

# *Metal complexes of flavonoids: their synthesis, characterization, and enhanced anti-oxidant and anti-cancer activities*

Article

Accepted Version

Khater, M., Ravishankar, D., Greco, F. and Osborn, H. ORCID: <https://orcid.org/0000-0002-0683-0457> (2019) Metal complexes of flavonoids: their synthesis, characterization, and enhanced anti-oxidant and anti-cancer activities. *Future Medicinal Chemistry*, 11 (21). ISSN 1756-8927 doi: <https://doi.org/10.4155/fmc-2019-0237> Available at <https://centaur.reading.ac.uk/86372/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

To link to this article DOI: <http://dx.doi.org/10.4155/fmc-2019-0237>

Publisher: Future Science Group

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the [End User Agreement](#).

[www.reading.ac.uk/centaur](http://www.reading.ac.uk/centaur)

**CentAUR**

Central Archive at the University of Reading

Reading's research outputs online

# **Metal complexes of Flavonoids: Their synthesis, characterization, and enhanced anti-oxidant and anti-cancer activities**

Mai Khater<sup>1,2</sup>, Divyashree Ravishankar<sup>1</sup>, Francesca Greco<sup>1</sup> and Helen M.I. Osborn<sup>1\*</sup>

<sup>1</sup> School of Pharmacy, University of Reading, Whiteknights, Reading, RG6 6AD. UK

<sup>2</sup>Therapeutic Chemistry Department, Pharmaceutical and Drug Industries Research Division, National Research Centre, Cairo, Egypt.

## **Abstract**

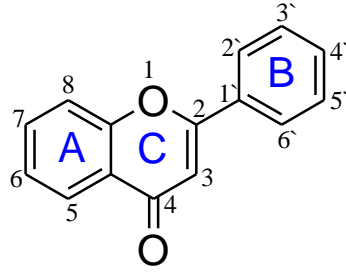
Flavonoids are polyphenolic compounds of natural origin. They are extensively studied within drug discovery programmes due to their wide ranging biological activities such as anti-microbial, anti-oxidant, anti-tumor, neuroprotective and cardioprotective properties. The ability of flavonoids to coordinate with metal atoms has provided new leads for drug discovery programmes, with better pharmacological activities and clinical profiles than the parent flavonoids. In this review, the enhanced anti-oxidant and anti-cancer activities of flavonoid metal complexes versus the parent flavonoids are discussed. Possible mechanisms of action for the metal complexes, such as DNA binding and apoptosis induction, are also presented alongside an overview of the synthesis of the metal complexes, and the different techniques used for their characterization.

## **Keywords**

Flavonoid, metal, flavonoid metal complex, anti-oxidant, anti-cancer.

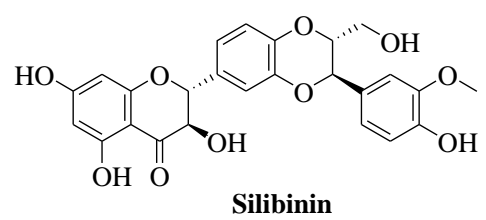
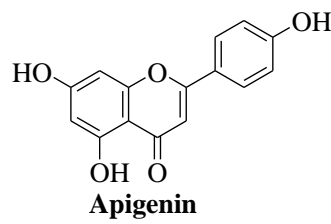
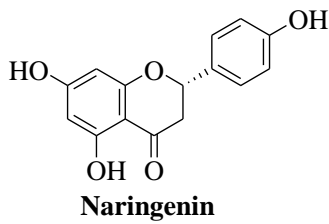
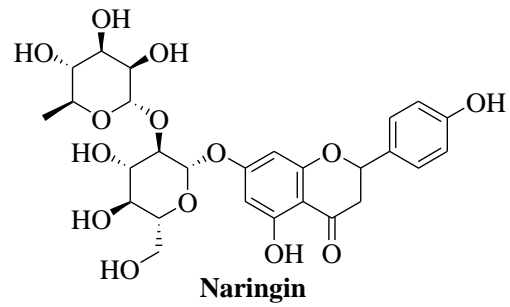
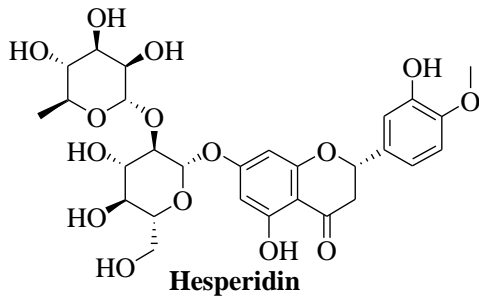
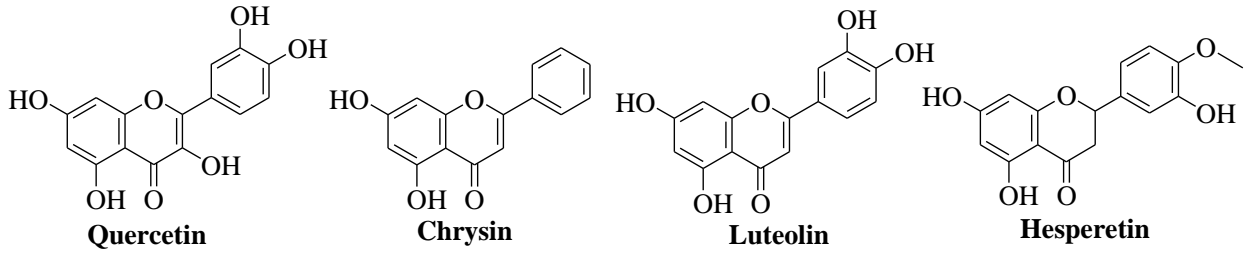
## **Introduction**

Flavonoids are phytochemicals that are mainly found in tea, citrus fruit, berries, apples and legumes. Chemically, they are polyphenolic compounds with a C<sub>6</sub>-C<sub>3</sub>-C<sub>6</sub> ring system (**Figure 1**) and they are often found in esterified or glycosylated forms [1]. It was not until the 1990's that research on flavonoids witnessed significant progress, with the number of publications increasing by approximately 6-fold, from 524 in 1990 to 3147 in 2017. As a result it is now well established that flavonoids have a wide and diverse range of biological activities [2, 3] such as anti-viral [4-6], anti-bacterial [7-10], neuroprotective [11-13], cardioprotective [14, 15], anti-oxidant [16-18] and anti-cancer [3, 19, 20] properties.

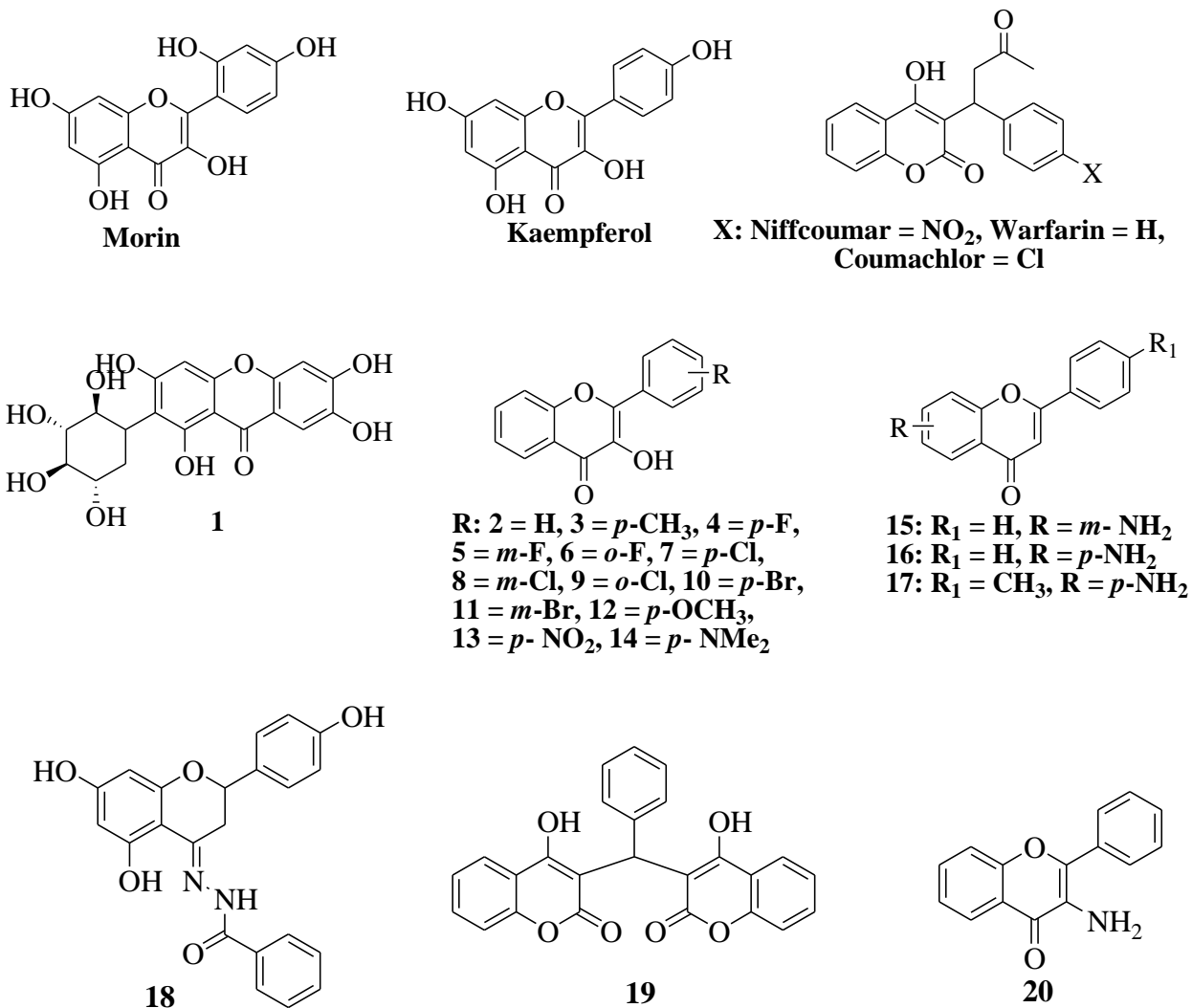


31

**Basic skeleton of flavonoids**



32



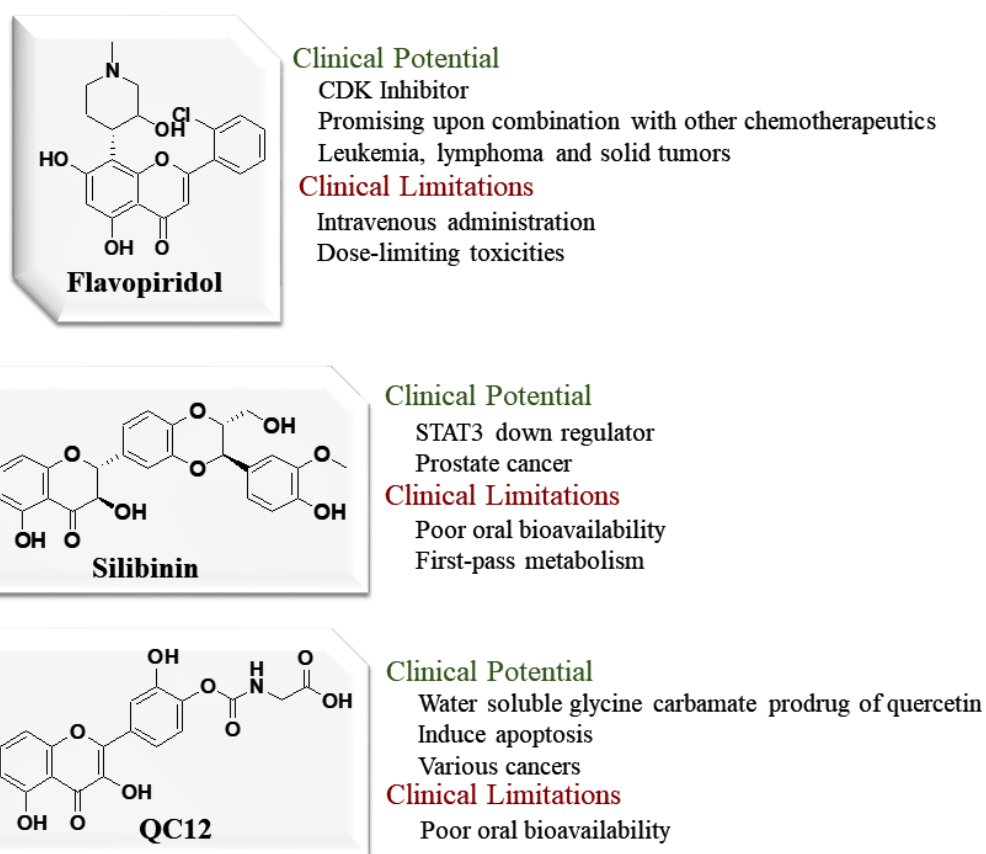
33

34

35 **Figure 1.** Chemical structures of flavonoids from which organometallic derivatives have been  
 36 prepared

37 With respect to the beneficial health effects of flavonoids, the greatest impact has been seen  
 38 within the anti-cancer field [2]. Flavonoids are known to interfere with an array of targets  
 39 affecting cancer growth and progression. For example, they have been shown to induce cell cycle  
 40 arrest and apoptosis [21] in addition to inhibiting mitotic spindle formation [22] and  
 41 angiogenesis [23, 24]. Despite the advantages of having a compound that can interact with  
 42 different targets, this can be a limitation due to limited selectivity. Indeed, this has been one of  
 43 the biggest obstacles in the use of flavonoids as potential drugs due to its direct relation with  
 44 adverse effects and poor *in vivo* toxicity profiles. However, despite the extensive research and  
 45 number of structures and the activity reported for them, no flavonoid has to date reached the

46 market and only a limited number are in clinical investigation as anti-cancer agents, eg  
 47 flavopiridol (phase-II) [25, 26], silibinin (phase-II) [27, 28], quercetin (phase-II) [29, 30] and the  
 48 quercetin derivative QC12 (phase-I) [31] (**Figure 2**). Arguably, the main drug development  
 49 challenge is the poor bioavailability of flavonoids, resulting from water insolubility and  
 50 susceptibility to glucourinidation and/or methylation by intestinal and liver metabolism (first  
 51 pass effect). There are several studies on the pharmacokinetics of flavonoids indicating their poor  
 52 bioavailabilities. Wu et al., for instance, reported the absolute oral bioavailability of silibinin in  
 53 rats to be approximately 0.95% [32]. In another study, flavopiridol's mean oral bioavailability  
 54 following bolus intra-gavage was shown to be 20% [33]. Moreover, a study by Gugler et al.  
 55 failed to detect any plasma quercetin concentrations in subjects receiving 4 g orally [34].



