Determination of Anticancer Zn(II)–Rutin Complex Structures in Solution through Density Functional Theory Calculations of $^1$H NMR and UV–VIS Spectra

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ABSTRACT: Coordination compounds formed by flavonoid ligands are recognized as promising candidates as novel drugs with enhanced antioxidant and anticancer activity. Zn(II)–Rutin complexes have been described in the literature and distinct coordination modes proposed based on $^1$H NMR/MS and IR/UV–VIS experimental spectroscopic data: 1:1/1:2 (Zn(II) binding to A-C rings) and 2:1 (Zn(II) binding to A-C-B rings) stoichiometry. Aiming to clarify these experimental findings and provide some physical insights into the process of complex formation in solution, we carried out density functional theory calculations of NMR and UV–VIS spectra for 25 plausible Zn(II)–Rutin molecular structures including solvent effect using the polarizable continuum model approach. The studied complexes in this work have 1:1, 1:2, 2:1, and 3:1 metal–ligand stoichiometry for all relevant Zn(II)–Rutin configurations. The least deviation between theoretical and experimental spectroscopic data was used as an initial criterion to select the probable candidate structures. Our theoretical spectroscopic results strongly indicate that the experimentally suggested modes of coordination (1:2 and 2:1) are likely to exist in solution, supporting the two distinct experimental findings in DMSO and methanol solution, which may be seen as an interesting result. Our predicted 1:2 and 2:1 metal complexes are in agreement with the experimental stoichiometry; however, they differ from the proposed structure. Besides the prediction of the coordination site and molecular structure in solution, an important contribution of this work is the determination of the OH–C5 deprotonation state of rutin due to metal complexation at the experimental conditions (pH = 6.7 and 7.20). We found that, in the two independent synthesis of metal complexes, distinct forms of rutin (OH$^–$C5 and O$^{2–}$–C5) are present, which are rather difficult to be assessed experimentally.

INTRODUCTION

Flavonoids belong to a broad subclass of natural polyphenols with relatively low molar mass, which are present in plants such as *Camellia sinensis* (popularly known as green tea), fruits like *Euterpe oleracea* (açai berry, typical fruit from Brazil), and plants seed such as *Glycine max* (soy). They consist of a large group of polyphenolic compounds that have a benzopyrone base structure, and their activities are dependent on structural aspects. Compounds derived from natural flavonoids are widely known for their antibacterial, antiviral, antioxidant, and anti-inflammatory biological activities. Although it is proven that most of the biological properties of these polyphenols are associated with its high antioxidant potential, they have shown to be promising anticancer agents. The chemical nature of flavonoids depends on their class, hydroxylation and polymerization degrees, chemical substitutions, and conjugations. A relationship between the molecular structure and biological activity is always a welcome piece of information; in this sense, structural characterization studies of polyphenols have been conducted, mainly through X-ray data analysis in the solid state. From a theoretical side, a recent review has been reported addressing the reliability of density functional theory (DFT) for the prediction of the main antioxidant properties, encompassing phenolic natural antioxidants, and reaction mechanisms involving free radicals. The base nucleus of the flavonoids (the benzopyrone group) may present several functional groups that give rise to the classes of flavonoids, making this family of compounds one of the most studied in the area of natural products. Rutin (Scheme 1), belonging to the flavonols class, possesses a total of 10 hydroxyl groups, with two attached to the A ring (positions 5 and 7), two at the B ring (positions 3′ and 4′), and six distributed on the glucopyranosyl (labeled G, positions...
produced two peaks with m/z ratio equivalent to 1:1 and 1:2 forms of Zn(II)–rutin complexes. No other site of ligand coordination was considered in ref 28.

In ref 12, a solution of [Zn(CH₃COO)₂]·2H₂O salt in distilled water was slowly added dropwise to a solution of dehydrated rutin (Dimorphandra mollis) in methanol. The mixture was stirred at 90–140 rpm for 24 h at 37–40 °C. The complex was filtered in a vacuum system, washed with methanol, and dried at room temperature. The complex characterization was made analyzing IR (solid-state sample), UV–VIS (in methanol solution), and 1H NMR (in DMSO-d₆ solution) spectroscopic data. Through the analysis of spectroscopic data, the authors proposed the formation of a 2:1 complex with the first Zn(II) ion binding at C5–OH (not deprotonated) and O=C4 positions, and the second metal ion binding at (−O=C3′ and (−O=C4′ (both hydroxyl groups deprotonated). Two water molecules completed the coordination sphere of the first Zn(II) ion (tetracoordinated) and four water molecules in the second Zn(II) ion (hexacoordinated). An important result reported in ref 12 is the enhanced biological activity of the synthesized Zn(II)–rutin complexes compared with free rutin. Zn(II)–Rutin complexes has not shown any cytotoxicity against normal cells or toxicity in BALB/c mice but has shown antioxidant activity and cytotoxicity against cancer cell lines in vitro and synergistic antitumor activity preventing side effects of chemotheraphy.

