, 582-595.
237. K. Amudha and L. Pari, *Chem.-Biol. Interact.*, 2011, **193**, 57-64.
238. L. Pari and K. Amudha, *Eur. J. Pharmacol.*, 2011, **650**, 364-370.
239. P. Hu, M. Wang, W.-H. Chen, J. Liu, L. Chen, S.-T. Yin, W. Yong, J.-T. Chen, H.-L. Wang and D.-Y. Ruan, *Naunyn Schmiedebergs Arch. Pharmacol.*, 2008, **378**, 43-51.
240. A. Solti, L. Gaspar, P. Vagi, G. Zaray, F. Fodor and E. Sarvari, O*MICS*, 2011, **15**, 811-818.
241. R. Tolra, J. Barcelo and C. Poschenrieder, *J. Inorg. Biochem.*, 2009, **103**, 1486-1490.
242. V. A. Okello, S. Mwilu, N. Noah, A. Zhou, J. Chong, M. T. Knipfing, D. Doetschman and O. A. Sadik, *Environ. Sci. Technol.*, 2012, **46**, 10743-10751.
243. L. G. Domratcheva, N. B. Mel'nikova and G. A. Domrachev, *Dokl. Biochem. Biophys.*, 2005, **401**, 108-110.
244. S. Selvaraj, S. Krishnaswamy, V. Devashya, S. Sethuraman and U. Maheswari Krishnan, *Med. Res. Rev.*, 2014, **34**, 677–702.
245. G. Zhai, W. Zhu, Y. Duan, W. Qu and Z. Yan, *Main Group Met. Chem.*, 2012, **35**, 103–109.
246. G. Dehghan, J. E. Dolatabi, A. Jouyban, K. A. Zeynali, S. M. Ahmadi, S. Kashnian, *DNA Cell Biol.*, 2011, **30**, 195-201.
247. B. D. Wang, Z. Y. Yang, Q. Wang, T. K. Cai and P. Crewdson, *Bioorg. Med. Chem.*, 2006, **14**, 1880-1888.
248. E. G. Ferrer, M. V. Salinas, M. J. Correa, L. Naso, D. A. Barrio, S. B. Etcheverry, L. Lezama, T. Rojo and P. A. M. Williams, *J. Biol. Inorg. Chem.*, 2006, **11**, 791-801.
249. B. Kosmider, E. Zyner, R. Osiecka and J. Ochocki, *Mutat Res*, 2004, **563**, 61-70.
250. B. Kosmider, R. Osiecka, E. Ciesielska, L. Szmigiero, E. Zyner and J. Ochocki, *Mutat Res*, 2004, **558**, 169-179.
251. K. Hiramoto, N. Ojima, K. Sako and K. Kikugawa, *Biol. Pharm. Bull.*, 1996, **19**, 558-563.
252. Y. Chashi, K. Yoshinaga, H. Yoshioka and H. Yoshioka, *Biosci., Biotechnol., Biochem.*, 2002, **66**, 770-776.
253. J. Tan, W. Bochu and Z. Liancai, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 1197-1199.
254. T. Jun, W. Bochu and Z. Liancai, *Colloids Surf., B*, 2007, **55**, 149-152.
255. Q. Wang, M. Huang, Y. Huang, J.-S. Zhang, G.-F. Zhou, R.-Q. Zeng and X.-B. Yang, *Med. Chem. Res.*, 2014, **23**, 2659-2666.
256. J. Zhou, J. Y. Wang and N. Tang, *Indian J. Chem., Sect. A: Inorg., Bio-inorg., Phys., Theor. Anal. Chem.*, 2001, **40**, 149-154.
257. Y. B. Zeng, N. Yang, W. S. Liu and N. Tang, *J. Inorg. Biochem.*, 2003, **97**, 258-264.
258. J. W. Kang, L. Zhuo, X. Q. Lu, H. D. Liu, M. Zhang and H. X. Wu, *J. Inorg. Biochem.*, 2004, **98**. 79-86.
259. Y. M. Song, J. W. Kang, J. Zhou, Z. H. Wang, X. Q. Lu, L. F. Wang and J. Z. Gao, *Spectrochim. Acta, Part A*, 2000, **56**, 2491-2497.