56

57

**Figure 2.** Flavonoids in clinical trials as anti-cancer agents

58 Due to the presence of hydroxyl and oxo groups within flavonoids, they possess metal chelating  
 59 abilities that can have profound effects on their pharmacokinetic and pharmacological properties  
 60 [35, 36]. The flavonoid structure, type of chelating metal and the pH of the surrounding medium

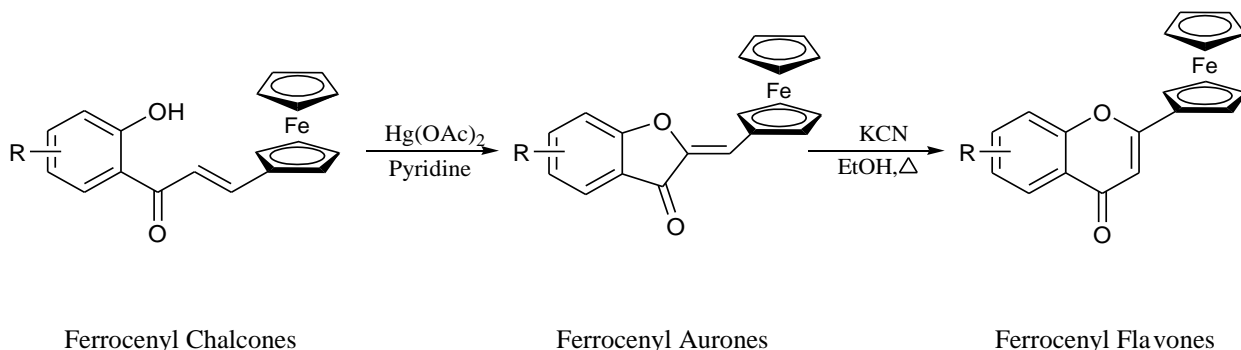
61 are determining factors of the preferred coordination site [37]. Metal atoms have beneficial  
62 biological properties such as chelating free radicals and binding to DNA [38]. These properties  
63 can cause synergistic effects when combined with other active chemical entities such as the  
64 flavonoids. Accordingly, metal complexation is reported to enhance the anti-oxidant, anti-  
65 proliferative and chemical properties of parent flavonoids. This review will therefore highlight  
66 important advances within the field of metal-flavonoid complexes with particular emphasis on  
67 their synthesis, methods of characterization, anti-oxidant and anti-proliferative activities.

68

69

## 70 **Synthesis and characterization of flavonoid metal complexes**

71 Flavonoid metal complexes are generally synthesized by dissolving a flavonoid salt in an  
72 alcoholic or aqueous solution, followed by the addition of the metal salt also in alcoholic or  
73 aqueous solution. The reaction can be carried out under different conditions of stirring and/or  
74 heating, normally a base is used to deprotonate the hydroxyl groups and facilitate metal  
75 coordination. The complex usually precipitates from solution and is then filtered and air dried  
76 (**Table 1**). Preparations of ferrocenyl flavonoid complexes, where the ferrocenyl moiety acts as a  
77 replacement for ring B, follow a different synthetic approach. Ferrocenyl aurones are obtained  
78 via a classic Claisen-Schmidt condensation of ferrocene carboxaldehyde and various 2-  
79 hydroxyacetophenones to obtain the corresponding ferrocene chalcones [39, 40]. The  
80 synthesized chalcones are then treated with mercury acetate in pyridine to form the ferrocenyl  
81 aurones that can be isomerized into their flavone counterparts by heating in ethanol at reflux with  
82 potassium cyanide (**Figure 3**) [39, 40].

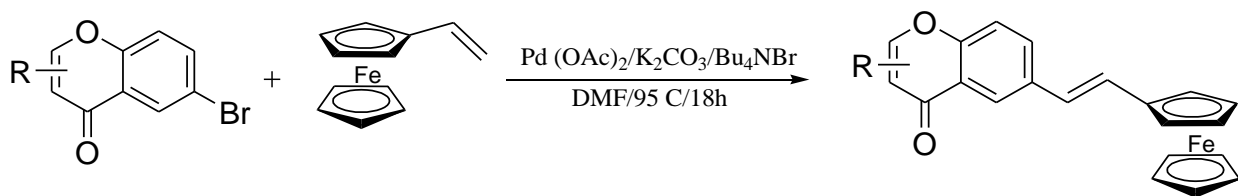


86

### Figure 3. Synthesis of ferrocenyl flavonoids

87 Kowalski et al. reported the synthesis of ferrocenyl flavonoids using the palladium-catalyzed

88 Heck cross-coupling reaction of vinyl ferrocene with 6-bromochromones (**Figure 4**) [41].



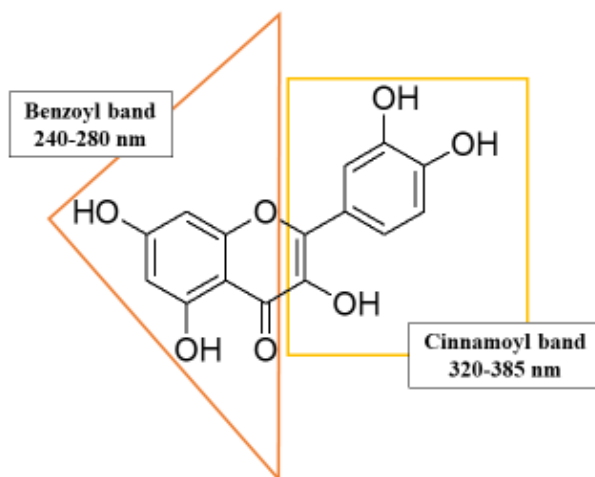
89

### Figure 4. Synthesis of ferrocenyl flavonoids using Pd

91 Structural characterization of metal complexes can be achieved using a number of methods  
92 (**Table 1**). Fourier transform infra-red (FT-IR) spectroscopy can indicate the coordination site of  
93 the metal. Complexation of the metal atom with the C=O group, for instance, causes an increase  
94 in the bond length which is manifested as a decrease in the frequency of the C=O peak.  
95 Coordination with OH groups leads to disappearance of their characteristic broad peaks  $\nu$  OH at  
96 3600-3200  $\text{cm}^{-1}$ . However, this disappearance can be masked by the presence of other OH  
97 groups in the flavonoid structure and in the H<sub>2</sub>O molecules incorporated in the metal sphere  
98 during complexation. Therefore, IR spectroscopy is not a helpful tool in determining which OH  
99 groups are specifically coordinated to the metal atom. <sup>1</sup>H NMR spectroscopic analysis can  
100 provide a better alternative where the disappearance of an OH peak from the <sup>1</sup>H NMR spectrum  
101 corresponds to coordination at this particular OH group. For example, the disappearance of the 5-  
102 OH peak at  $\delta$  10.52 ppm of the **lanthanum-18** complex proved coordination at this OH in ring  
103 A [42]. Heteroatom NMR spectroscopic analysis is also extremely useful. For example,  
104 Tabassum et al. used <sup>119</sup>Sn NMR spectroscopic analysis to prove the presence of stannous atoms  
105 in the synthesized bi-metallic complexes **31** and **32** (**Figure 6**) [43]. <sup>195</sup>Pt NMR spectroscopic  
106 analysis was also used to prove the structure of complex **68** [44]. Electron Paramagnetic  
107 Resonance (EPR) spectroscopy is also a helpful technique which is similar to NMR spectroscopy  
108 but more sensitive as it explores the interaction between an external magnetic field and unpaired  
109 electrons rather than nuclei. This is attributed to the fact that the amount of energy absorbed by a  
110 spinning electron is higher than that absorbed by a nucleus. This technique is more difficult to  
111 use as it requires background knowledge of subjects like quantum mechanics and mathematical  
112 techniques [45]. However it was used by Tabassum and Naso et al. groups to elucidate structures



113 of complexes (**31** and **32**) and (**33**, **37**, **VOhespiridin**, **VOsilibinin** and **VOmorin**), respectively  
114 [43, 46-49].



115

116 **Figure 5.** UV absorption bands using quercetin as an illustrative example

117 Flavonoids display absorption bands in the UV-Vis region due to electronic  $\pi - \pi^*$  transitions  
118 [50, 51]. These transitions are responsible for the two characteristic benzoyl and cinnamoyl  
119 bands of flavonoids at 240-280 nm and 320-385 nm, respectively (**Figure 5**) which are  
120 bathochromically shifted after chelating with metals [52]. The observation of a red shift in one  
121 band rather than the other verifies chelation with that particular ring [53-55]. Although the shift  
122 is small in many cases, it gives a good insight when overlapped with the parent's spectrum. UV-  
123 Vis absorption bands are also characteristic to binding in other sites. Analysis of the spectra of  
124 **VOhespiridin** showed complexation with the rutinose sugar part [46]. Its electronic spectra  
125 showed bands at 358, 520 and 702 nm in agreement with the three band pattern at ca. 420, 500  
126 and 700 nm observed for similar sugar vanadyl(IV) complexes coordinated through deprotonated  
127 cis-hydroxyl groups [56]. UV-Vis spectra can also help in detecting the stoichiometry of the  
128 complex. This is achieved by monitoring the absorption intensity of the ligand through a range of  
129 concentrations until the reaction is completed [46, 47, 57, 58]. Different methods can then be  
130 used to represent the data, e.g. Job's method, from which ligand-metal ratio can be interpreted.  
131 Similarly, fluorescence of free parents can be compared to their complexes at a specific  
132 wavelength. Peaks from complexes are higher in intensity and are shifted to lower wavelengths  
133 ca. 15-20 nm [55]. Elemental analysis and mass spectrometry provide complementary

134 information about the molecular formulae of the resulting complexes. In a recent study by Wang  
135 et al. the elemental analysis of a lead-luteolin complex was determined by scanning electron  
136 microscope (SEM) equipped with energy dispersive spectroscopy (EDS) [59]. The experimental  
137 values of weight and atomic number percentages of carbon, oxygen and lead atoms favored a 2:1  
138 rather than 1:1 complex [59]. Analysis of the fragmentation pattern in mass spectrometry  
139 specifies how many metal atoms are coordinated in the complex [43, 60-64]. Roy et al. and Naso  
140 et al. reported the synthesis of two different complexes (**36** and **37** respectively) using the same  
141 ligand (**Luteolin**) and metal (**Vanadium**) [49, 65]. The two complexes are reported to differ in  
142 their stoichiometries and metal coordination sites which may have resulted from different  
143 ligand/metal ratios added during the synthesis process. Thus for complex **36**, two luteolin  
144 molecules are reported to chelate to the oxidovanadium(VI) group via the 4-C=O and 5-OH  
145 functionalities, whilst **37** is reported to contain only one luteolin chelated to the 3' and 4' cis-OH  
146 groups in ring B. Among the characterization techniques provided by Roy and his group, mass  
147 spectrometry data supported their structural hypothesis for complex **36**. Thus peaks at m/z  
148 623.11, 639.89 and 658.21 represented two luteolin + one vanadium, two luteolin + vanadium  
149 oxide and two luteolin + vanadium oxide with one molecule of water, respectively. When  
150 deducing the structure of complex **37**, Nato et al. demonstrated that the FT-IR spectrum showed  
151 no change in the C=O and the C2=C3 bands, in addition to an increase of 44 cm<sup>-1</sup> of the 4'-OH  
152 band, indicating chelation with ring B. EPR and UV-Vis spectra of complex **37** also supported  
153 the same findings. Thermal gravimetric studies provide information on the heat-induced  
154 decomposition of the complex as a function of temperature or time. Dehydration happens during  
155 the early stages at temperatures ranging from 100-250 °C. This is followed by decomposition of  
156 the ligand at higher temperatures (400-600 °C). At temperatures as high as 900 °C, the complex  
157 decomposes completely leaving the metal oxide [42, 43, 55, 60, 62, 66].

158 Nevertheless, the best proven technique to confirm a theoretical structure is X-ray  
159 crystallography. Unfortunately, it is not feasible to apply this to all synthesized molecules due to  
160 the specific crystal requirements needed to run an X-ray. Only structures of complexes **49**, **51**, **53**  
161 and **55** [67, 68] were proven by X-ray crystallography.

162  
163 In general, all the used characterization methods can provide helpful information of varying  
164 importance. NMR and UV-Vis spectroscopy are the most insightful if X-ray is not feasible.

165 Other methods like IR spectroscopy, elemental analysis and mass spectrometry can complement  
166 the results obtained and offer the advantages of being easy to interpret and available in most  
167 research facilities.

168 **Table 1.** Summary of synthetic conditions and characterization methods for flavonoid metal complexes

<i>Flavonoid Solution</i>	<i>Metal</i>	<i>Base used and pH</i>	<i>Final product Formula</i>	<i>Characterization</i>	<i>Ref</i>
<i>Quercetin.2H<sub>2</sub>O in EtOH</i>	NiCl <sub>2</sub> ·6H <sub>2</sub> O	NaOEt 6-7	Ni(Que) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub>	UV IR Elemental Analysis	[54]
	CuCl <sub>2</sub> ·2H <sub>2</sub> O	NaOEt 6-7	Cu(Que) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub>	UV IR Elemental Analysis	[53]
	La acetate Nd acetate Eu acetate Gd acetate Tb acetate Dy acetate Tm acetate Y acetate	NaOEt <i>N.A.</i>	La(Que) <sub>3</sub> (H <sub>2</sub> O) <sub>6</sub> Nd(Que) <sub>3</sub> (H <sub>2</sub> O) <sub>6</sub> Eu(Que) <sub>3</sub> (H <sub>2</sub> O) <sub>6</sub> Gd(Que) <sub>3</sub> (H <sub>2</sub> O) <sub>6</sub> Tb(Que) <sub>3</sub> (H <sub>2</sub> O) <sub>6</sub> Dy(Que) <sub>3</sub> (H <sub>2</sub> O) <sub>6</sub> Tm(Que) <sub>3</sub> (H <sub>2</sub> O) <sub>6</sub> Y(Que) <sub>3</sub> (H <sub>2</sub> O) <sub>6</sub>	UV IR <sup>1</sup> H NMR TG-DTA Fluorescence analysis Electrochemistry Elemental analysis	[55]

<i>[Cu(Que)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>]</i> <i>in MeOH</i>	SnCl <sub>4</sub>	N.A.	[Cu(Que) <sub>2</sub> (H <sub>2</sub> O) <sub>6</sub> -Sn <sub>2</sub> Cl <sub>4</sub> ]	IR <sup>1</sup> H, <sup>13</sup> C & <sup>119</sup> Sn NMR EPR ESI-MS TG-DTG	[43]
<i>[Zn(Que)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>]</i> <i>in MeOH</i>	SnCl <sub>4</sub>	N.A.	[Zn(Que) <sub>2</sub> (H <sub>2</sub> O) <sub>6</sub> -Sn <sub>2</sub> Cl <sub>4</sub> ]	IR <sup>1</sup> H, <sup>13</sup> C & <sup>119</sup> Sn NMR EPR ESI-MS TG-DTG	[43]
<i>Chrysin in EtOH</i>	Vanadyl acetylacetonate	N.A. 5	VO(Chry) <sub>2</sub> EtOH	UV-Vis IR EPR Spectrophotometric titrations	[58]
	Ph <sub>3</sub> GeBr	Na <sub>2</sub> CO <sub>3</sub> N.A.	Chry-Ge. C <sub>2</sub> H <sub>6</sub> O	IR <sup>1</sup> H & <sup>13</sup> C NMR Elemental analysis	[69]

	La acetate	NaOH N.A.	La(Chry) <sub>2</sub> .OAc(H <sub>2</sub> O) <sub>7</sub>	IR <sup>1</sup> H NMR Elemental analysis TG-DTG Spectrophotometric titrations	[57]
<i>Luteolin in H<sub>2</sub>O</i>	VOSO <sub>4</sub> .H <sub>2</sub> O	NaOH 6	VO(Lut) <sub>2</sub>	UV-Vis IR <sup>1</sup> H NMR ESI-MS	[65]
<i>Luteolin</i>	50% aqueous solution of VOCl <sub>2</sub>	NaOH 5	[VO(Lut)(H <sub>2</sub> O) <sub>2</sub> ]Na·3H <sub>2</sub> O	UV-Vis IR EPR	[49]
<i>Luteolin in EtOH</i>	Mn(CH <sub>3</sub> COO) <sub>2</sub>	N.A. 4	MnO-Lut	Elemental analysis UV-Vis IR Elemental analysis TG-DTG	[70]
<i>Hesperidin in H<sub>2</sub>O</i>	50% aqueous solution of VOCl <sub>2</sub>	NaOH 12	[VO(Hesp)(OH) <sub>3</sub> ]. Na <sub>4</sub> (H <sub>2</sub> O) <sub>3</sub>	UV-Vis IR EPR Spectrophotometric	[46]