We found these dissimilar experimental assignments of the preferred complex molecular structures in solution12,28 very interesting and intriguing. We thought that it would be relevant to investigate this complex at a molecular level using quantum chemical methods since experimental data are reported for macroscopic samples in solution and no direct access to the molecular structure or conformation of the metal–flavonoid complex is available to the experimentalists. The only experimental source of information is the spectroscopic data, and according to the analysis of experimental data, it seems that distinct modes of complexation are present in the two reported preparations12,28 of Zn(II)–Rutin complexes in DMSO and methanol solution using different zinc ion reactants.

The understanding of biological activity involves the knowledge of metal–flavonoid complex structure and binding sites. Therefore, the use of quantum chemical calculations of molecular properties combined with available experimental spectroscopic data can be of great help for the determination of the molecular structure in solution (which is hard to be attained experimentally). Theoretical results may contribute to the investigation of the mechanism of action at a molecular level that involves the study of interaction of metal–flavonoid complexes with specific target sites in a biological environment. In this article, we investigated the molecular structure of Zn(II)–Rutin complexes in solution through DFT calculations of spectroscopic properties (1H NMR, UV–VIS, and IR). From comparison of theoretical results with experimental spectroscopic data obtained, the most probable structures of Zn(II) complexes with rutin ligand in solution was obtained. Our results strongly suggest that metal complexes having a distinct metal:ligand stoichiometry (1:2 and 2:1) can be present in the two experimental preparations reported recently12,28 for the metal complex.

2′′, 3′′, and 4′′′) and rhamnopyranosyl (labeled R, positions 2′′′, 3′′′′, and 4′′′′) units. The relevant rutin torsion angles (ϕ) are also shown in Scheme 1, where the high conformational flexibility of the molecule can be promptly seen. Therefore, the rutin molecule has a high capacity of coordination to metal ions through available oxygen atoms, including the carbonyl group at the C4 position of ring C. There are various reports on the synthesis and characterization of metal–Rutin complexes as, for example, Zn(II)12,28 Sn(II)13, Al(III),14 Cu(II),15 Fe(II),16 and other metal ions, with coordination compounds exhibiting various ligand–metal stoichiometries (1:1, 1:2, and 2:1) and spatial conformations being predicted. According to Ikeda et al.,12 the formation of coordination compounds with rutin greatly increases the antioxidant power and, consequently, other properties (especially antitumor activity). The authors showed that the Zn(II)–Rutin complexes have significantly higher antioxidant activity than free rutin, no toxicity against normal cells of the rat organ, and potential cytotoxicity against the cancer cells.

Various experimental studies addressing the metal complex formation with flavonoid ligands in solution and highlighting the enhancement of biological activity compared with free flavonoids have been reported over the past years (see, for example, refs 17–21). A recent review on the properties and applications of metal–flavonoid complexes can be found in ref 22. The improvement of the drug efficacy due to complex formation is a well-known fact, and an understanding of this experimental result at a molecular level is certainly of relevance and gives a great motivation for the use of computational chemistry methods to investigate these molecular systems. The utility of theoretical calculations of metal–flavonoid complexes to assist experimental investigations is evident from various DFT studies reported on computation of molecular structures, relative energies, and spectroscopic quantities that can be compared with experimental data (see, for example, refs 23–27).

Two independent synthesis of Zn(II)–Rutin complexes have been reported in the literature12,28 and conducted in DMSO and methanol solution using ZnCl₂ and [Zn-(CH₃COO)₂]·2H₂O as reactants, respectively. In ref 28, the preparation of the sample for NMR experiment studies were conducted on 5 mM solutions of flavonoids in mixture solvents of DMSO/Tris-HCl (pH 7.20). The solutions were titrated with increasing concentration of zinc chloride (1/4, 1/2, 1, and 2 equiv of Zn(II) ions). Through the analysis of 1H NMR and MS spectra in DMSO-d₆/D₂O, two possible complex structures were proposed in ref 28, with the hydroxy group at the C5 position of A ring deprotonated and Zn(II) binding at O(1′′′–C5 and O==C4′′′ positions to form 1:1 and 1:2 metal:ligand complexes. The assignment of the two structures was based on the analysis of MS data in DMSO, which
**RESULTS AND DISCUSSION**

Figure 1 shows the B3LYP/6-31G(d,p) optimized free rutin structure predicted to exist in DMSO solution\(^3\) with relevant torsion angles \(\phi_1\) and \(\phi_2\), atomic labels, and coordination sites indicated. Eight sites of coordination were considered in this work, with a total of 25 Zn(II)–Rutin complex structures optimized, encompassing mono-, bi-, and tri-OH deprotonated structures besides the neutral rutin (fully protonated) as shown in Figure 1. All Zn(II)–Rutin optimized complex structures investigated in this work are shown in Figure 2.