260. Y. Song, J. Kang, Z. Wang, X. Lu, J. Gao and L. Wang, *J. Inorg. Biochem.*, 2002, **91**, 470-474.
261. A. A. Ensafi, R. Hajian and S. Ebrahimi, *J. Braz. Chem. Soc.*, 2009, **20**, 266-276.
262. A. A. Jamali, A. Tavakoli and J. E. N. Dolatabadi, *Eur. Food Res. Technol.*, 2012, **235**, 367-373.
263. S. Niu, B. Han, W. Cao and S. Zhang, *Anal. Chim. Acta*, 2009, **651**, 42-47.
264. Y. Ni, S. Du and S. Kokot, *Anal. Chim. Acta*, 2007, **584**, 19-27.
265. L. D. Mello, R. M. S. Pereira, A. C. H. F. Sawaya, M. N. Eberlin and L. T. Kubota, *J. Pharm. Biomed. Anal.*, 2007, **45**, 706–713.
266. J. Tan, L. Zhu and B. Wang, *Dalton Trans.*, 2009, **24**, 4722-4728.
267. M. S. Ahmed, V. Ramesh, V. Nagaraja, J. H. Parish and S. M. Hadi, *Mutagenesis*, 1994, **9**, 193-197.
268. J. Tan, L. Zhu and B. Wang, *Biometals*, 2010, **23**, 1075–1084.
269. A. Constantinou, R. Mehta, C. Runyan, K. Rao, A. Vaughan and R. Moon, *J. Nat. Prod.*, 1995, **58**, 217-225.
270. S. K. Singh, S. Joshi, A. R. Singh, J. K. Saxena and D. S. Pandey, *Inorg. Chem.*, 2007, **46**, 10869-10876.
271. A. Kurzwernhart, W. Kandioller, C. Bartel, S. Baechler, R. Trondl, G. Muehlgassner, M. A. Jakupec, V. B. Arion, D. Marko, B. K. Keppler and C. G. Hartinger, *Chem. Commun.*, 2012, **48**, 4839-4841.
272. A. Kurzwernhart, W. Kandioller, E. A. Enyedy, M. Novak, M. A. Jakupec, B. K. Keppler and C. G. Hartinger, *Dalton Trans.*, 2013, **42**, 6193-6202.
273. S. Tabassum, M. Zaki, M. Afzal and F. Arjmand, *Dalton Trans.*, 2013, **42**, 10029-10041.
274. A. Cremieux, *Revue Du Praticien*, 1980, **30**, 2185-2195.
275. R. H. Kampmeier, *Sex. Transm. Dis.*, 1981, **8**, 224-226.
276. T. P. T. Cushnie and A. J. Lamb, *Int. J. Antimicrob. Agents*, 2011, **38**, 99-107.
277. M. K. Hamdy and S. R. Wheeler, *Bull. Environ. Contam. Toxicol.*, 1978, **20**, 378-386.
278. S. X. Wang, F. J. Zhang, Q. P. Feng and Y. L. Li, *J. Inorg. Biochem.*, 1992, **46**, 251-257.
279. Y. Liu, X. He, X. Liu, H. Zuo, Z. Li, Z. Wu, C. Xiang and X. Lai, *Zhongguo Zhong yao za zhi = Zhongguo zhongyao zazhi = China journal of Chinese materia medica*, 2012, **37**, 1296-1302.
280. L. Mishra and A. K. Singh, *Indian J. Chem., Sect. A: Inorg., Bio-inorg., Phys., Theor. Anal. Chem.*, 2001, **40**, 1288-1294.
281. L. Mishra, A. K. Singh, S. K. Trigun, S. K. Singh and S. M. Pandey, *Indian J. Exp. Biol.*, 2004, **42**, 660-666.
282. M. Kopacz, E. Woznicka and J. Gruszecka, *Acta Pol. Pharm.*, 2005, **62**, 65-67
283. L. Mishra, A. K. Singh and Y. Maeda, *J. Indian Chem. Soc.*, 2005, **82**, 879-885.
284. H. S. Lee, K.-s. Park, C. Lee, B. Lee, D.-E. Kim and Y. Chong, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 5709-5712.
285. P. Borissova, S. Valcheva and A. Belcheva, *Acta Physiol. Pharmacol. Bulg.*, 1994, **20**, 25-30.
286. J. Li, L. Wang, H. Bai, B. Yang and H. Yang, *Med. Chem. Res.*, 2011, **20**, 88-92.
287. M. Badea, R. Olar, V. Uivarosi, D. Marinescu, V. Aldea, S. F. Barbuceanu and G. M. Nitulescu, *J. Therm. Anal. Calorim.*, 2011, **105**, 559–564.