<i>Hesperetin in EtOH</i>	CuCl <sub>2</sub> ·2H <sub>2</sub> O	NH <sub>3</sub> solution 7-8	[Cu(Hespt) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ] · H <sub>2</sub> O	titrations	[66]
				UV-Vis IR ESI-MS TG-DTG	
<i>Naringin in MeOH</i>	Cu acetate in distilled H <sub>2</sub> O	N.A.	[Cu (Nar)] <sup>+</sup> [CH <sub>3</sub> COO] <sup>-</sup> · (H <sub>2</sub> O) <sub>5</sub>	UV-Vis	[71]
				IR <sup>1</sup> H NMR ESI-MS Elemental analysis	
<i>Naringenin in EtOH</i>	CuCl <sub>2</sub> ·2H <sub>2</sub> O	NH <sub>3</sub> solution 7-8	[Cu(Narg) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ] · H <sub>2</sub> O	UV-Vis	[66]
				IR ESI-MS TG-DTG	
<i>Apigenin in EtOH</i>	CuCl <sub>2</sub> ·2H <sub>2</sub> O	NH <sub>3</sub> solution 7-8	[Cu(Apg) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ] · H <sub>2</sub> O	UV-Vis	[66]
				IR ESI-MS TG-DTG	
<i>Silibinin in EtOH</i>	50% aqueous solution of VOCl <sub>2</sub>	NaOCH <sub>3</sub> 9	Na <sub>2</sub> [VO(Sil) <sub>2</sub> ] · (H <sub>2</sub> O) <sub>6</sub>	IR	[47]
				EPR Spectrophotometric titrations	

<i>Morin in MeOH</i>	50% aqueous solution of VOCl <sub>2</sub>	NaOCH <sub>3</sub> 5	[VO(Mor) <sub>2</sub> H <sub>2</sub> O] · (H <sub>2</sub> O) <sub>5</sub>	UV-Vis IR EPR Spectrophotometric titrations	[48]
<i>Kaempferol in EtOH</i>	ZnCl <sub>2</sub> ·2H <sub>2</sub> O	NaCl 8-10	[Zn(Kaem) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> ] · H <sub>2</sub> O	UV-Vis IR <sup>1</sup> H NMR ESI-MS Elemental analysis	[64]
<i>Nifflcoumar sodium salt in H<sub>2</sub>O</i>	Ce(NO <sub>3</sub> ) <sub>3</sub> ·6H <sub>2</sub> O La(NO <sub>3</sub> ) <sub>3</sub> ·6H <sub>2</sub> O Nd(NO <sub>3</sub> ) <sub>3</sub> ·6H <sub>2</sub> O	N.A. 4-5	Ce(NS) <sub>3</sub> · (H <sub>2</sub> O) <sub>4</sub> La(NS) <sub>3</sub> · (H <sub>2</sub> O) <sub>4</sub> Nd(NS) <sub>3</sub> · (H <sub>2</sub> O) <sub>6</sub>	IR <sup>1</sup> H NMR Elemental analysis	[72]
<i>Nifflcoumar in H<sub>2</sub>O</i>	Aqueous solution of ZrCl <sub>4</sub>	NaOH 5	Zr(Niff) <sub>2</sub> (OH) <sub>4</sub> (H <sub>2</sub> O) <sub>5</sub>	IR <sup>1</sup> H NMR Elemental analysis TG-DTG	[73]
<i>Warfarin in H<sub>2</sub>O</i>	Aqueous solution of ZrCl <sub>4</sub>	NaOH 5	Zr(War) <sub>2</sub> (OH) <sub>4</sub> (H <sub>2</sub> O) <sub>2</sub>	IR <sup>1</sup> H NMR Elemental analysis TG-DTG	[73]



<i>Coumachlor in H<sub>2</sub>O</i>	Aqueous solution of ZrCl <sub>4</sub>	NaOH 5	Zr(Coum) <sub>2</sub> (OH) <sub>4</sub> (H <sub>2</sub> O) <sub>6</sub>	IR <sup>1</sup> H NMR Elemental analysis TG-DTG	[73]
<i>1 in 50% EtOH</i>	GeO <sub>2</sub> in deionized H <sub>2</sub> O	NaOH 7	<i>N.A.</i>	UV-Vis IR <sup>1</sup> H NMR MS Elemental analysis TG-DTG	[62]
<i>2-11 in MeOH</i>	[Ru(η <sup>6</sup> -p- cymene)Cl <sub>2</sub> ] <sub>2</sub> in CH <sub>2</sub> Cl <sub>2</sub>	NaOMe <i>N.A.</i>	<i>N.A.</i>	<sup>1</sup> H & <sup>13</sup> C NMR Elemental analysis X-ray for (49, 51, 53, 55)	[67, 68]
<i>15-18 in CH<sub>2</sub>Cl<sub>2</sub></i>	[Ru(η <sup>6</sup> -p- cymene)Cl <sub>2</sub> ] <sub>2</sub> in CH <sub>2</sub> Cl <sub>2</sub>	<i>N.A.</i>	<i>N.A.</i>	UV-Vis spectra IR <sup>1</sup> H NMR FAB/EI-MS	[61, 68, 74]
<i>7, 12-14 in EtOH</i>	[Ru(DMSO) <sub>4</sub> Cl <sub>2</sub> ] in EtOH	TEA <i>N.A.</i>	[Ru(DMSO) <sub>2</sub> (7) <sub>2</sub> ] <sub>2</sub> NaNO <sub>3</sub> (H <sub>2</sub> O) <sub>2</sub> [Ru(DMSO) <sub>2</sub> (12) <sub>2</sub> ] <sub>2</sub> NaNO <sub>3</sub> . H <sub>2</sub> O [Ru(DMSO) <sub>2</sub> (13) <sub>2</sub> ]. (NO <sub>3</sub> ) <sub>2</sub> (H <sub>2</sub> O) <sub>2</sub> [Ru(DMSO) <sub>2</sub> (14) <sub>2</sub> ] <sub>2</sub> NaNO <sub>3</sub> (H <sub>2</sub> O) <sub>5</sub>	IR <sup>1</sup> H NMR ESI-MS Elemental analysis	[63]

<i>18 in EtOH</i>	La(NO <sub>3</sub> ) <sub>3</sub> .6H <sub>2</sub> O	TEA N.A.	N.A.	UV-Vis spectra IR <sup>1</sup> H NMR Elemental analysis TG-DAT	[42]
<i>19 in H<sub>2</sub>O</i>	Aqueous solution of Ce La Nd	NaOH 5	Ce(19)(OH)(H <sub>2</sub> O) <sub>2</sub> La(19)(OH).H <sub>2</sub> O Nd(19)(OH).H <sub>2</sub> O	IR <sup>1</sup> H & <sup>13</sup> C NMR Elemental analysis	[75]
<i>20 in EtOH</i>	Aqueous solution of K <sub>2</sub> PtCl <sub>4</sub>	N.A.	cis - [Pt(20) <sub>2</sub> Cl <sub>2</sub> ]	IR <sup>1</sup> H & <sup>195</sup> Pt NMR	[44]

169

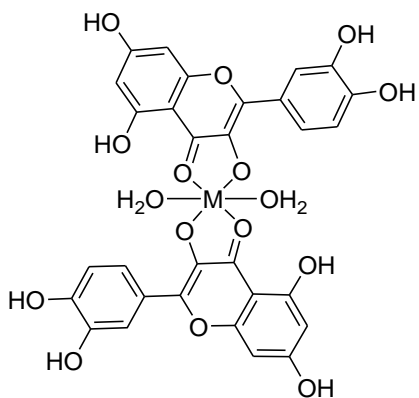
170 N.A.: Not available

171

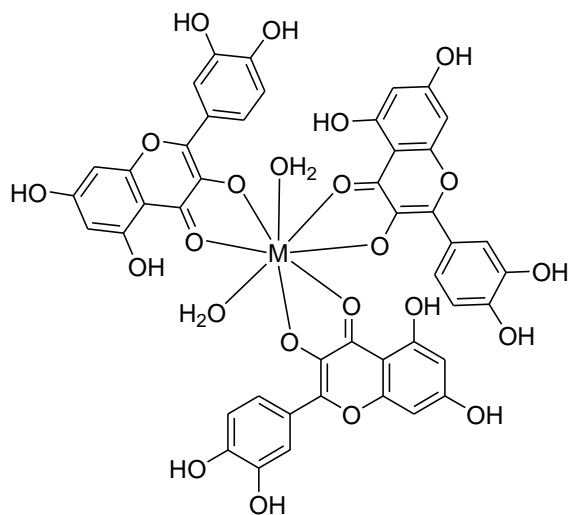
172

173

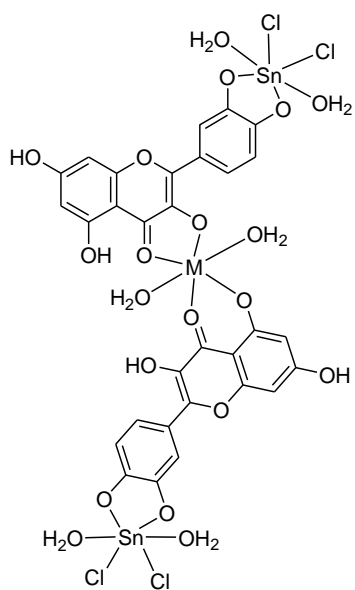
174



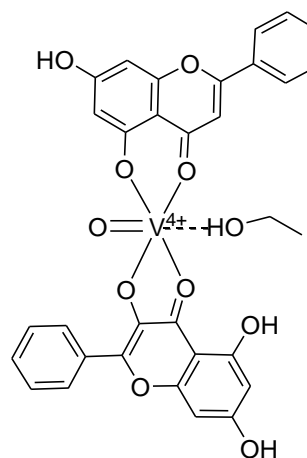
**21, M = Ni; 22, M = Cu**



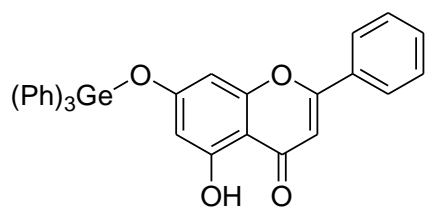
**23, M = La; 24, M = Nd; 25, M = Eu;  
26, M = Gd; 27, M = Tb; 28, M = Dy;  
29, M = Tm; 30, M = Y**