Experimental \(^1\)H NMR chemical shift (in DMSO-\(d_6/\)D\(_2\)O solution) was reported for Zn(II)–Rutin complexes,\(^2\) which are used as a reference for the determination of plausible candidate complex structures based on the comparison with DFT-PCM calculated NMR spectra in DMSO. As reported previously,\(^3\) in a comprehensive conformational analysis of flavonoid rutin, rotation of \(\phi_1\) and \(\phi_2\) torsion angles is required to reach an agreement with \(^1\)H NMR data in DMSO solution for H2\’, H5\’, and H6\’ B-ring protons of free rutin. The best dihedral angle values are \(\phi_1 = -150^\circ\) and \(\phi_2 = 130^\circ\) with the fully optimized torsion angles being 175\(^\circ\) (close to planarity) and 119\(^\circ\), respectively. These torsion angle values were used as a guide for the rotation of the B ring in the metal complex structures. Figure 3a shows deviation between B3LYP-PCM-DMSO and experimental (in DMSO-\(d_6\))\(^2\) \(^1\)H NMR chemical shift, and Figure 3b gives the RMSD values for selected Zn(II)–Rutin complex structures. The best fit with experimental data is highlighted in rectangle in Figure 3 where it can be seen that the RMSD value for complex 3 is only 0.085. Corresponding MAE, slope standard error, and Adj. \(R^2\) squared deviation values for all 25 metal complex structures are given in the Supporting Information (Figure S1) as well as \(^1\)H NMR spectra for representative complex structures showing reasonable agreement with experimental NMR data (Figure S2).

Figure 4 shows experimental \(^1\)H NMR spectra\(^2\) measured in DMSO-\(d_6\) and B3LYP/6-31G(d,p)-PCM-DMSO \(^1\)H NMR spectra for selected Zn(II)–Rutin \(\phi_1/\phi_2\) rotated complex structures. Complexes 17, 20, and 25 are included to assess the effect of deprotonation at OH–C5 on the NMR profile. It is worth mentioning that B3LYP \(^1\)H NMR spectra for free rutin and complex 2 structures optimized in DMSO (PCM) are given in Figures S3 and S4 (Supporting Information) just to show the effect of inclusion of solvent effect on geometry optimization (DFT-PCM). It can be seen that there is no significant change on the molecular structure, with the new \(^1\)H NMR spectra (PCM optimized geometries) being virtually identical to the spectra calculated with geometries optimized in the gas phase, and so, no geometry optimization in DMSO (PCM) is required. The best match between experimental and theoretical \(^1\)H NMR profiles can be used as a criterion to select the candidate structure to be present in DMSO solution. It should be said that all magnetic tensors were scaled to reproduce the HS\’ proton (as has been done previously for rutin\(^3\)) only to facilitate comparison with experimental NMR pattern (this is just a translation of the theoretical spectrum without changing the relative position of each proton signal). This procedure has been adopted in previous works.\(^27\)–\(^30\),\(^33\)–\(^39\),\(^41\)

It can be seen from Figure 4 that complex 3 (1:2) exhibits the best agreement with the experimental NMR profile and the lowest statistics indices according to the results reported in Figure 3 followed by complex 1 (1:1) and 24 (3:1), which also show a reasonable deviation from experimental NMR data. Based on the analysis of experimental NMR and mass spectra data reported in ref \(^28\), the authors proposed two structures as candidates to be present in DMSO solution (Figure 2b,d): complex 2 (1:1) and 4 (1:2), which are equivalent to the structures 1 and 3 but deprotonated at HO–C5 (see Figure 2). The mass spectra revealed two major fragments species at \(m/z\) 673.2 and 1283.6 assigned to 1:1 complex denominated \([\text{Zn}(\text{L}^-)\text{H})]\)\(^+\) and 1:2 complex \([\text{Zn}(\text{L}^-)\text{H})_2\]\(^+\), respectively. The fragment species assigned in the mass spectra could also be obtained considering complexes 1 (1:1) and 3 (1:2) calculated in this work; therefore, they cannot be ruled out based only on the analysis of the mass spectra. However, the mass spectra data eliminate the possibility of the existence of 2:1 (structures 15–23) and 3:1 (structures 24 and 25) complexes in the experimental preparation of Zn(II)–Rutin complexes in DMSO solution reported in ref \(^28\).
Figure 2. B3LYP/6-31G(d,p)/Lanl2DZ optimized Zn(II)–Rutin complex geometries with the following charges: (a) +2, (b) +1, (c) +2, (d) 0, (e) +2, (f) +1, (g) 0, (h) 0, (i) +1, (j) 0, (k) 0, (l) 0, (m) 0, (n) +1, (o) +4, (p) +3, (q) +2, (r) +2, (s) +1, (t) +1, (u) +3, (v) +2, (w) +3, and (y) +3. Hydrogen atoms relevant for $^1$H NMR analysis (C–H protons) are highlighted.

Figure 3. Statistics of B3LYP/6-31G(d,p)-PCM-DMSO $^1$H NMR chemical shift deviation from experimental data (in DMSO-$d_6$). The best fit is highlighted in rectangle. (a) Deviation from each experimental signal for $\phi_1$ and $\phi_2$ rotated Zn(II)–Rutin complex structures. (b) RMSD results.
Experimental 1H NMR patterns show an overall best match with the experiment. It can be seen from Figure 4 that complex proton is preventing an almost exact match for complex ppm and precisely 1.1 ppm for complex the complex structures present in DMSO solution (only H8 experimental spectrum, while that for complexes ppb and 2.8 rotated complex structures (, proposed in ref , respectively. This mode of Zn(II) coordination shifts the H6,H8 NMR signals away from the experimental pattern. In addition, the separation between H6 and H-1G protons signals is 1.13 ppm in the experimental spectrum, while that for complexes 2 and 4 is 0.6 ppm and precisely 1.1 ppm for complex 3, again in agreement with the experiment. It can be seen from Figure 4 that complex 3 NMR patterns show an overall best match with the experimental 1H NMR profile, strongly indicating that this is the complex structures present in DMSO solution (only H8 proton is preventing an almost exact match for complex 3 by ca. 0.2 ppm).