288. R. Shukla, V. Barve, S. Padhye and R. Bhonde, *Biometals*, 2006, **19**, 685-693.
289. L. H. Cazarolli, L. Zanatta, A. P. Jorge, E. de Sousa, H. Horst, V. M. Woehl, M. G. Pizzolatti, B. Szpoganicz and F. Silva, *Chem.-Biol. Interact.*, 2006, **163**, 177-191.
290. S. O. Emdin, G. G. Dodson, J. M. Cutfield and S. M. Cutfield, *Diabetologia*, 1980, **19**, 174-182.
291. K. Vijayaraghavan, S. I. Pillai and S. P. Subramanian, *Eur. J. Pharmacol.*, 2012, **680**, 122-129.
292. V. Kuntic, I. Filipovic and Z. Vujic, *Molecules*, 2011, **16**, 1378-1388.
293. K. Ono, Y. Yoshiike, A. Takashima, K. Hasegawa, H. Naiki and M.Yamada, *J. Neurochem.*, 2003, **87**, 172-181.
294. X. He, H. M. Park, S.-J. Hyung, A. S. DeToma, C. Kim, B. T. Ruotolo and M. H. Lim, *Dalton Trans.*, 2012, **41**, 6558-6566.
295. W. Maung Tay, G. F. Z. da Silva and L.-J. Ming, *Inorg. Chem.*, 2013, **52**, 679-690.
296. R. Liu, F. Meng, L. Zhang, A. Liu, H. Qin, X. Lan, L. Li and G. Du, *Molecules*, 2011, **16**, 2084-2096.
297. A. S. DeToma, J. S. Choi, J. J. Braymer and M. H. Lim, *ChemBioChem*, 2011, **12**, 1198-1201.
298. Y. Yang, L. Zhu, M. Cui, R. Tang and H. Zhang, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 5337-5344.



299. M. Ono, R. Ikeoka, H. Watanabe, H. Kimura, T. Fuchigami, M. Haratake, H. Saji and M. Nakayama, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 5743-5748.
300. Y. S. Tarahovsky, E. A. Yagolnik, E. N. Muzafarov, B. S. Abdrasilov and Y. A. Kim, *Biochim. Biophys. Acta - Biomembranes*, 2012, **1818**, 695-702.
301. S. Selvaraj, S. Krishnaswamy, V. Devashya, S. Sethuraman and U. M. Krishnan, *Langmuir*, 2011, **27**, 13374-13382.
302. G. Cetta, G. Pallavicini, A. Calatroni, G. Fossati and A. A. Castellani, *Ital. J. Biochem.*, 1975, **24**, 46-47.
303. G. Cetta, G. Pallavicini, R. Tenni and C. Bisi, *Ital. J. Biochem.*, 1977, **26**, 317-327.
304. Y.-J. Hu, H.-L. Yue, X.-L. Li, S.-S. Zhang, E. Tang and L.-P. Zhang, *J. Photochem. Photobiol., B*, 2012, **112**, 16-22.
306. S. Cao, X. Jiang and J. Chen, *J. Inorg. Biochem.*, 2010, **104**, 146-152.
306. A. S. Roy, D. R. Tripathy, A. K. Ghosh and S. Dasgupta, *J. Lumin.*, 2012, **132**, 2943–2951.
307. A. H. Hegde, B. Sandhya and J. Seetharamappa, *Mol. Biol. Rep.*, 2011, **38**.
308. S. Shi, Y. Zhang, X. Chen and M. Peng, *J. Agric. Food Chem.*, 2011, **59**, 8499-8506.
309. H. O. Gutzeit, Y. Henker, B. Kind and A. Franz, *Biochem. Bioph. Res. Commun.*, 2004, **318**, 490-495.
310. J. Tian, J. Liu, X. Tian, Z. Hu and X. Chen, *J. Mol. Struct*., 2004, **691**, 197-202.
311. A. Papadopoulou, R. J. Green and R. A. Frazier, *J. Agric. Food Chem*., 2005, **53**, 158-163.
312. H. M. Rawel, K. Meidtner and J. Kroll, *J. Agric. Food Chem*., 2005, **53**, 4228-4235.
313. S. Martini, C. Bonechi and C. Rossi, *J. Nat. Prod*., 2008, **71**, 175-178.
314. J. Xiao, M. Suzuki, X. Jiang, X. Chen, K. Yamamoto, F. Ren, and M. Xu, *J. Agric. Food Chem*., 2008, **56**, 2350-2356 and refs therein.