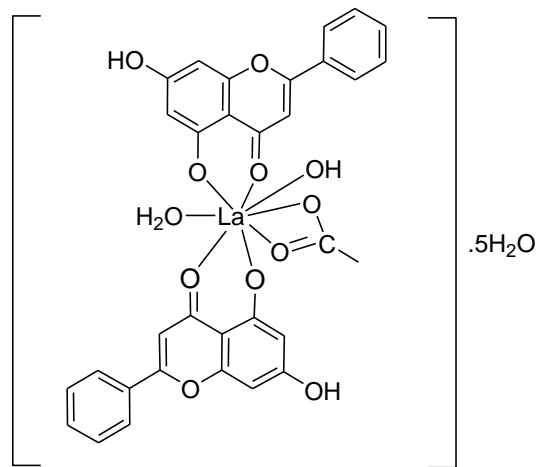
**31, M = Cu; 32, M = Zn**



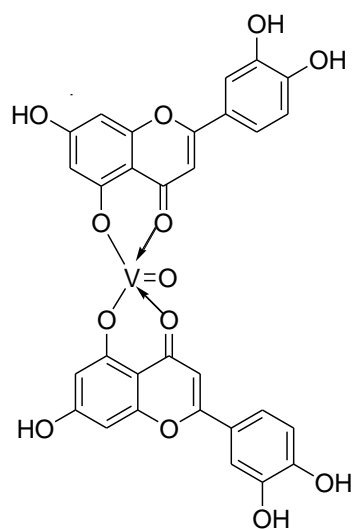
**33**



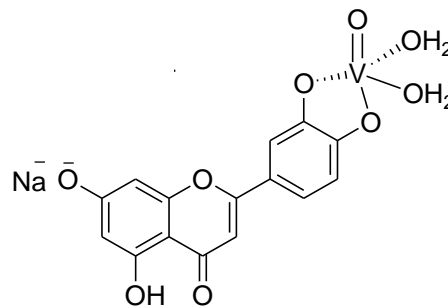
34



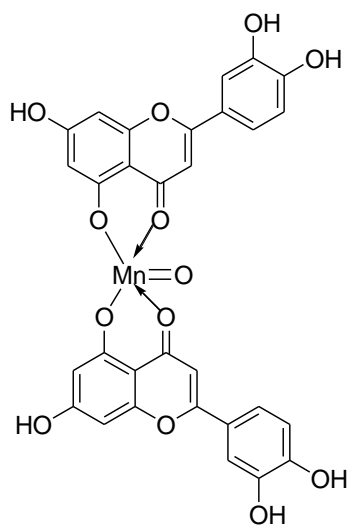
35



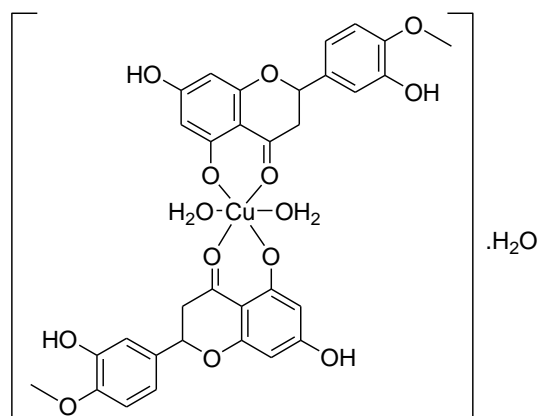
36



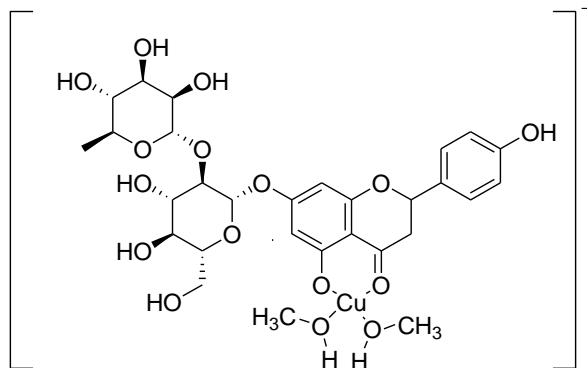
37



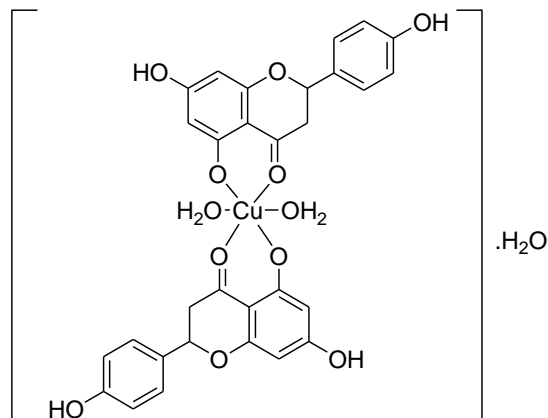
38



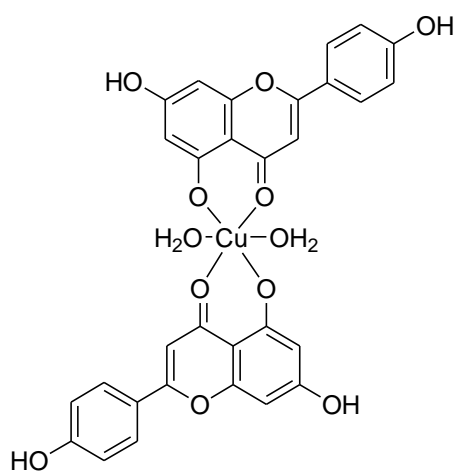
39



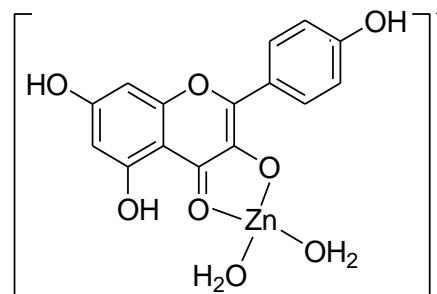
40



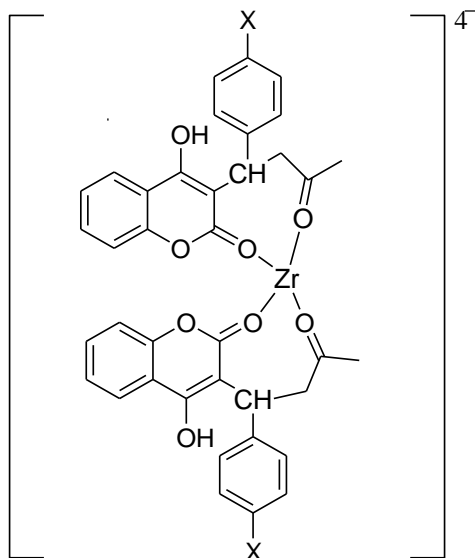
41



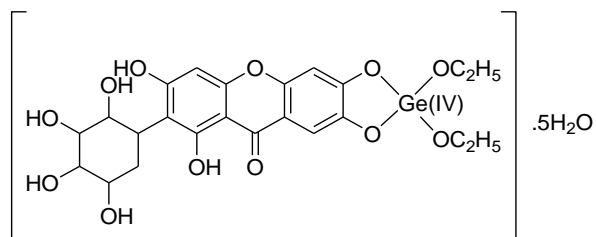
42



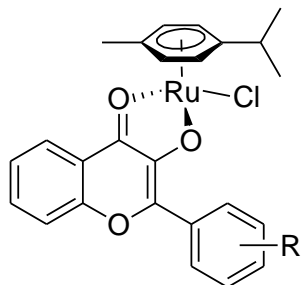
43



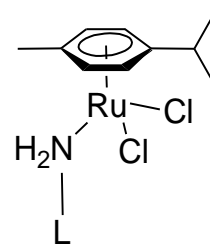
44, X = NO<sub>2</sub>; 45, X = H; 46, X = Cl



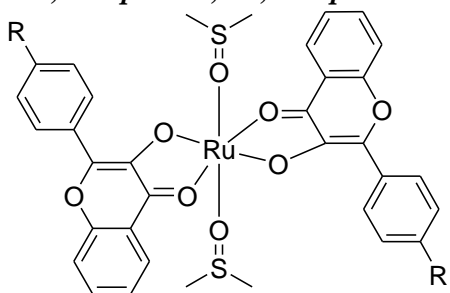
47



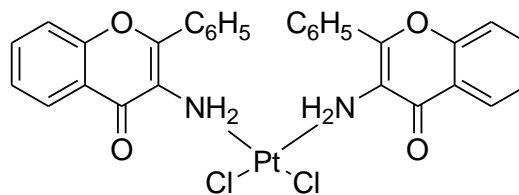
48, R = H; 49, R = *p*-CH<sub>3</sub>; 50, R = *p*-F;  
 51, R = *m*-F; 52, R = *o*-F; 53, R = *p*-Cl;  
 54, R = *m*-Cl; 55, R = *o*-Cl; 56, R = *p*-Br;  
 57, R = *m*-Br; 58, R = *p*-OCH<sub>3</sub>;  
 59, R = *p*-NO<sub>2</sub>; 60, R = *p*-NMe<sub>2</sub>



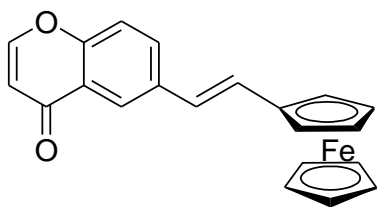
61, L = Cpd 15; 62, L = Cpd 16;  
 63, L = Cpd 17



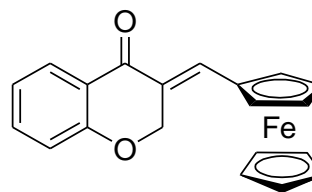
64, R = Cl; 65, R = OCH<sub>3</sub>; 66, R = NO<sub>2</sub>;  
 67, R = NMe<sub>2</sub>



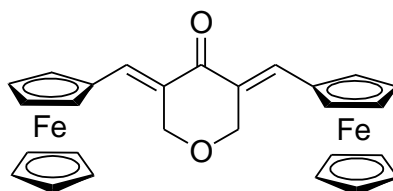
68



69



70



71

175

176

**Figure 6.** Chemical structures of flavonoid metal complexes

177 **Anti-oxidant activities of flavonoid metal complexes**

178 Complexation of flavonoids with metals often increases the anti-oxidant activity of the parent  
 179 flavonoids as shown in table 2. Metal complexes demonstrate enhanced activities in free radical  
 180 scavenging assays such as DPPH· (1,1-diphenyl-2-picrylhydrazyl radical), ABTS<sup>+</sup> [2,20-azino-  
 181 bis(3-ethylbenzothiazoline-6-sulfonic acid diammonium salt)], H<sub>2</sub>O<sub>2</sub>, OH· and O<sub>2</sub><sup>·-</sup>. Superoxide  
 182 dismutase (SOD) like activity is often used to measure the anti-oxidant activities of the  
 183 complexes as it is considered to be one of the body's first line free radical defense enzymes.

184 However, it is important to note that even where the complexes showed better SOD like activity  
 185 than their free parents they are still far above the limit for good SOD like activity which is IC<sub>50</sub> ≤  
 186 20 μM.

187 **Table 2.** Summary of anti-oxidant activities of reviewed flavonoids and their metal complexes

Complex	Assay	Results	Ref
		Enhanced activity at 120 μM,	
<b>23</b>		90%	
<b>24</b>		85%	
<b>25</b>	O <sub>2</sub> <sup>·-</sup>	84%	
<b>26</b>		97%	[55]
<b>27</b>		77%	
<b>28</b>		92%	
<b>29</b>		87%	
<b>30</b>		88%	
		<i>quercetin = 42%</i>	
	DPPH·	Enhanced activity at 100 μM, 45%	
<b>33</b>		<i>chrysin = 18%</i>	[58, 76]
	ABTS <sup>+</sup>	Enhanced activity 3.96 mM	
		<i>chrysin = 0.9 mM</i>	

	SOD	IC <sub>50</sub> = 157 $\mu$ M <i>chrysin = no activity</i>	
	OH $\cdot$	Enhanced activity at 100 $\mu$ M, 75% <i>chrysin = 45%</i>	
36	DPPH $\cdot$	Enhanced activity at 100 $\mu$ M, 40% <i>luteolin = 25%</i>	[65]
	ABTS <sup>+</sup>	Enhanced activity at 10 $\mu$ M, 64% <i>luteolin = 40%</i>	
37	DPPH $\cdot$	Lower activity IC <sub>50</sub> = >100 $\mu$ M <i>luteolin = 66.7 <math>\mu</math>M</i>	[49]
	OH $\cdot$	Enhanced activity IC <sub>50</sub> = 17 $\mu$ M <i>luteolin = 50.7 <math>\mu</math>M</i>	
	O <sub>2</sub> $\cdot^-$	Lower activity IC <sub>50</sub> = 417 $\mu$ M <i>luteolin = 384 <math>\mu</math>M</i>	
38	DPPH $\cdot$	Enhanced activity at 1 mM, 90% <i>luteolin = 75%</i>	[70]
	OH $\cdot$	Enhanced activity at 1 mM, 80% <i>luteolin = 74%</i>	
VOhesperidin	DPPH $\cdot$ ABTS <sup>+</sup>	No enhancement in activity	[46]
40	DPPH $\cdot$	Enhanced activity at 10 $\mu$ M, 35.5% <i>naringin = 2%</i>	[71]

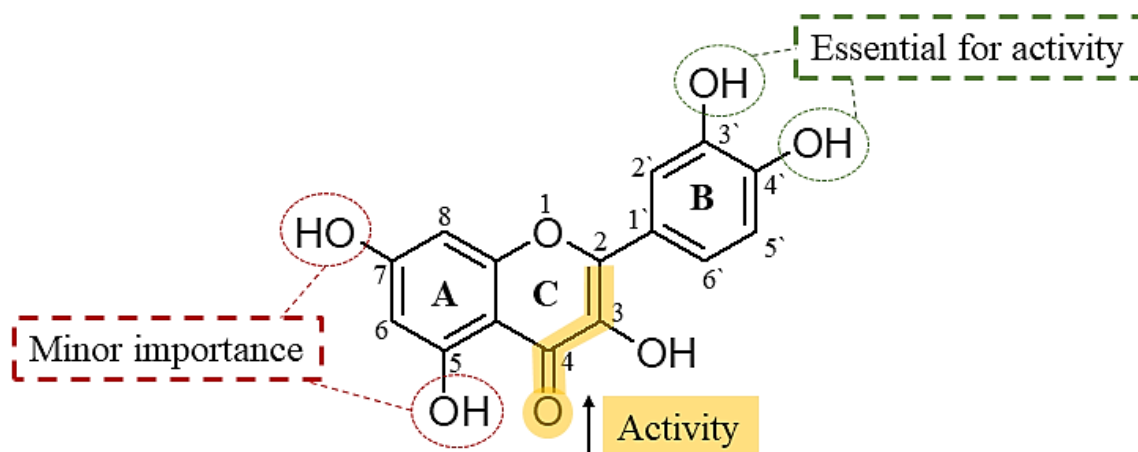


<b>VMorin</b>	SOD	Slightly enhanced activity IC <sub>50</sub> = 45 μM <b><i>morin</i> = 66 μM</b>	[48]
	DPPH·	Similar activity at 10 μM, 22% <b><i>morin</i> = 15%</b>	
	ABTS <sup>+</sup>	Similar activity at 10 μM, 81% <b><i>morin</i> = 76%</b>	
	OH·	Enhanced activity at 10 μM, 26% <b><i>morin</i> = 2%</b>	
	ROO·	No effect on radical	
<b>47</b>	OH·	Enhanced activity at 100 μM, 65% <b><i>2</i> = 43%</b>	[62]
	DPPH·	Lower activity at 80 μM, 60% <b><i>2</i> = 95%</b>	

188

189 As shown in table 2, rare earth metal complexes (**23-30**) increased O<sub>2</sub><sup>-</sup> scavenging activity of  
 190 quercetin by, on average, 2-fold [55]. The difference in activity between the eight metal  
 191 complexes is not significant, however, the gadolinium complex (**26**) showed the best activity.  
 192 Whether this increase in activity is attributed to incorporation of the metal atom or to the higher  
 193 molar ratio of quercetin present in the complex (as opposed to 1 quercetin molecule) needs  
 194 further investigation. **Figure 7** illustrates the main structural features responsible for the anti-  
 195 oxidant activity of flavonoids. The 3', 4' *ortho*-dihydroxyl group is the most significant  
 196 contributor to flavonoids' anti-oxidant activity [3]. These two catechol moieties form *ortho*-  
 197 semiquinone radicals that are highly stabilized by the electron delocalization and intra-molecular  
 198 hydrogen bonding. The combination of C2=C3 and 4-C=O group in ring C also assists in the

199 delocalization of the  $\pi$ -electrons in ring B. This in turn influences the dissociation of phenolic  
 200 hydroxyl groups as well as the stability of the formed phenoxy radicals in ring B [3]. Ring A  
 201 *meta*-hydroxyl groups are less important than ring B dihydroxyl groups which are oxidized more  
 202 readily [77]. The highest increase in activity can be seen upon complexation of metals with  
 203 flavonoids lacking the essential structural features of anti-oxidant activity (**Figure 7**), such as  
 204 chrysin and naringin [78]. For instance, complexation of vanadium metal with chrysin (**33**)  
 205 increased ABTS<sup>+</sup> scavenging activity from 0.9 mM for chrysin to 3.96 mM [58]. This value is  
 206 close to that of quercetin (4.7 mM) [79] that fulfills anti-oxidant structural activity requirements  
 207 (**Figure 7**) and is therefore one of the best flavonoid anti-oxidants.

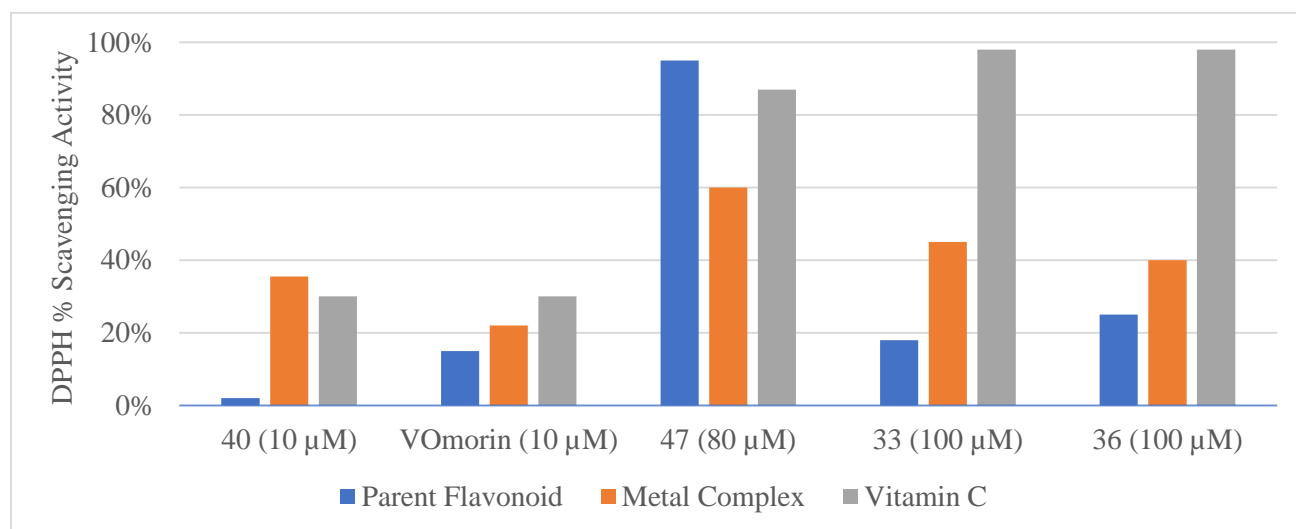


208

209 **Figure 7.** Summary of structural features responsible for anti-oxidant activity of flavonoids

210 As illustrated in **Figure 8**, metal complexation demonstrated a positive impact on the DPPH<sup>·</sup>  
 211 scavenging activity of ligands which was higher than that of vitamin C in only one case, the Cu-  
 212 Naringin complex **40**. Despite the witnessed increase in DPPH<sup>·</sup> scavenging activity of chrysin  
 213 (18%) upon complexation with vanadium (**33**) (45%) at 100  $\mu$ M, it is still far less than that of  
 214 ascorbic acid (98%) at the same concentration [58]. However, metal complexation did not  
 215 always result in enhanced DPPH<sup>·</sup> scavenging activity. The vanadium-luteolin complex **37**  
 216 showed a loss in the DPPH<sup>·</sup> scavenging activity of luteolin due to chelation of the vanadium  
 217 metal with the 3' and 4'-OH groups that are essential for anti-oxidant activity of flavonoids [49].  
 218 Pi et al. attributed the loss of the polyphenol ligand's (2) high DPPH<sup>·</sup> scavenging activity (95%)

219 after coordination with germanium (**47**) to steric hindrance factors [62]. This might be caused by  
220 the presence of the two ethyl ether groups rather than the germanium atom.