The deprotonation of OH—C5 at the A ring, proposed in ref 28, has a measurable effect on H8 and H6 protons signals, shifting to a high field region, which can be explained with the use of valence-bond theory, which reveals resonance hybrids with a negative charge distributed over C6 and C8 carbons (Scheme 2), justifying the bigger shielding in their hydrogen atoms (and the same behavior is observed for OH—C5 deprotonated free rutin, showed in Figure S3d,e). The calculated H6 and H8 1H NMR profiles for complexes 2 and 4 strongly indicate that they are not likely to be present in the experiment reported in DMSO solution.28

At this point, it is pertinent to establish how much the theoretical NMR pattern deviation from the experimental profile must be to safely discard a given Zn(II)—Rutin complex structure. According to the results reported in Figure 3, it can be seen that complexes 1 and 24 exhibit relatively small deviation from experimental data, with the difference from the RMSD values calculated for complex 1 being 0.044 and 0.035 ppm, respectively, which are within the DFT precision for the calculation of 1H NMR chemical shifts. Therefore, complexes 1 and 24 may not be totally discarded based on the analysis of 1H NMR data. All theoretical—experimental deviation data reported in Figure 3 pointed out to complex 3 as the predominant structure in DMSO solution, with complexes 1 and 24 coming next. In spite of that, the molecular mass of complexes 24 (908.0 amu) is incompatible with the data from the mass spectrum regarding the fragments of m/z 673.2 and 1283.6 reported in ref 28. It should be mentioned that, in ref 28, the proposal of complexes 2 and 4 was based on the analysis of MS spectra and the possibility of the protonated OH—C5 site was not considered. These structures are equivalent to 1 and 3, respectively, with the only difference being the deprotonation at OH—C5. Our calculated NMR results provide strong evidence that complex 3 (and also complex 1 within a given uncertainty range) should be present in the NMR experiment conducted in DMSO solution, not complexes 2 and 4 as proposed in ref 28.

In another experiment reported12 for the preparation of Zn(II)—Rutin complexes, a solution of [Zn(CH3COO)2]·2H2O salt in distilled water was slowly added dropwise to a solution of dehydrated rutin in methanol. The experimental complex characterization was done through IR and UV–VIS analysis, and a 2:1 complex involving the OC4—OC5 (site 1) and O(−)C3’—O(−)C4’ (site 2) modes of complexation was proposed (complex 20 from Figure 2t). We have investigated various possibilities for 2:1 Zn(II)—Rutin complexes (and also complex 1 within a given uncertainty range) with the DFT optimized structures given in Figure 2 (structures 15–25). Figure 5 shows B3LYP/6-31G(d,p)-PCM-Methanol Band-I and Band-II UV–VIS shifts due to complex formation (in nm) for these structures as well as 1:1 and 1:2 complexes. UV–VIS excitation energies (in nm) for selected metal–flavonoid complex structures are given in Table 1.

It can be seen from Figure 5 that the best fit with the experimental UV–VIS profile reported in ref 12 was attained by the 2:1 complex 17 (site 1 (O(−)C5—OC4) and site 2...

Scheme 2. Resonance Scheme Showing the Bigger H6 and H8 Shielding
Experimental UV–VIS complexes can be eliminated as candidate structures based on complexes 17 and 25, which are similar to the one proposed experimentally in ref 12 but deprotonated at the OS moiety. It can be seen from Figure 5 that the complex structure proposed in ref 12, structure 25, shows a poor agreement with experimental UV–VIS data (Band I) and can be ruled out from the second experimental work (the same holds for structure 18, which is similar but with Zn tetracoordinated). It should be mentioned that our DFT results corroborate the experimentally proposed complex stoichiometry (2:1) and also shows good agreement with experimental UV–VIS data. The same good agreement with experimental band shifts due to complexation is found for complex 25 (also having OH−C5 deprotonated).

Analyzing the IR data from ref 12, the C=O stretching shift of –28 cm−1 due to deprotonation can be used to rule out all 1:1 complexes with the Zn(II) metal ion not coordinated to site 1. Thus, the complexes 6, 13, and 14 (and also 5, 7, 8, 10, 11, and 12) were ruled out because a small positive shift is predicted, which is not in agreement with experimental data from refs 12, 28 lead us to affirm that there is a great possibility that distinct modes of complexation are operating in these two experimental works: the first using ZnCl2 in DMSO, and the second using [Zn(CH3COO)2]2·H2O salt and methanol as a solvent. The most probable structures predicted to exist in DMSO and methanol solution based on 1H NMR and UV–VIS spectroscopic analysis are shown in Figure 6, along with ϕ1, ϕ2 torsion angle values.