315. F. Yang, J. Wang, C. Liu, X. Xu, S. Shan, Z. Xia and X. Sun, *J. Solution Chem.*, 2012, **41**, 976-993.
316. D. Li, M. Zhu, C. Xu and B. Ji, *Eur. J. Med. Chem.*, 2011, **46**, 588-599.
317. F. Yang, J. Wang, C. Liu, X. Xu, W. Li, Z. Xia and J. Xiao, *Arch. Biol. Sci.*, 2011, **63**, 623-634.
318. M. Peng, S. Shi and Y. Zhang, *Environ. Toxicol. Pharmacol.*, 2012, **33**, 327-333.
319. D. Kim, J. Park, J. Kim, C. Han, J. Yoon, N. Kim, J. Seo and C. Lee, *J. Agric. Food Chem.*, 2006, **54**, 935-941.
320. Y.-X. Si, Z.-J. Wang, D. Park, H. Y. Chung, S.-F. Wang, L. Yan, J.-M. Yang, G.-Y. Qian, S.-J. Yin and Y.-D. Park, *Int. J. Biol. Macromol.*, 2012, **50**, 257-262.
321. Y. Wang, G. Zhang, J. Yan and D. Gong, *Food Chem.*, 2014, **163**, 226-233.
322. Y.-X. Si, S.-J. Yin, S. Oh, Z.-J. Wang, S. Ye, L. Yan, J.-M. Yang, Y.-D. Park, J. Lee and G.-Y. Qian, *J. Biomol. Struct. Dyn.*, 2012, **29**, 999-1012.
323. N. Al Shukor, J. Van Camp, G. B. Gonzales, D. Staljanssens, K. Struijs, M. J. Zotti, K. Raes and G. Smagghe, *J. Food Chem.*, 2013, **61**, 11832-11839.
324. A. Berger, S. Venturelli, M. Kallnischkies, A. Boecker, C. Busch, T. Weiland, S. Noor, C. Leischner, T. S. Weiss, U. M. Lauer, S. C. Bischoff and M. Bitzer, *J. Nutr. Biochem.*, 2013, **24**, 977-985.
325. W.-F. Peng and C.-Y. Huang, *Biochimie*, 2014, **101C**, 113-122.
326. A. L. Perryman, S. Forli, G. M. Morris, C. Burt, Y. Cheng, M. J. Palmer, K. Whitby, J. A. McCammon, C. Phillips and A. J. Olson, *J. Mol. Biol.*, 2010, **397**, 600-615.
327. B.-W. Li, F.-H. Zhang, E. Serrao, H. Chen, T. W. Sanchez, L.-M. Yang, N. Neamati, Y.-T. Zheng, H. Wang and Y.-Q. Long, *Bioorg. Med. Chem.*, 2014, **22**, 3146-3158.
328. D. R. Tripathy, A. S. Roy and S. Dasgupta, *FEBS Letters*, 2011, **585**, 3270-3276.
329. J. Kaizer, I. Ganszky, G. Speier, A. Rockenbauer, L. Korecz, M. Giorgi, M. Reglier and S. Antonczak, *J. Inorg. Biochem.*, 2007, **101**, 893-899.
330. I. Lippai and G. Speier, *J. Mol. Catal. A: Chem.*, 1998, **130**, 139-148.
331. J. S. Pap, A. Matuz, G. Barath, B. Kripli, M. Giorgi, G. Speier and J. Kaizer, *J. Inorg. Biochem.*, 2012, **108**, 15-21.
332. K. Grubel, B. J. Laughlin, T. R. Maltais, R. C. Smith, A. M. Arif and L. M. Berreau, *Chem. Commun.*, 2011, **47**, 10431-10433.
333. J. Kaizer, E. Balogh-Hergovich, M. Czaun, T. Csay and G. Speier, *Coord. Chem. Rev.*, 2006, **250**, 2222-2233.
334. J. S. Pap, J. Kaizer and G. Speier, *Coord. Chem. Rev.*, 2010, **254**, 781-793.