221

222 **Figure 8.** DPPH scavenging activity of metal complexes compared to their parent flavonoids  
223 and vitamin C at different concentrations

## 224 Cytotoxic activities of flavonoid metal complexes

225 One of the milestones in the history of chemotherapy was the discovery of cisplatin in 1969 [80].  
226 Cisplatin is effective and still widely used against various types of cancers, such as testicular,  
227 ovarian, breast, bladder, lung cancer and brain tumors. It demonstrates remarkable curing rates  
228 for testicular cancer (over 90% and near 100% with early discovery) [81]. Inside the body,  
229 cisplatin crosslinks DNA and initiates apoptosis. Despite this promising profile, cisplatin suffers  
230 from limitations such as nephrotoxicity, neurotoxicity and drug resistance [82, 83]. This ongoing  
231 interest in the design and development of metal-based anti-cancer drugs has also influenced the  
232 flavonoid field, where a number of flavonoid-metal complexes have been synthesized and  
233 evaluated as anti-cancer agents.

234

235

236

237

238 **Cytotoxic activity of quercetin metal complexes**239 **Table 3.** Summary of *in vitro* anti-tumor activity of quercetin metal complexes

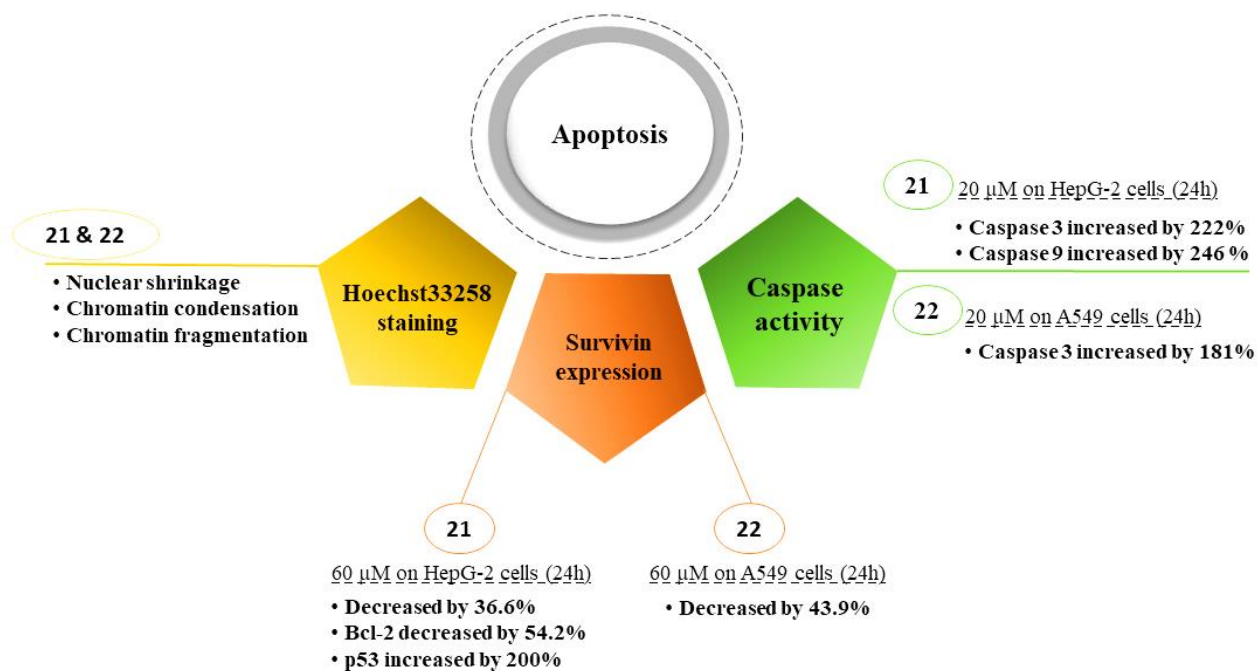
Complex	Cell line	Activity ( $\mu\text{M}$ ) ( $\text{IC}_{50}$ or suppression % or $\text{GI}_{50}$ )	Quercetin activity ( $\mu\text{M}$ )	Ref.
<b>21</b>	HepG2	$14.4 \pm 0.9$ *	$33.7 \pm 1.0$ *	[84]
	SMMC7721	$19.6 \pm 0.6$ *	$42.1 \pm 1.0$ *	
	A549	$35.6 \pm 0.8$ *	$72.1 \pm 1.2$ *	
<b>22</b>	A549	$21.5 \pm 0.5$ *	$34.9 \pm 1.0$ *	[53]
<b>23</b>	Bel-7402	46.03% ♦	20% ♦	[55]
<b>25</b>	Bel-7402	62.32% ♦	20% ♦	
<b>26</b>	Bel-7402	45.30% ♦	20% ♦	
<b>31</b>	U373MG, PC3, Hop62, HL60, HCT15 and HeLa	$< 8.7$ •	N.A.	[43]
<b>32</b>	HeLa	7.7 •	N.A.	

240 \* =  $\text{IC}_{50}$ , ♦ = Suppression % at 10  $\mu\text{M}$ , • =  $\text{GI}_{50}$ 241 Hepatocellular carcinoma (HepG2), hepatoma (SMMC7721), lung carcinoma (A549), liver cancer (Bel-  
242 7402), central nervous system (U373MG), prostate cancer (PC3), lung cancer (Hop62), leukemia  
243 (HL60), colon carcinoma (HCT15) and cervical cancer (HeLa).

244

245 It is evident that complexation with metals enhances the anti-proliferative activity of quercetin.

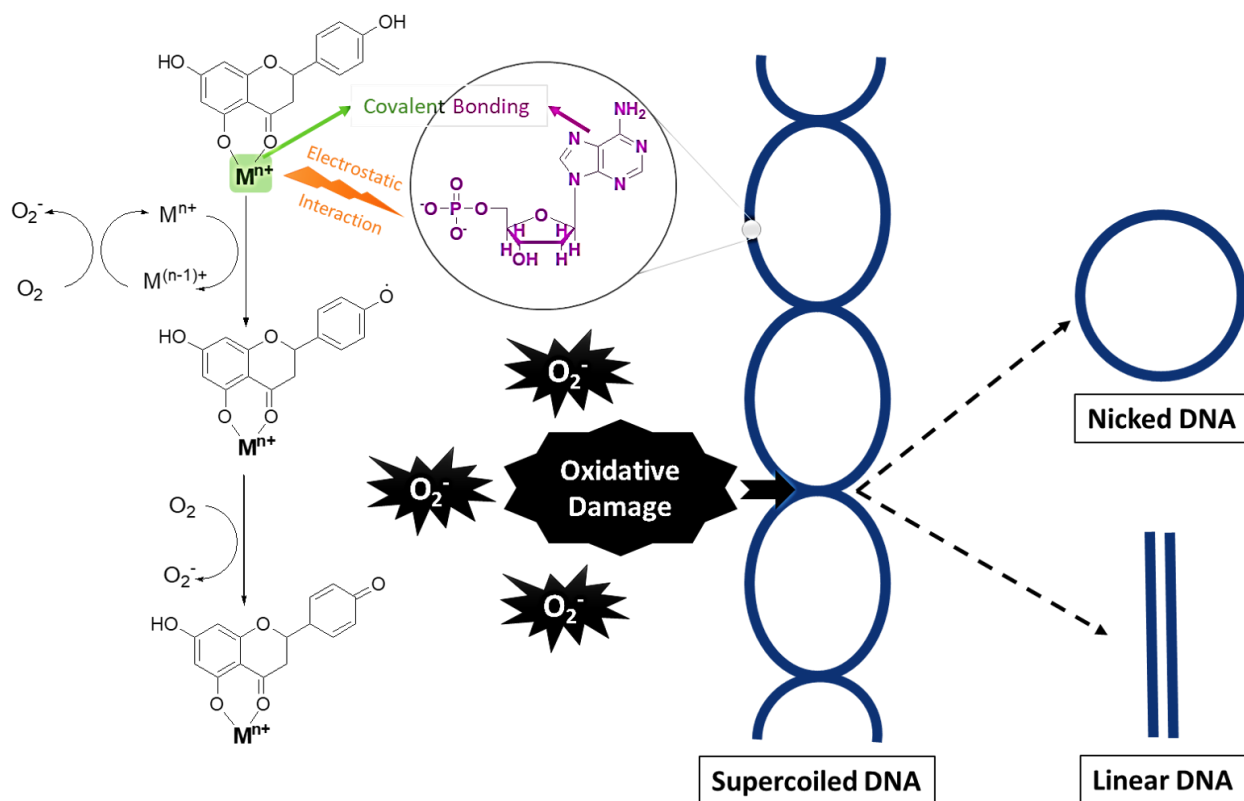
246 Compound **21** (Ni-quercetin) had better activity on the hepatic cancer cell lines (HepG2 and  
247 SMMC7721) than on the lung carcinoma cell line (A549). Pro-apoptotic ability has been  
248 investigated for complexes **21** and **22** (Cu-quercetin) as a possible cytotoxic mechanism (**Figure**  
249 **9**) [53, 84]. This is indicated by changes in the levels of key apoptotic proteins like survivin and  
250 caspases along with morphological changes observed using the DNA-sensitive Hoechst33258  
251 stain. Possible mechanisms of action of **21** on the A549 cell line were not evaluated. This might  
252 have given a better idea of the behavior of complexes in different cell lines and the reasons  
253 behind the witnessed difference in activities.



254

255 **Figure 9.** Summary of pro-apoptotic studies done on complexes 21 and 22. Survivin expression  
 256 was measured by immunocytochemistry and caspase activity by absorption spectra.  
 257

258 Metal atoms in flavonoid complexes can interact with DNA nucleotides by either electrostatic  
 259 interactions with the phosphate groups, or covalent binding to nucleobases (**Figure 10**). The  
 260 ROS and free radicals resulting from flavonoids' auto-oxidation are thus generated in proximity  
 261 to the DNA [37]. This leads to oxidative damage of DNA and induces apoptosis (**Figure 10**) [85,  
 262 86]. Flavonoid metal complexes are also more planar than the free ligands which facilitates their  
 263 binding to DNA via intercalation [37]. Tan et al. reported the selective binding of the nickel  
 264 quercetin complex (**21**) to GC-rich DNA sequences using fluorescence emission and molecular  
 265 modelling. This, in addition to decreased survivin activity, led them to conclude that down  
 266 regulation of survivin via interference with the GC-rich survivin protein promoter gene might be  
 267 a plausible mechanism by which the complex **21** exerts its anti-tumor activity [84].



268

269 **Figure 10.** Plausible mechanism of oxidative DNA damage by flavonoid metal complexes

270 The quercetin rare earth metal complexes (**23**, **25** and **26**) showed increased activities compared  
 271 with free quercetin on the liver cancer cell line (Bel-7402) (**Table 3**) [55]. Complex **23** (La-  
 272 quercetin) can intercalate with DNA as evidenced by DNA fluorescence studies. Tabassum and  
 273 her colleagues were able to synthesize two unique hetero-bimetallic complexes quercetin- $Cu^{II}$ -  
 274  $Sn_2^{IV}$  (**31**) and quercetin- $Zn^{II}$ - $Sn_2^{IV}$  (**32**) [43]. The Cu complex (**31**) showed significant activity  
 275 on various human cancer cell lines (**Table 3**) except for ovarian cancer (A2780). The Zn  
 276 complex (**32**), however, did not demonstrate good activity except on the HeLa cell line (**Table**  
 277 **3**). Complexes **22**, **31** and **32** were able to promote the conversion of substrate DNA to cleaved  
 278 forms (nicked and linear) as studied by gel electrophoresis [43, 53, 54]. To better understand  
 279 whether this DNA cleavage is promoted through an oxidative pathway, the effect of different  
 280 radical scavengers was evaluated where only  $H_2O_2$  markedly promoted plasmid DNA breakage  
 281 caused by the complexes. Increased level of thiobarbituric acid reactive species (TBARS) with  
 282 increasing concentrations of complex **22** backs up the role of oxidative damage as a mechanism  
 283 of CT-DNA cleavage [53, 54]. At 30  $\mu M$  of complex **31**, DNA relaxation activity of

284 Topoisomerase-I was significantly inhibited. This does not necessarily correlate with direct  
 285 inhibition of the enzyme and may instead result from strong binding with DNA which prevents  
 286 the enzyme from exercising its function [43]. To exploit binding of complexes **31** and **32** with  
 287 the minor groove of DNA, the complexes were docked into the DNA duplex of sequence  
 288 d(CGCGAATTCGCG)<sub>2</sub> dodecamer (PDB ID: 1BNA). Both complexes bound to the narrow  
 289 minor groove region of DNA within the GC rich region and were stabilized by hydrogen  
 290 bonding (2.8–3.0 Å) between the –OH groups of quercetin with O4/N2 atoms of C8/G8 bases in  
 291 DNA.

292 Complex **31** also showed good binding modes with the human-DNA–Topo-I complex (PDB ID:  
 293 1SC7) and bovine erythrocyte Cu, Zn superoxide dismutase (PDB ID: 2SOD) [43].

#### 294 Cytotoxic activity of chrysin, luteolin and silibinin metal complexes

295 **Table 4.** Summary of *in vitro* anti-tumor activity of metal complexes of chrysin, luteolin and  
 296 silibinin

Complex	Cell line	Activity (μM) (IC <sub>50</sub> or suppression %)	Flavonoid activity (μM)	Ref.
<b>33</b>	MG-63	16 *	>100 *	[76]
	HT-29	45% ◊	No effect ◊	[87]
<b>VOsilibinin</b>	HT-29	45% ◊	No effect ◊	
<b>34</b>	HepG2	30% ♦	20% ♦	[69]
	Colo205	25% ♦	10% ♦	
	MCF-7	45% ♦	20% ♦	
	MCF-7 10A	15% ♦	10% ♦	
<b>35</b>	A549	73.5 % ♦	4.9 % ♦	[57]
<b>37</b>	A549	60.5 *	66.3 *	[49]
	MDAMB-231	17 *	88.3 *	

297 \* = IC<sub>50</sub>, ♦ = Suppression % at 10 μM, ◊ = Suppression % at 100 μM  
 298 Osteosarcoma (MG-63), colon adenocarcinoma (HT-29), hepatocellular carcinoma (HepG2), caucasian  
 299 colon adenocarcinoma (Colo205), breast cancer (MCF-7), breast cell line (MCF-7 10A), lung carcinoma  
 300 (A549) and triple negative breast cancer (MDAMB-231).  
 301

302 The vanadium chrysin complex (**33**) showed significant cytotoxic activity on a human  
303 osteosarcoma cell line (MG-63) with an  $IC_{50}$  value of 16  $\mu$ M (**Table 4**) [76]. This activity is  
304 higher than the reported activity of cisplatin on the same cell line ( $IC_{50} = 28.5 \mu$ M), yet lower  
305 than that of doxorubicin ( $IC_{50} = 3.35 \mu$ M) [88]. According to Leon et al. complex **33** showed a  
306 multi-factorial anti-tumor mechanism of action. For example, it demonstrated cytotoxicity to  
307 lysosomes and mitochondria of MG-63 cell line (at concentrations from 2.5 to 25  $\mu$ M) as  
308 evidenced using neutral red (NR) uptake and the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-  
309 diphenyltetrazolium bromide (MTT) assays, respectively. Genotoxicity was also evident through  
310 induced micronuclei formation and DNA breaks using the Comet assay, in the concentration  
311 range 2.5-5  $\mu$ M. In addition to that, compound **33** lowered GSH/GSSG ratio in MG-63 cell line  
312 especially at higher doses (40%, 50 and 100  $\mu$ M). In a protein array platform test, **33** upregulated  
313 82 proteins and downregulated 9 such as PKB/AKT, Cdk 4, 6 and 7, NAK, and JNK [89]. It  
314 induced apoptosis via:

- 315 • Externalization of the phosphatidyl serine (PS), at the outer plasma membrane leaflet  
316 measured by Annexin V-FITC fluorescent probe.  
317
- 318 • Alteration of the mitochondria membrane potential (MMP) measured by Rhodamine 123 that  
319 leads to the release of cytochrome c and finally to DNA fragmentation.  
320
- 321 • Activation of caspase pathway measured by caspase 3 activation (350% basal at 100  $\mu$ M).  
322

323 In another report the anti-tumor activities of vanadium complexes of chrysin (**33**) and silibinin  
324 (**VOsil**) were tested on the HT-29 cell line (**Table 4**) [87]. **33** and **VOsil** showed better anti-  
325 proliferative activities than cisplatin at 100  $\mu$ M (inhibition rate = 12 %) on the same cell line.  
326 However, **33** did not show pro-apoptotic effects in the MG-63 cell line despite it causing cell  
327 cycle arrest in the G2/M phase after 24 and 48 hours incubation. **VOsil**, on the other hand,  
328 increased the percentages of apoptotic/necrotic cells, induced caspase 3 activation and reduced  
329 nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) that controls DNA  
330 transcription and is aberrantly activated in many cancers. Moreover, **VOsil** fully inhibited  
331 topoisomerase-IB using plasmid relaxation assay at 100  $\mu$ M. The germanium complex of  
332 chrysin, (**34**) also had activity on several targets. Its cytotoxic effect on HepG2, Colo250 and  
333 MCF-7 cell lines was not significantly high nor much better than chrysin [69, 90]. The mean  
334 apoptotic population detected on MCF-7 and Colo205 cell lines by Annexin-V were 19.5% and



335 12.5% for 20 µg/ml, respectively. Additionally, cellular ROS production increased in MCF-7  
336 cell line upon addition of high doses of complex **34**. This could be a possible etiology for  
337 apoptosis alongside with caspase-3, 8 and 9 activation whose levels increased from 100% to  
338 214.5%, 181.1% and 286.0%, respectively at 20 µg/ml. Some caspase activation was also  
339 observed on the Colo205 cell line with an increase from 100% to 129.3%, 146.5 and 119.3%, for  
340 caspase-3, 8 and 9, respectively. Cell cycle arrest at the G2/M phase was detected on MCF-7 and  
341 Colo205 (high doses) cell lines and at the S phase with low doses of the complex on the Colo205  
342 cell line. Complex **35** (La-chrysin) showed a significant increase (73.5%) in the anti-proliferative  
343 activity of chrysin (4.9%) on A549 cell lines. The complex intercalated with DNA base pairs as  
344 illustrated by an increase in viscosity and fluorescence of DNA upon addition of complex **35**  
345 [57]. The vanadium luteolin complex (**37**) significantly improved the cytotoxic activity of its  
346 parent on the MDAMB-231 cell line (IC<sub>50</sub> = 17 µM) as shown in table 4 [49]. However, it  
347 showed a trivial improvement on the A549 cell line (IC<sub>50</sub> = 60.5 µM, luteolin IC<sub>50</sub> = 66.3 µM).  
348 Naso et al. carried out high content cytotoxicity assays on complex **37** to investigate its  
349 mechanism of action on the MDAMB-231 cell line. The tests included ROS production, MMP,  
350 plasmatic membrane damage, nuclear membrane damage and mitotic arrest. Complex **37** showed  
351 significant increase in the levels of ROS production (650%) and percentage of depolarized cells  
352 (20%). Lactate dehydrogenase (LDH) levels in the culture media were measured as an indication  
353 for plasmatic membrane damage. Complex **37** demonstrated 212% increase compared with the  
354 basal levels as opposed to 98% for luteolin. The vanadium complex (**37**) also showed DNA  
355 damage and mitotic arrest activities using H2AX and PHH3 assays, respectively.

356 **Cytotoxic activity of hesperidin, naringin, hesperitin, naringenin, apigenin, morin,**  
357 **kaempferol, warfarin and mendiaxon metal complexes**  
358

359 **Table 5.** Summary of *in vitro* anti-tumor activity of hesperidin, naringin, hesperitin, naringenin,  
360 apigenin, morin, kaempferol, warfarin and mendiaxon metal complexes.

Complex	Cell line	Activity (suppression %)	Flavonoid Activity	Ref.
<b>VOhesperidin</b>	Caco-2	60% (at 100 µM)	No effect	[46]
<b>40</b>	K562	38.4% (at 100 µM)	13.2% (at 100 µM)	[71]
<b>La-18</b>	HL60	50% (at 0.01 µM)	N.A.	[42]
	A549	100% (at 0.003 µM)	N.A.	

<b>39</b>	HeLa	22.5%	15%	
	SGC-7901	45%	30%	
	HepG2	45%	30%	
<b>41</b>	HeLa	24%	27%	[66]
	SGC-7901	25%	33%	
	HepG2	35%	15%	
<b>42</b>	HeLa	25%	10%	
	SGC-7901	45%	5%	
	HepG2	35%	5%	
<b>VOmorin</b>	T47D	43% (at 10 $\mu$ M)	No effect	[48]
	SKBR3	38% (at 10 $\mu$ M)	14% (at 10 $\mu$ M)	
<b>43</b>	EC9706	72% (at 30 $\mu$ g/ml)	45 % (at 30 $\mu$ g/ml)	[64]
<b>45</b>	HL60	30% (at 100 $\mu$ M)	<i>N.A.</i>	[73]

361 Colon adenocarcinoma (Caco-2), chronic myeloid leukemia (K562), leukemia (HL60), lung carcinoma  
362 (A549), cervical carcinoma (HeLa), gastric carcinomas (SGC-7901) and hepatocellular carcinoma  
363 (HepG2), breast cancer (T47D), breast cancer overexpressing Herceptin-2 (SKBR3) and oesophageal  
364 cancer cell line (EC9706).

365  
366 As shown in table 5, **VOhesperidin** showed 60% inhibition in cellular proliferation of the colon  
367 adenocarcinoma cell line (Caco-2) while its parent hesperidin showed no effect at all on the same  
368 cell line at 100  $\mu$ M [46]. The Cu naringin complex (**40**) exhibited better inhibition of the K562  
369 cell lines (**Table 5**). Cell cycle analysis showed a 50% decrease in viable cells at the S/G2/M  
370 phases after 24 h. The number of hypodiploid cells tremendously increased from 5.5% (control)  
371 to 38.4% indicating the complex led to cell death in the first 24 h [71]. The lanthanum complex  
372 with ligand **18** exhibited a stronger suppression rate (100% inhibition at 0.003  $\mu$ M) than cisplatin  
373 (100 % inhibition at 0.004  $\mu$ M) on the A549 cell line [42]. Lanthanum complexes like **35** and  
374 **La-18** have significantly high suppression rates on A549 cell line.

375 As shown in table 5, complexation of copper with the flavonoids hesperetin (**39**) and apigenin  
376 (**42**) did enhance the anti-proliferative activity of their free flavonoids whilst the naringenin  
377 complex (**41**) did not show any improvement except on the HepG2 cell line [66]. **41** and **42** had  
378 the same suppression rate (35%) on the HepG2 cell line. This indicates that unsaturation of ring  
379 C has no effect on the activity whilst the addition of an OH group on ring B, as in complex **39**,  
380 results in 30% increase in the activity. This may be due to the enhanced anti-oxidant activity of

381 compounds featuring two *m*-hydroxyl groups on ring B (**Figure 7**). Several DNA binding studies  
 382 (UV-Vis spectral, fluorescence and CD measurements) were performed with the complex **39** that  
 383 indicated DNA binding via intercalation with higher affinity than the free ligand.

384 The vanadium morin complex (**VOmor**) showed promising cytotoxic activity on human breast  
 385 cancer cell lines (**Table 5**). It did not have deleterious effects on the non-tumorigenic breast  
 386 epithelial mammal cells indicating its selectivity towards cancer cells [48]. Naso et al. suggest  
 387 that the **VOmor** complex causes cell death by induction of apoptosis. This can be correlated to  
 388 perturbation of the mitochondrial membrane potential which results in release of cytochrome c  
 389 from the mitochondria to cytosol. This process leads to the activation of caspases-9, 3 and 7.  
 390 However, mitochondrial membrane potential was only observed in the SKBR3 cell line which  
 391 suggests the apoptotic potential on the T47D is achieved via a different mechanism that needs  
 392 further investigation. The MTT assay was used to determine the anti-proliferative activity of the  
 393 Kaempferol Zn complex (**43**) on EC9706 cells (**Table 5**) [64]. Cell viability decreased nearly by  
 394 half in the EC9706 cell line while no significant effect was observed on the normal kidney cells  
 395 (HK-2 cells). Atomic force microscopy (AFM) morphological data showed that complex **43**  
 396 could deform and shrink EC9706 cells at the nanoscale. To measure apoptosis induction ability,  
 397 Annexin V-FITC/PI was used. The apoptosis ratio of EC9706 cells increased from  $3.4 \pm 0.9\%$   
 398 for control cells to  $33 \pm 7.6\%$  upon raise of **43** concentrations from 0 to 30  $\mu\text{g/mL}$ , respectively.  
 399 Complex **43** was able to increase the intracellular calcium ion level in cancer cells that can  
 400 mediate cell death.

#### 401 **Cytotoxic activity of metal complexes 47-71**

402 **Table 6.** Summary of *in vitro* anti-tumor activity of complexes **47-71**

Complex	Cell line	Activity ( $\mu\text{M}$ ) (IC <sub>50</sub> or suppression%)	Flavonoid activity ( $\mu\text{M}$ )	Ref.
<b>47</b>	HepG2	65% $\blacklozenge$	35% $\blacklozenge$	
<b>51</b>		1.5 *	N.A.	
<b>53</b>	CH1	0.86 *	0.6 *	[67, 68]
<b>54</b>		1 *	N.A.	
<b>55</b>		1.2 *	N.A.	
<b>61</b>	DMBC12	0.96 *	N.A.	[61]

<b>62</b>		1.13 *	<i>N.A.</i>	
<b>63</b>		2.53 *	<i>N.A.</i>	
	DMBC11	4.14 *	<i>N.A.</i>	
<b>64</b>		16 *	17.2 *	
<b>65</b>	MCF-7	28 *	29.5 *	[63]
<b>66</b>		32.1 *	35.4 *	
<b>67</b>		36.2 *	38.4 *	
<b>Ce-19</b>	HL60	21.37 *	<i>N.A.</i>	[75]
<b>69</b>	CCRF-CEM	37.5 *	<i>N.A.</i>	[41]
<b>70</b>	Jurkat	2.97 *	<i>N.A.</i>	
		7.23 *	<i>N.A.</i>	[91]
<b>71</b>	HeLa	7.4 *	<i>N.A.</i>	

403 \* = IC<sub>50</sub>, ♦ = Suppression % at 160 μM

404 Hepatocellular carcinoma (HepG2), ovarian cancer (CH1), patient derived (not-established) melanoma  
 405 (DMBC11 and DMBC12), breast cancer (MCF-7), lung carcinoma (A549), cervical carcinoma (HeLa),  
 406 chronic myelogenous leukemia (K562), leukemia (HL60), T lymphoblast-like polymorph cancerous cell  
 407 line (CCRF-CEM) and acute T-lymphoblastic leukemia (Jurkat)

408  
 409 Pi et al. reported the DNA binding of the germanium complex of compound **1** (**47**) [62].  
 410 Compound **47** showed an increase in fluorescence emission by 46% (at 550 nm) while that of the  
 411 free parent **1** showed insignificant increase by 8% (at 536 nm). Intercalation with DNA was also  
 412 confirmed by a UV absorption method through the hypochromism of complex **47** upon addition  
 413 of CT-DNA. The anti-proliferative evaluation of **47** on the HepG2 cell line (**Table 6**) was  
 414 followed by AFM that showed deformation of HepG2 cells and increase in the size of cell  
 415 membrane particles. The effect on cell cycle, measured by flow cytometry, demonstrated that  
 416 complex **47** causes cell cycle arrest at the G<sub>0</sub>/G<sub>1</sub> phase.

417 The ruthenium complexes **48-57** were tested on a number of human cancer cell lines; CH1  
 418 (ovarian carcinoma), SW480 (colon carcinoma), and A549 (non-small cell lung carcinoma),  
 419 human urinary bladder (5637), human large cell lung (LCLC-103H), and human pancreatic  
 420 carcinoma cell lines (DAN-G) [67, 68]. The most significant anti-proliferative activities were  
 421 observed on the CH1 cell line with IC<sub>50</sub> values ranging from 0.89 to 7.9 μM (**Table 6**) [67]. The  
 422 SW480 cell line also showed high sensitivity to these complexes (IC<sub>50</sub> from 3.4 - 26 μM) while  
 423 A549 was the least affected with moderate activities (IC<sub>50</sub> from 8.6 - 30 μM). Kurzwehnart et al.

424 attempted to determine the mode of action of complexes **48-57** via CDK2 and topoisomerase II $\alpha$   
425 inhibition assays as well as flow cytometry cell cycle analysis. Despite showing inhibition of  
426 CDK2 comparable with the standard reference roscovitine, CDK2 inhibition was excluded by the  
427 authors as a mechanism of action. This is due to miscorrelation with the *in vitro* anti-tumor assay  
428 activity pattern and poor influence on the G1/S transition of the cell cycle in which CDK2 is  
429 involved. On the other hand, complexes **48-57** showed good inhibition of topoisomerase II $\alpha$   
430 catalytic activity at  $\geq 10 \mu\text{M}$ , which correlated well with the cytotoxic activity (compounds with  
431 lowest IC<sub>50</sub> in MTT assay showed the highest topoisomerase II $\alpha$  inhibition).

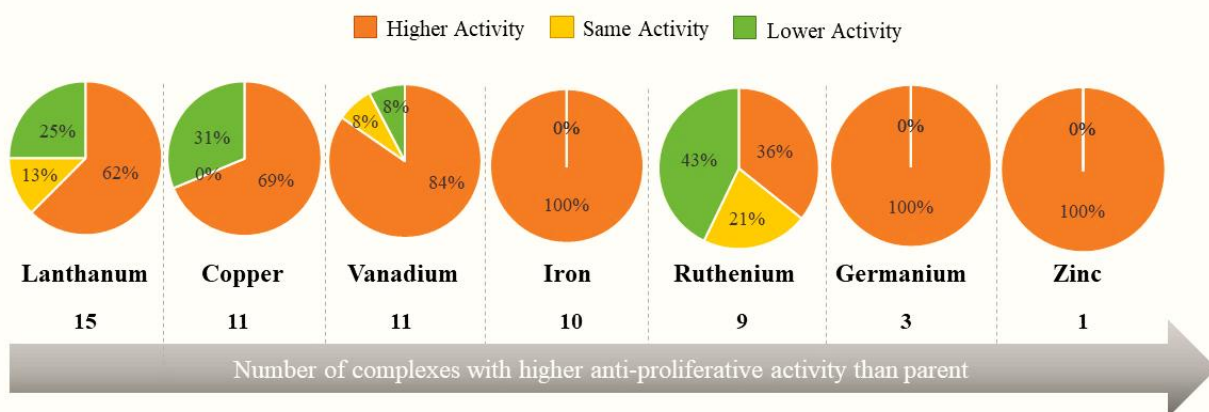
432 Compounds **61-63** showed promising IC<sub>50</sub> values on the patient derived (not-established)  
433 melanoma cell lines (DMBC11 and DMBC12) as shown in table 6 [61]. Only complex **63**  
434 exhibited potency on both DMABC11 and DMBC12 cell lines (IC<sub>50</sub> = 2.53 and 4.14  $\mu\text{M}$ ,  
435 respectively). Singh et al. developed four flavonoid ruthenium DMSO complexes (**64-67**) and  
436 tested their anti-proliferative activities on the MCF-7 cell line (**Table 6**) in addition to their  
437 action on different phases of the cell cycle [63]. Both the free ligands, and their ruthenium  
438 complexes, showed similar activities with complex **64** being the most potent with IC<sub>50</sub> = 16  $\mu\text{M}$ .  
439 Analysis of the cell cycle showed that compound **64** dramatically increased the G1 phase and  
440 decreased the S phase which might be explained by G1 arrest via inhibition of DNA synthesis in  
441 the S phase.