B3LYP/6-31G(d,p)/Lanl2DZ metal–ligand bond distance and bond angles for 16 representative complex structures (Figure 2) are given in Table S2 along with the de definition of geometrical parameters (Scheme 3), with results for four selected structures shown in Figure 6 given in Table 2. The Zn–O distances range from 1.9 to 2.1 Å for the tetracoordinated (site 1) and hexacoordinated (site 2) with rutin deprotonated at O′(O)H3 and O′(O)H4 positions (site 2), has very poor agreement with experimental UV–VIS data (Band-I), complex 17, a similar structure with rutin deprotonated at O′(O)H5 (site 1) and O′(O)H4 (site 2) positions and Zn(II) ions tetracoordinated, shows nice agreement with experimental UV–VIS data. The same good agreement with experimental band shifts due to complexation is found for complex 25 (also having OH−C5 deprotonated). It is also worth noting that the number of water molecules coordinated to Zn at site 2 does not change the UV spectral profile as shown for structures 7 (two water molecules) and 8 (four water molecules) (Figure S6, Supporting Information).

Table 1. Experimental and B3LYP/6-31G(d,p)/Lanl2DZ Infrared C=O Frequency (Wavenumber in cm−1) and UV–VIS (PCM-Methanol, in nm) Data for Relevant Zn(II)–Rutin Complex Structures

| ZnII–Rutin complex | υ_{C=O} | shift | Band-I deviation | Band-II deviation |
|---------------------|---------|-------|----------------|------------------|
| Experimental        | 1627    | −28   | 393            | 269              |
| Cpx-1               | 1558    | −70   | 424            | 31               |
| Cpx-2               | 1555    | −73   | 372            | −21              |
| Cpx-3               | 1562    | −66   | 418            | 25               |
| Cpx-4               | 1586    | −42   | 355            | −37              |
| Cpx-6               | 1650    | 22    | 372            | −21              |
| Cpx-13              | 1651    | 23    | 369            | −24              |
| Cpx-15              | 1643    | 15    | 372            | −21              |
| Cpx-16              | 1563    | −65   | 373            | −20              |
| Cpx-17              | 1656    | −62   | 427            | 34               |
| Cpx-18              | 1568    | −60   | 403            | 10               |
| Cpx-19              | 1558    | −70   | 568            | 175              |
| Cpx-20              | 1542    | −86   | 508            | 115              |
| Cpx-21              | 1559    | −69   | 559            | 166              |
| Cpx-22              | 1548    | −80   | 427            | 34               |
| Cpx-23              | 1546    | −82   | 427            | 34               |
| Cpx-25              | 1559    | −69   | 408            | 15               |
Following the protocol from ref 28 for the preparation of experimental conditions of complex synthesis must be known. Reaction for the formation of Zn(II) observed molecular structure. In order to propose a model considered as a useful quantity to assess its plausibility as larger (2.0 Å). Hexacoordinated Zn(II) (Cpx−1) being shorter than the Zn$t^\text{2+}$ tetracoordinated metal center, with the Zn−O(−) distance being shorter than the Zn−OH one as expected. For hexacoordinated Zn(II) (Cpx-20, see Table S2), the distances are larger (2.0−2.3 Å). In addition, the O−Zn−O bond angles involving the oxygen atoms from rutin (around 90°−100°) are smaller than the corresponding angles involving the water molecules coordinated to the metal. The geometries are distorted from the ideal tetrahedral form (and also octahedral for complex 20) with the coordinated water molecules occupying spatial positions in order to minimize the interaction energy without preserving symmetry.

Calculated energy of complex formation (ΔE) is often considered as a useful quantity to assess its plausibility as observed molecular structure. In order to propose a model reaction for the formation of Zn(II)−Rutin complexes, the experimental conditions of complex synthesis must be known. Following the protocol from ref 28 for the preparation

![Diagram](https://dx.doi.org/10.1021/acsomega.9b04174)

**Scheme 3.** (a) Bond Lengths ($r_e$) and (b) Bond Angles between Two Bonds ($\theta_n$) in the Coordinating Spheres with a Distorted Tetrahedral Geometry$^a$

$^a$All data were obtained from calculations at the B3LYP/6-31G(d,p)/LanL2DZ level of theory.

![Table](https://dx.doi.org/10.1021/acsomega.9b04174)

**Table 2.** B3LYP/6-31G(d,p)/LanL2DZ Optimized Geometrical Parameters for Selected Zn(II)−Rutin Complexes (See Figure 6)$^a$

| symbol | Cpx-1 | Cpx-3 | Cpx-24 | Cpx-25 |
|--------|-------|-------|-------|-------|
| Bond length (Å) | | | | |
| $r_1$ | 2.11$^b$ | 2.15$^{b,c}$ | 2.08$^d$/2.15$^d$/2.08$^e$ | 1.98$^f$/2.10$^f$/2.06$^f$ | 1.89$^g$/2.10$^g$/2.06$^g$ |
| $r_2$ | 1.87$^b$ | 1.88$^{b,c}$ | 1.89$^d$/1.90$^d$/1.87$^e$ | 1.96$^f$/1.89$^f$/1.89$^f$ | 1.96$^g$/1.89$^g$/1.89$^g$ |
| $r_3$ | 2.04$^b$ | 2.04$^d$/2.03$^d$/2.07$^e$ | 2.07$^f$/2.05$^f$/2.07$^f$ | 2.07$^g$/2.05$^g$/2.07$^g$ |
| $r_4$ | 2.05$^b$ | 2.03$^d$/2.04$^d$/2.08$^e$ | 2.07$^f$/2.06$^f$/2.09$^f$ | 2.07$^g$/2.06$^g$/2.09$^g$ |
| Bond angle (°) | | | | |
| $\theta_1$ | 89.5 | 87 | 90.8$^h$/81.6$^i$/87.8$^j$ | 99.1$^k$/84.0$^k$/88.8$^k$ | 99.1$^k$/84.0$^k$/88.8$^k$ |
| $\theta_2$ | 129.5 | 114.9 | 128.2$^d$/118.6$^d$/122.6$^e$ | 127.7$^f$/104.0$^f$/95.3$^g$ | 127.7$^f$/104.0$^f$/95.3$^g$ |
| $\theta_3$ | 118.9 | 116.3 | 123.9$^d$/117.6$^d$/121.1$^e$ | 123.5$^f$/116.3$^f$/121.1$^f$ | 123.5$^f$/116.3$^f$/121.1$^f$ |
| $\theta_4$ | 98.14 | 83.3 | 98.9$^d$/104.9$^d$/85.4$^e$ | 125.2$^f$/116.6$^f$/119.2$^g$ | 125.2$^f$/116.6$^f$/119.2$^g$ |

$^a$Definition of bond distance ($r_e$ in Å) and bond angle ($\theta_n$ in degrees) is given in Scheme 3. $^b$Coordination site 1. $^c$The same value was observed for the bond between the metal and the corresponding groups of the two ligand molecules. $^d$Coordination site 2. $^e$Coordination to the 2" and 3" oxygen atoms of site 8 (from the sugar fraction).
In addition, binding energy calculations employing eq 5 from ref 25, used in the DFT study of Zn(II) coordination by quercetin and luteolin leading to the determination of the preferred chelation site, were also done, which is rather different from eqs 1–4. In eq 5, the change in the flavonoid structure due to the removal of hydrogen atoms from OH groups where the zinc atom was bound (deprotonation) was accomplished using H2O as a relevant chemical potential for the abstraction of H atoms. In eq 5, $E_{\text{total}}$ is the total energy of the complex, $E_X$ and $n_X$ are the energy and number of species X involved in the complexation reaction (X = Zn, Rut, H), and $n_H$ is the number of hydrogen atoms missing from the neutral rutin molecule after complexation, with the abstracted H atoms becoming part of a H2O molecule.25 Complexes solvated by H2O or chlorine molecules are calculated adding the appropriate species in eq 5.

For the calculation of Zn(II)—Rutin interaction energy, we used the M06-2x DFT functional with a triple-zeta quality 6-311+G(2d,p) basis set.
311+G(2d,p) basis set. M06-2x is a highly parameterized approximate exchange-correlation energy functional based on the meta-GGA approximation developed by the Truhlar group. There are various reports in the literature supporting the use of the M06-2x functional in quantum chemical calculations of molecular systems. An example is the study of a corannulene–cisplatin model complex where the B3LYP interaction energy was shown to be considerably underestimated compared to post-Hartree–Fock MP2 result, while the M06-2x functional showed fine agreement and so was recommended for the calculation of the intermolecular interaction in the cisplatin–nanohorn system and also cisplatin–carbon nanotube inclusion and adsorbed complexes. Therefore, this seems appropriate for the calculation of Zn(II)–Rutin interaction energy.

M06-2x/6-311+G(2d,p)//B3LYP/6-31G(d,p)/Lanl2DZ energy of Zn(II)–Rutin complex formation (ΔE) and Gibbs free energy (ΔG), both in the gas phase, using eqs 1–4 named Model-1, Model-2, Model-3, and Model-4 reactions, respectively, are given in Figure 7a, along with binding energies calculated using eq 5. Corresponding ΔG values in DMSO, water, and methanol solvents (M06-2x-PCM results) are reported in Figure 7b–d. The double slash means that geometries were optimized at the B3LYP level and energies were calculated using the M06-2x functional. As we might have expected, the chosen model reaction of complex formation can influence significantly the calculated complex stabilization energy (Figure 7a) and may not be very helpful to assign the most probable complex structure. However, looking at the energy profiles shown in Figure 7a, it can be seen that the vacuum and gas phase profiles of model-3 of formation reaction matches very well the corresponding pattern using eq 5 from ref 25, with complex 25 predicted as the most favorable followed by 24, 20, and 19. Therefore, it seems reasonable to use the model-3 reaction to analyze our results. Model-3 reactions of complex formation equations for representative complex structures are given in Scheme 4.