442 Kostova et al. tested the cytotoxicity of three metal complexes (Cerium, lanthanum and  
443 neodymium) with a bis-coumarin (**19**) [75]. Only the cerium complex demonstrated promising  
444 anti-proliferative effect on the HL-60 cell line (**Table 6**).

445 Among four ferrocenylvinyl-flavone derivatives synthesized by Kowalski et al., compound **69**  
446 was the most potent (**Table 6**) [41]. The mechanism of action was induction of apoptosis and  
447 necrosis as shown by double staining of cells with Hoechst 33258 and propidium iodide.  
448 Although anti-tumor activity was evaluated on four human cancer cell lines (HepG2, MCF-7,  
449 MDA-MB-231 and CCRF-CEM), CCRF-CEM was the only affected cell line.

450 Janka and his colleagues also developed a number of ferrocenyl chalcones of which compound  
451 **70** showed high activity on Jurkat cell line with IC<sub>50</sub> = 2.97  $\mu\text{M}$  (**Table 6**) [91]. Complex **71** has  
452 an additional ferrocenyl-vinyl group in its structure, however, it didn't show increase in the

453 activity on the HeLa cell line ( $IC_{50} = 7.4 \mu M$ ) when compared with complex **70** ( $IC_{50} = 7.23$   
454  $\mu M$ ).



455  
456 **Figure 11.** Summary of the effect of chelating different metals on the anti-proliferative activities  
457 of parent flavonoids

458 It can be deduced from **Figure 11** that 64% of the complexes showed enhanced anti-proliferative  
459 activities when compared to their parents while 11% maintained the same activity and 25% had  
460 lower activity. It should be noted that not all of the complexes' activities were compared to their  
461 respective parent flavonoid, depending on the availability of data. All of iron, zinc and  
462 germanium complexes showed higher activity than the free flavonoids. However, the total  
463 number of zinc complexes is too low to establish a clear view on the effect of complexation. On  
464 the other hand, 84%, 69% and 62% of the vanadium, copper and lanthanum complexes,  
465 respectively, exhibited better anti-proliferative activities than the free flavonoid. Ruthenium  
466 metal complexes showed inconsistent results as 36% showed enhanced activity while 21% had  
467 the same activity and 43% showed lower activities than their parent flavonoids.

## 468 469 **Future perspectives**

470  
471 Flavonoids have gained significant interest from researches since the early times. This is due to  
472 their therapeutic activities in a broad range of fields alongside their natural abundance, with the  
473 anti-oxidant and anti-cancer effects of flavonoids being of the greatest impact. Consequently,  
474 flavonoids like quercetin and flavopiridol have reached clinical trials as anti-cancer agents.  
475 However, none of them are available on the market as anti-cancer drugs due to their poor

476 pharmacokinetic profiles. The structural ability of flavonoids to coordinate with metal atoms has  
477 provided a new strategy for the development of flavonoid metal complexes with better  
478 pharmacological profiles. Although chelation of flavonoids with metals enhanced the anti-  
479 oxidant and anti-proliferative activities of their free parents in the majority of cases reported  
480 herein, more research is needed to confirm if this is a predictable and general phenomenon.  
481 Additionally, more research is required to probe whether this enhancement of activity is  
482 sufficient to afford new drugs that offer enhanced clinical outcomes when compared to drugs  
483 present in the market for the same indications. Larger scale studies probing the impact of  
484 complexing different metal atoms with sets of structurally similar and/or different flavonoids on  
485 anti-oxidant and anti-proliferative activities are therefore greatly needed. This would pave the  
486 way for a better understanding of their structural activity relationships. Furthermore, the use of  
487 computational studies like molecular modelling and quantitative SAR (QSAR) can provide  
488 valuable information about possible targets and mechanisms of action of these complexes. This  
489 will in turn lead to well informed and targeted structural designs.

## 490 **Executive summary**

### 491 **Introduction**

- 492 • Flavonoids are polyphenolic compounds with a C<sub>6</sub>-C<sub>3</sub>-C<sub>6</sub> ring system.
- 493 • Flavopiridol, silibinin and quercetin flavonoids have reached clinical trials as anti-cancer  
494 agents for various tumors.
- 495 • Flavonoids have poor pharmacokinetic properties which limit their clinical applications.
- 496 • Flavonoids have the ability to form metal complexes that can change their pharmacological  
497 and pharmacokinetic properties.

### 498

### 499 **Synthesis and characterization of flavonoid metal complexes**

- 500 • Flavonoid metal complexes are synthesized by reacting alcoholic or aqueous solutions of both  
501 the flavonoid and the metal under stirring and/or heating conditions.
- 502 • Structural characterization techniques of flavonoid metal complexes are versatile and are often  
503 complementary to one another.

### 504

### 505 **Anti-oxidant activities of flavonoid metal complexes**

- 506 • Flavonoid metal complexes demonstrate better radical scavenging activities than their free  
507 parents.
- 508 • Anti-oxidant activity is measured by the ability to scavenge free radicals like DPPH<sup>•</sup>, ABTS<sup>+</sup>,  
509 H<sub>2</sub>O<sub>2</sub>, OH<sup>•</sup> and O<sub>2</sub><sup>•-</sup> in addition to measuring SOD like activity.

- 510 • Some flavonoid metal complexes showed DPPH· scavenging activity higher or comparable to  
511 vitamin C.

512

### 513 **Cytotoxic activities of flavonoid metal complexes**

- 514 • The discovery of cisplatin had an important role in directing researchers' focus on the  
515 development of metal based drugs.
- 516 • Flavonoid metal complexes showed promising results on various human cancer cell lines.
- 517 • Cytotoxic activity of flavonoid metal complexes is caused by induction of apoptosis and/or  
518 oxidative damage of DNA.
- 519 • 64% of the flavonoid metal complexes showed better anti-proliferative activities than their  
520 parents, 11% maintained the same activity and 25% had lower activity.

521

### 522 **Financial disclosure**

523 MK is grateful to the Newton-Mosharafa Fund for a scholarship that has funded  
524 her PhD studies.

### 525 **References**

- 526 1. Isoda H, Motojima H, Onaga S, Samet I, Villareal MO, Han J. Analysis of the erythroid  
527 differentiation effect of flavonoid apigenin on K562 human chronic leukemia cells.  
528 *Chem. Biol. Interact.* 220, 269-277 (2014).
- 529 2. Perez-Vizcaino F, Fraga CG. Research trends in flavonoids and health. *Arch. Biochem.*  
530 *Biophys.* 646, 107-112 (2018).
- 531 3. Wang T-Y, Li Q, Bi K-S. Bioactive flavonoids in medicinal plants: Structure, activity  
532 and biological fate. *Asian J. Pharm. Clin. Res.* 13(1), 12-23 (2018).
- 533 4. Jeong HJ, Ryu YB, Park S-J *et al.* Neuraminidase inhibitory activities of flavonols  
534 isolated from *Rhodiola rosea* roots and their in vitro anti-influenza viral activities.  
535 *Bioorg. Med. Chem.* 17(19), 6816-6823 (2009).
- 536 5. Kwon H-J, Kim H-H, Ryu YB *et al.* In vitro anti-rotavirus activity of polyphenol  
537 compounds isolated from the roots of *Glycyrrhizauralensis*. *Bioorg. Med. Chem.* 18(21),  
538 7668-7674 (2010).
- 539 6. Sithisarn P, Michaelis M, Schubert-Zsilavec M, Cinatl Jr J. Differential antiviral and  
540 anti-inflammatory mechanisms of the flavonoids biochanin A and baicalein in H5N1  
541 influenza A virus-infected cells. *Antivir. Res.* 97(1), 41-48 (2013).



- 542 7. Guz NR, Stermitz FR, Johnson JB *et al.* Flavonolignan and Flavone Inhibitors of a  
543 Staphylococcus aureus Multidrug Resistance Pump: Structure– Activity Relationships. *J.*  
544 *Med. Chem.* 44(2), 261-268 (2001).
- 545 8. Kim YS, Ryu YB, Curtis-Long MJ *et al.* Flavanones and rotenoids from the roots of  
546 *Amorpha fruticosa* L. that inhibit bacterial neuraminidase. *Food Chem. Toxicol.* 49(8),  
547 1849-1856 (2011).
- 548 9. Ohemeng K, Schwender C, Fu K, Barrett J. DNA gyrase inhibitory and antibacterial  
549 activity of some flavones (1). *Bioorg. Med. Chem. Lett.* 3(2), 225-230 (1993).
- 550 10. Wu T, He M, Zang X *et al.* A structure–activity relationship study of flavonoids as  
551 inhibitors of *E. coli* by membrane interaction effect. *Biochim. Biophys. Acta (BBA)-*  
552 *Biomembr.* 1828(11), 2751-2756 (2013).
- 553 11. Eghorn LF, Hoestgaard-Jensen K, Kongstad KT *et al.* Positive allosteric modulation of  
554 the GHB high-affinity binding site by the GABAA receptor modulator monastrol and the  
555 flavonoid catechin. *Eur. J. Pharmacol.* 740, 570-577 (2014).
- 556 12. Gopinath K, Sudhandiran G. Naringin modulates oxidative stress and inflammation in 3-  
557 nitropropionic acid-induced neurodegeneration through the activation of nuclear factor-  
558 erythroid 2-related factor-2 signalling pathway. *Neuroscience.* 227, 134-143 (2012).
- 559 13. Spencer JP, Vafeiadou K, Williams RJ, Vauzour D. Neuroinflammation: modulation by  
560 flavonoids and mechanisms of action. *Mol. Aspects Med.* 33(1), 83-97 (2012).
- 561 14. Guo L, Hu W-R, Lian J-H *et al.* Anti-hyperlipidemic properties of CM108 (a flavone  
562 derivative) in vitro and in vivo. *Eur. J. Pharmacol.* 551(1-3), 80-86 (2006).
- 563 15. Li G, Zhu Y, Zhang Y, Lang J, Chen Y, Ling W. Estimated daily flavonoid and stilbene  
564 intake from fruits, vegetables, and nuts and associations with lipid profiles in Chinese  
565 adults. *J. Acad. Nutr. Diet.* 113(6), 786-794 (2013).
- 566 16. Greeff J, Joubert J, Malan SF, Van Dyk S. Antioxidant properties of 4-quinolones and  
567 structurally related flavones. *Bioorg. Med. Chem.* 20(2), 809-818 (2012).
- 568 17. Hyun J, Woo Y, Hwang D-S *et al.* Relationships between structures of hydroxyflavones  
569 and their antioxidative effects. *Bioorg. Med. Chem. Lett.* 20(18), 5510-5513 (2010).
- 570 18. Xie P-J, Huang L-X, Zhang C-H, Zhang Y-L. Phenolic compositions, and antioxidant  
571 performance of olive leaf and fruit (*Olea europaea* L.) extracts and their structure–activity  
572 relationships. *J. Funct. Foods.* 16, 460-471 (2015).

- 573 19. Ravishankar D, Rajora AK, Greco F, Osborn HM. Flavonoids as prospective compounds  
574 for anti-cancer therapy. *Int. J. Biochem. Cell. Biol.* 45(12), 2821-2831 (2013).
- 575 20. Ravishankar D, Watson KA, Greco F, Osborn HM. Novel synthesised flavone derivatives  
576 provide significant insight into the structural features required for enhanced anti-  
577 proliferative activity. *RSC Adv.* 6(69), 64544-64556 (2016).
- 578 21. Liu-Smith F, Meyskens FL. Molecular mechanisms of flavonoids in melanin synthesis  
579 and the potential for the prevention and treatment of melanoma. *Mol. Nutr. Food Res.*  
580 60(6), 1264-1274 (2016).
- 581 22. Klimaszewska-Wiśniewska A, Hałas-Wiśniewska M, Izdebska M, Gagat M, Grzanka A,  
582 Grzanka D. Antiproliferative and antimetastatic action of quercetin on A549 non-small  
583 cell lung cancer cells through its effect on the cytoskeleton. *Acta Histochem.* 119(2), 99-  
584 112 (2017).
- 585 23. Mirossay L, Varinská L, Mojžiš J. Antiangiogenic effect of flavonoids and chalcones: An  
586 update. *Int. J. Mol. Sci.* 19(1), 27 (2017).
- 587 24. Ravishankar D, Watson KA, Boateng SY, Green RJ, Greco F, Osborn HM. Exploring  
588 quercetin and luteolin derivatives as antiangiogenic agents. *Eur. J. Med. Chem.* 97, 259-  
589 274 (2015).
- 590 25. Senderowicz AM. Flavopiridol: the first cyclin-dependent kinase inhibitor in human  
591 clinical trials. *Invest. New Drugs.* 17(3), 313-320 (1999).
- 592 26. A Study of Venetoclax and Alvocidib in Patients With Relapsed/Refractory Acute  
593 Myeloid Leukemia <https://ClinicalTrials.gov/show/NCT03441555> (April, 22).
- 594 27. Loguercio C, Festi D. Silybin and the liver: from basic research to clinical practice.  
595 *World J Gastroenterol.* 17(18), 2288 (2011).
- 596 28. A Phase II Study to Assess Efficacy of Combined Treatment With Erlotinib (Tarceva)  
597 and Silybin-phytosome (Siliphos) in Patients With EGFR Mutant Lung Adenocarcinoma  
598 <https://ClinicalTrials.gov/show/NCT02146118> (May, 23).
- 599 29. Ferry DR, Smith A, Malkhandi J *et al.* Phase I clinical trial of the flavonoid quercetin:  
600 pharmacokinetics and evidence for in vivo tyrosine kinase inhibition. *Clin. Cancer Res.*  
601 2(4), 659-668 (1996).
- 602 30. Effect of Quercetin on Green Tea Polyphenol Uptake in Prostate Tissue From Patients  
603 With Prostate Cancer Undergoing Surgery <https://ClinicalTrials.gov/show/NCT01912820>  
604 (May, 7).