Figure 7b–d shows us that complex 17 (2:1) is the preferred one in solution according to model-3 reactions (highlighted) followed by complexes 6 and 14, with ΔG values relative to complex 17 being 16.5 and 18.1 kcal mol−1 (DMSO), 13.3 and 15.3 kcal mol−1 (water), and 17.9 and 19.3 kcal mol−1 (methanol), respectively. Complex 17 is also a good candidate structure based on comparison of theoretical and experimental UV–VIS data (in methanol) reported in ref 12 (Figure 5). However, complexes 1, 2, 3, and 4, which were considered candidate structures according to the analysis of 1H NMR spectra in DMSO, have very low energy of formation compared to the lowest energy complex, with values relative to complex 17, being 65.9, 51.7, 68.1, and 50.1 kcal mol−1, respectively, and so may be discarded from a thermodynamic analysis of reaction of complex formation. Based only on energetic grounds, complex 17 should be predominant in solution (DMSO, water, and methanol solvents), which is in agreement with the analysis of UV–VIS spectra (Figure 5).

According to comparison between theoretical and experimental 1H NMR data obtained in DMSO solution from ref 28, it can be ruled out from that first experimental preparation of Zn(II)–Rutin complexes since it exhibits much large deviation from the experimental NMR profile (see Figure 3) and also a wrong NMR profile regarding H6, H8, and H1G protons (see Figure 4).

An important point to be addressed is the solubility of flavonoids in a polar solvent, such as water, which is present in biological media, that is fundamental for efficient use as an anticancer and antioxidant agent. It has been reported in various experimental works that complexion with transition metal ions enhances considerably the biological activity of flavonoids, and our results corroborate to the role played by solvent effects on the increasing activity of these polyphenol compounds. Solubility (mole fraction x 10^6) of free rutin in various solvents taken from ref 42 and negative solvation energy (M06-2x/6-311+G(2d,p)) PCM value in kcal mol−1 for representative Zn(II)–Rutin complexes in three solvents (DMSO, water, and methanol) at room temperature are shown in Figure 8a. It can be seen from Figure 8a that there is a rough correlation between solubility and DFT-calculated solvation energy, with the lowest solubility found in water solution. The discrepancy between experimental and solvation energy trend for ethanol and 1-butanol can be seen in the light of the PCM model used to describe solvent effects, which does not include explicit solute–solvent interaction that certainly is relevant for the prediction of solubility in polar solvents. Nevertheless, our results provide evidence that DFT solvation energies can be used as an estimate of the solubility tendency of a series of structural related chemical compounds.

The DFT solvation energy results (negative values are used to easy to make connection with solubility) reported in Figure 8b reveal that metal complexion indeed improves solubility, except for complex 4 where complexion with Zn(II) did not cause a positive effect. This fact indicates that complex 4 may not be effective in biological applications as well as complexes 2. As expected, charged species have larger solvation energies in polar solvents, which is reflected by the high solvation energy values of complexes 1 (charge = 2+), 3 (charge = 2+), and 17 (charge = 2+) and also much larger values for complexes 15 (charge = 4+), 16 (charge = 3+), 22 (charge = 3+), 24 (charge = 4+), and 25 (charge = 3+). Therefore, these structures may be considered good candidates as the Zn(II)–Rutin molecular structure responsible for the enhanced biological activity compared to the free rutin reported in the experimental work based on solvation energy analysis. The solubility of rutin in polar solvents can be enhanced enormously through complexion with metal ions as shown in Figure 8b, with charged complexes being substantially

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**Scheme 4. Model-3 Reaction Equations for Complex Formation EnergiesShown in Figure 7 (highlighted)**