- 605 31. Mulholland P, Ferry D, Anderson D *et al.* Pre-clinical and clinical study of QC12, a  
606 water-soluble, pro-drug of quercetin. *Ann. Oncol.* 12(2), 245-248 (2001).
- 607 32. Wu J-W, Lin L-C, Hung S-C, Chi C-W, Tsai T-H. Analysis of silibinin in rat plasma and  
608 bile for hepatobiliary excretion and oral bioavailability application. *J. Pharmaceut.*  
609 *Biomed. Anal.* 45(4), 635-641 (2007).
- 610 33. Deep A, Marwaha RK, Marwaha MG, Nandal R, Sharma AK. Flavopiridol as cyclin  
611 dependent kinase (CDK) inhibitor: a review. *New J. Chem.* 42(23), 18500-18507 (2018).
- 612 34. Gugler R, Leschik M, Dengler H. Disposition of quercetin in man after single oral and  
613 intravenous doses. *Eur. J. Clin. Pharmacol.* 9(2-3), 229-234 (1975).
- 614 35. Samsonowicz M, Regulska E, Kalinowska M. Hydroxyflavone metal complexes-  
615 molecular structure, antioxidant activity and biological effects. *Chem. Biol. Interact.* 273,  
616 245-256 (2017).
- 617 \*\* This article reviews the synthetic procedures, spectroscopic properties, biological activities  
618 and mechanisms of action of hydroxyflavone metal complexes.
- 619 36. Kasprzak MM, Szmigiero L, Zyner E, Ochocki J. Proapoptotic activity in vitro of two  
620 novel ruthenium (II) complexes with flavanone-based ligands that overcome cisplatin  
621 resistance in human bladder carcinoma cells. *J. Inorg. Biochem.* 105(4), 518-524 (2011).
- 622 37. Kasprzak MM, Erxleben A, Ochocki J. Properties and applications of flavonoid metal  
623 complexes. *RSC Adv.* 5(57), 45853-45877 (2015).
- 624 \*\* This review discusses the different metal coordination patterns, structural properties and  
625 the various applications of flavonoid metal complexes.
- 626 38. Grazul M, Budzisz E. Biological activity of metal ions complexes of chromones,  
627 coumarins and flavones. *Coord. Chem. Rev.* 253(21-22), 2588-2598 (2009).
- 628 \* This review gives details about the mechanisms of action of some metals and flavonoid  
629 metal complexes in the treatment of several pathological conditions.
- 630 39. Monserrat J-P, Tiwari KN, Quentin L *et al.* Ferrocenyl flavonoid-induced morphological  
631 modifications of endothelial cells and cytotoxicity against B16 murine melanoma cells. *J.*  
632 *Organomet. Chem.* 734, 78-85 (2013).
- 633 40. Tiwari KN, Monserrat J-P, Hequet A *et al.* In vitro inhibitory properties of ferrocene-  
634 substituted chalcones and auronones on bacterial and human cell cultures. *Dalton Trans.*  
635 41(21), 6451-6457 (2012).

- 636 41. Kowalski K, Koceva-Chyła A, Szczupak Ł *et al.* Ferrocenylvinyl-flavones: Synthesis,  
637 structure, anticancer and antibacterial activity studies. *J. Organomet. Chem.* 741, 153-161  
638 (2013).
- 639 42. Wang B-D, Yang Z-Y, Wang Q, Cai T-K, Crewdson P. Synthesis, characterization,  
640 cytotoxic activities, and DNA-binding properties of the La (III) complex with Naringenin  
641 Schiff-base. *Bioorg. Med. Chem.* 14(6), 1880-1888 (2006).
- 642 43. Tabassum S, Zaki M, Afzal M, Arjmand F. New modulated design and synthesis of  
643 quercetin–Cu II/Zn II–Sn 2 IV scaffold as anticancer agents: in vitro DNA binding  
644 profile, DNA cleavage pathway and Topo-I activity. *Dalton Trans.* 42(27), 10029-10041  
645 (2013).
- 646 \* This article offers the novel synthesis of bimetallic quercetin complexes. It provides a  
647 detailed explanation of their anti-cancer mechanism of action supported by the relevant  
648 biological assays and computational studies.
- 649 44. Ciesielska E, Studzian K, Zyner E, Ochocki J, Szmigiero L. DNA damage and apoptosis  
650 induction in L1210 cells by cis-diamminedichloroplatinum (II) and its new aminoflavone  
651 analogue. *Cellular Mol. Biol. Lett.* 5(2), 235-235 (2000).
- 652 45. Weil JA, Bolton JR. *Electron paramagnetic resonance: elementary theory and practical*  
653 *applications.* John Wiley & Sons, (2007).
- 654 46. Etcheverry SB, Ferrer EG, Naso L, Rivadeneira J, Salinas V, Williams PaM. Antioxidant  
655 effects of the VO (IV) hesperidin complex and its role in cancer chemoprevention. *J.*  
656 *Biol. Inorg. Chem.* 13(3), 435 (2008).
- 657 47. Naso LG, Ferrer EG, Butenko N *et al.* Antioxidant, DNA cleavage, and cellular effects of  
658 silibinin and a new oxovanadium (IV)/silibinin complex. *J. Biol. Inorg. Chem.* 16(4),  
659 653-668 (2011).
- 660 48. Naso LG, Lezama L, Rojo T *et al.* Biological evaluation of morin and its new  
661 oxovanadium (IV) complex as antio-xidant and specific anti-cancer agents. *Chem Biol*  
662 *Interact.* 206(2), 289-301 (2013).
- 663 49. Naso LG, Lezama L, Valcarcel M *et al.* Bovine serum albumin binding, antioxidant and  
664 anticancer properties of an oxidovanadium (IV) complex with luteolin. *J. Inorg.*  
665 *Biochem.* 157, 80-93 (2016).
- 666 50. Jurd L, Geissman T. Absorption spectra of metal complexes of flavonoid compounds. *J.*  
667 *Org. Chem.* 21(12), 1395-1401 (1956).

- 668 51. Selvaraj S, Krishnaswamy S, Devashya V, Sethuraman S, Krishnan UM. Flavonoid–  
669 metal ion complexes: a novel class of therapeutic agents. *Med. Res. Rev.* 34(4), 677-702  
670 (2014).
- 671 \* This review summarizes the structural characterization techniques and biological  
672 significance of flavonoid metal complexes.
- 673 52. Ren J, Meng S, Lekka CE, Kaxiras E. Complexation of flavonoids with iron: structure  
674 and optical signatures. *J. Phys. Chem. B.* 112(6), 1845-1850 (2008).
- 675 53. Tan J, Wang B, Zhu L. DNA binding and oxidative DNA damage induced by a quercetin  
676 copper (II) complex: potential mechanism of its antitumor properties. *J. Biol. Inorg.*  
677 *Chem.* 14(5), 727-739 (2009).
- 678 54. Tan J, Zhu L, Wang B. DNA binding and cleavage activity of quercetin nickel (II)  
679 complex. *Dalton Trans.* (24), 4722-4728 (2009).
- 680 55. Zhou J, Wang L-F, Wang J-Y, Tang N. Synthesis, characterization, antioxidative and  
681 antitumor activities of solid quercetin rare earth (III) complexes. *J. Inorg. Biochem.*  
682 83(1), 41-48 (2001).
- 683 56. Etcheverry SB, Barrio DA, Williams PA, Baran EJ. On the interaction of the vanadyl  
684 (IV) cation with lactose. *Biol. Trace. Elem. Res.* 84(1-3), 227-238 (2001).
- 685 57. Zeng Y-B, Yang N, Liu W-S, Tang N. Synthesis, characterization and DNA-binding  
686 properties of La (III) complex of chrysin. *J. Inorg. Biochem.* 97(3), 258-264 (2003).
- 687 58. Naso L, Ferrer EG, Lezama L, Rojo T, Etcheverry SB, Williams P. Role of oxidative  
688 stress in the antitumoral action of a new vanadyl (IV) complex with the flavonoid chrysin  
689 in two osteoblast cell lines: relationship with the radical scavenger activity. *J. Biol. Inorg.*  
690 *Chem.* 15(6), 889-902 (2010).
- 691 59. Wang Q, Zhao L, Zhao H *et al.* Complexation of luteolin with lead (II): Spectroscopy  
692 characterization and theoretical researches. *J. Inorg. Biochem.* 193, 25-30 (2019).
- 693 60. Kostova I, Manolov I, Nicolova I, Konstantinov S, Karaivanova M. New lanthanide  
694 complexes of 4-methyl-7-hydroxycoumarin and their pharmacological activity. *Eur. J.*  
695 *Med. Chem.* 36(4), 339-347 (2001).
- 696 61. Pastuszko A, Majchrzak K, Czyz M, Kupcewicz B, Budzisz E. The synthesis,  
697 lipophilicity and cytotoxic effects of new ruthenium (II) arene complexes with chromone  
698 derivatives. *J. Inorg. Biochem.* 159, 133-141 (2016).

- 699 62. Pi J, Zeng J, Luo J-J, Yang P-H, Cai J-Y. Synthesis and biological evaluation of  
700 Germanium (IV)–polyphenol complexes as potential anti-cancer agents. *Bioorg. Med.*  
701 *Chem. Lett.* 23(10), 2902-2908 (2013).
- 702 63. Singh AK, Saxena G, Arshad M. Synthesis, characterization and biological evaluation of  
703 ruthenium flavanol complexes against breast cancer. *Spectrochim. Acta A Mol. Biomol.*  
704 *Spectrosc.* 180, 97-104 (2017).
- 705 64. Tu L-Y, Pi J, Jin H, Cai J-Y, Deng S-P. Synthesis, characterization and anticancer  
706 activity of kaempferol-zinc (II) complex. *Bioorg. Med. Chem. Lett.* 26(11), 2730-2734  
707 (2016).
- 708 65. Roy S, Mallick S, Chakraborty T *et al.* Synthesis, characterisation and antioxidant  
709 activity of luteolin–vanadium (II) complex. *Food Chem.* 173, 1172-1178 (2015).
- 710 66. Tan M, Zhu J, Pan Y *et al.* Synthesis, cytotoxic activity, and DNA binding properties of  
711 copper (II) complexes with hesperetin, naringenin, and apigenin. *Bioinorg. Chem. Appl.*  
712 2009, (2009).
- 713 67. Kurzwernhart A, Kandioller W, Bächler S *et al.* Structure–activity relationships of  
714 targeted RuII ( $\eta^6$ -p-Cymene) anticancer complexes with flavonol-derived ligands. *J.*  
715 *Med. Chem.* 55(23), 10512-10522 (2012).
- 716 68. Kurzwernhart A, Kandioller W, Bartel C *et al.* Targeting the DNA-topoisomerase  
717 complex in a double-strike approach with a topoisomerase inhibiting moiety and covalent  
718 DNA binder. *Chem. Commun.* 48(40), 4839-4841 (2012).
- 719 69. Yang F, Jin H, Pi J *et al.* Anti-tumor activity evaluation of novel chrysin–  
720 organogermanium (IV) complex in MCF-7 cells. *Bioorg. Med. Chem. Lett.* 23(20), 5544-  
721 5551 (2013).
- 722 70. Dong H, Yang X, He J, Cai S, Xiao K, Zhu L. Enhanced antioxidant activity,  
723 antibacterial activity and hypoglycemic effect of luteolin by complexation with  
724 manganese (II) and its inhibition kinetics on xanthine oxidase. *RSC Adv.* 7(84), 53385-  
725 53395 (2017).
- 726 71. Pereira R, Andrades NE, Paulino N *et al.* Synthesis and characterization of a metal  
727 complex containing naringin and Cu, and its antioxidant, antimicrobial, antiinflammatory  
728 and tumor cell cytotoxicity. *Molecules.* 12(7), 1352-1366 (2007).
- 729 72. Manolov I, Kostova I, Konstantinov S, Karaivanova M. Synthesis, physicochemical  
730 characterization and cytotoxic screening of new complexes of cerium, lanthanum and  
731 neodymium with Nifflcoumar sodium salt. *Eur. J. Med. Chem.* 34(10), 853-858 (1999).

- 732 73. Kostova I, Manolov I, Karaivanova M. Synthesis, physicochemical characterization, and  
733 cytotoxic screening of new zirconium complexes with coumarin derivatives. *Arch.*  
734 *Pharm.* 334(5), 157-162 (2001).
- 735 74. Pastuszko A, Niewinna K, Czyz M, Józwiak A, Małecka M, Budzisz E. Synthesis, X-ray  
736 structure, electrochemical properties and cytotoxic effects of new arene ruthenium (II)  
737 complexes. *J. Organomet. Chem.* 745, 64-70 (2013).
- 738 75. Kostova I, Kostova R, Momekov G, Trendafilova N, Karaivanova M. Antineoplastic  
739 activity of new lanthanide (cerium, lanthanum and neodymium) complex compounds. *J.*  
740 *Trace Elem. Med. Biol.* 18(3), 219-226 (2005).
- 741 76. Leon IE, Di Virgilio AL, Porro V *et al.* Antitumor properties of a vanadyl (IV) complex  
742 with the flavonoid chrysin [VO (chrysin) 2 EtOH] 2 in a human osteosarcoma model: the  
743 role of oxidative stress and apoptosis. *Dalton Trans.* 42(33), 11868-11880 (2013).
- 744 77. Chen Y-H, Yang Z-S, Wen C-C *et al.* Evaluation of the structure–activity relationship of  
745 flavonoids as antioxidants and toxicants of zebrafish larvae. *Food Chem.* 134(2), 717-724  
746 (2012).
- 747 78. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of  
748 flavonoids and phenolic acids. *Free Radic. Biol. Med.* 20(7), 933-956 (1996).
- 749 \* This review compares the anti-oxidant potential of a range of flavonoid structures giving a  
750 detailed idea about the impact of each functional group on the anti-oxidant activity
- 751 79. Pietta P-G. Flavonoids as antioxidants. *J. Nat. Prod.* 63(7), 1035-1042 (2000).
- 752 80. Rosenberg B, Vancamp L, Trosko JE, Mansour VH. Platinum compounds: a new class of  
753 potent antitumour agents. *Nature.* 222(5191), 385 (1969).
- 754 81. Jung Y, Lippard SJ. Direct cellular responses to platinum-induced DNA damage. *Chem.*  
755 *Rev.* 107(5), 1387-1407 (2007).
- 756 82. Pil P. Cisplatin and related drugs. *Encyclopedia of Cancer.* 1, 391-410 (1997).
- 757 83. Fuertes MA, Alonso C, Pérez JM. Biochemical modulation of cisplatin mechanisms of  
758 action: enhancement of antitumor activity and circumvention of drug resistance. *Chem.*  
759 *Rev.* 103(3), 645-662 (2003).
- 760 84. Tan J, Zhu L, Wang B. From GC-rich DNA binding to the repression of survivin gene for  
761 quercetin nickel (II) complex: implications for cancer therapy. *Biometals.* 23(6), 1075-  
762 1084 (2010).

- 763 85. Kaizer J, Baráth G, Pap J, Speier G, Giorgi M, Réglér M. Manganese and iron  
764 flavonolates as flavonol 2, 4-dioxygenase mimics. *Chem. Commun.* (48), 5235-5237  
765 (2007).
- 766 86. Sahu SC, Gray GC. Interactions of flavonoids, trace metals, and oxygen: nuclear DNA  
767 damage and lipid peroxidation induced by myricetin. *Cancer Lett.* 70(1-2), 73-79 (1993).
- 768 87. León I, Cadavid-Vargas J, Tiscornia I *et al.* Oxidovanadium (IV) complexes with chrysin  
769 and silibinin: anticancer activity and mechanisms of action in a human colon  
770 adenocarcinoma model. *J. Biol. Inorg. Chem.* 20(7), 1175-1191 (2015).
- 771 88. Naruse T, Nishida Y, Ishiguro N. Synergistic effects of meloxicam and conventional  
772 cytotoxic drugs in human MG-63 osteosarcoma cells. *Biomed. Pharmacother.* 61(6), 338-  
773 346 (2007).
- 774 89. León IE, Díez P, Etcheverry SB, Fuentes M. Deciphering the effect of an oxovanadium  
775 (IV) complex with the flavonoid chrysin (VOChrys) on intracellular cell signalling  
776 pathways in an osteosarcoma cell line. *Metallomics.* 8(8), 739-749 (2016).
- 777 90. Yang F, Gong L, Jin H *et al.* Chrysin–organogermanium (IV) complex induced Colo205  
778 cell apoptosis-associated mitochondrial function and anti-angiogenesis. *Scanning.* 37(4),  
779 246-257 (2015).
- 780 91. Janka V, Žatko D, Ladislav V, Pál P, Janka P, Gabriela M. Some ferrocenyl chalcones as  
781 useful candidates for cancer treatment. *In Vitro Cell. Dev. Biol. Anim.* 51(9), 964-974  
782 (2015).
- 783
- 784
- 785