Cps-1: Zn2+ + 2H2O + Rut → [Zn(H2O)2(Rut)]2+

Cps-2: Zn2+ + 2Cl− + 2H2O + Rut → [Zn(H2O)2(Rut)Cl2]+ + HCl + Cl−

Cps-3: Zn2+ + 2H2O + 2Rut → [Zn(Rut)2]+

Cps-4: Zn2+ + 2Cl− + 2H2O + Rut → [Zn(Rut)2]Cl2 + 2HCl

Cps-6: Zn2+ + 2Cl− + 2H2O + Rut → [Zn(H2O)2(Rut)Cl4]+ + 2HCl + Cl−

Cps-8: Zn2+ + 2Cl− + 2H2O + Rut → [Zn(H2O)2(Rut)2Cl2]+ + 2HCl + Cl−

Cps-10: Zn2+ + 4Cl− + 2H2O + Rut → [Zn2H2O4(Rut)2Cl2]+ + 2HCl + 2Cl−

Cps-11: Zn2+ + 4Cl− + 2H2O + Rut → [Zn2H2O4(Rut)2Cl2]+ + 2HCl + 2Cl−

Cps-16: Zn2+ + 4Cl− + 4H2O + Rut → [Zn2H2O4(Rut)4]+ + HCl + 3Cl−

Cps-17: Zn2+ + 4Cl− + 4H2O + Rut → [Zn2H2O4(Rut)4]+ + HCl + 3Cl−

Cps-18: Zn2+ + 4Cl− + 4H2O + Rut → [Zn2H2O4(Rut)4]+ + HCl + 3Cl−

Cps-19: Zn2+ + 4Cl− + 4H2O + Rut → [Zn2H2O4(Rut)4]+ + HCl + 3Cl−

Cps-20: Zn2+ + 4Cl− + 6H2O + Rut → [Zn2H2O4(Rut)4]+ + 2HCl + 2Cl−

Cps-22: Zn2+ + 4Cl− + 4H2O + Rut → [Zn2H2O4(Rut)4]+ + 2HCl + 2Cl−

Cps-24: Zn2+ + 6Cl− + 6H2O + Rut → [Zn2H2O4(Rut)3]+ + 3HCl + 3Cl−

Cps-25: Zn2+ + 6Cl− + 6H2O + Rut → [Zn2H2O4(Rut)3]+ + 3HCl + 3Cl−
stabilized by the solvent effect. It can be inferred from Figure 8b that the active form of the drug may not be complex 4, as proposed in ref 28, due to too low solubility in aqueous media. As it could be expected, the protonated rutin (Rut−OH) is moderately destabilized in DMSO solution (and also water), which corroborates to the lower solubility in water observed experimentally, with the opposite happened with the OH−C5 deprotonated form of rutin (Rut−O−), which is substantially stabilized in DMSO, water, and methanol solution (solvation energy values of −36.8, −33.9, and −46.9 kcal mol−1, respectively). 1H NMR spectra analysis indicates that the deprotonated rutin species is not present in the experimental DMSO sample of free rutin and consequently in water solution (see Supporting Information, Figure S2) unless basic conditions are satisfied, which is also not the case of biological media. Therefore, the formation of the Zn(II)−Rutin complexes with deprotonated species in neutral pH aqueous solution should be viewed with caution, and determination of a mechanism of deprotonation is required. Our theoretical 1H NMR results for complex 3 [Zn(Rut)2]OH+ , having the rutin molecule protonated at the OH−C5 position, are in agreement with this common sense argument. For the existence of complexes 2 and 4, there must be a viable mechanism for deprotonation of Rut−OH to allow complexation with Zn(II). Basic media could be a possibility; however, this condition is not met at biological media where the pH is close to 7, not favoring OH deprotonation.

An experimental and theoretical study of deprotonation mechanism and acidity constants in aqueous solution of flavonols was published recently. pKα values for quercetin were reported and may be useful to analyze our theoretical results since quercetin has A, B, and C ring protons similar to rutin, with the only difference being a hydroxyl group at the O−C3 position of ring C replacing the glucopyranosyl and rhamnopyranosyl units of rutin. According to the results (ΔG of deprotonation) reported in ref 47, the order of deprotonation sites of quercetin in aqueous solution is as follows: OH−C4′ (pKα = 6.59), OH−C7 (pKα = 8.60), OH−C3′ (pKα = 14.40) (which is replaced by sugar groups in rutin), OH−C3 (pKα = 19.11), and OH−C5 (pKα = 20.91), values obtained at 298.15 K from the standard equation that defines the thermodynamic equilibrium constant (ΔG = −RTlnK). The deprotonation position (OH−C5) assumed in ref 28 is the least favorable deprotonation hydroxyl group of quercetin, which should also hold for rutin. Regarding the OH protons situated at the sugar moieties, a variable pH NMR spectroscopic study reported for β-cyclodextrin in alkaline aqueous solution revealed that it does not deprotonate at pH < 12.0, with pKα values for OH groups adjacent to C-2 and C-3 carbon atoms being 13.5 ± 0.02 (22.5 °C). We can use the results from ref 48 as an estimate of pKα values for sugar OH protons of rutin, which is not so far away from the second pKα value determined for quercetin. These reported pKα values provide support (except for deprotonations in the glycidic region) for our predicted Zn(II)−Rutin complexes structures based on spectroscopic analysis, having deprotonation at the OH−C4′ position and not at the OH−C5 site (proposed in ref 28).

However, our good agreement with experimental UV−VIS data in methanol solution for complexes 17 and 25 (deprotonated at the OH−C5 position) makes us believe that the Zn(II) ion play an important role on the whole process somehow activating deprotonation at the OH−C5 of free rutin, which would not happen in the absence of the metal ion.

### CONCLUSIONS

In this work, we reported DFT calculations of energy of formation and spectroscopic properties (1H NMR, IR, and UV−VIS data) in DMSO, methanol, and water solution using the PCM model to describe solvent effects for 25 plausible structures of Zn(II)−Rutin complexes, which showed enhanced biological activity as compared to the free rutin molecule. There are two independent experimental preparations of the rutin complex with zinc ion, and distinct molecular structures were proposed, 1:2 (and also 1:1) and 2:1 model of complexation, based on the analysis of 1H NMR (and mass spectra data in DMSO solution) and UV−VIS data (in methanol), respectively.
Comparison of our calculated DFT spectroscopic results in solution with experimental data revealed that different metal complex structures are likely to be present in the two Zn(II)–Rutin synthesis conducted in DMSO28 (complex 3, [Zn-(Rut)]123, 1:2) and methanol solution15 (complex 17, [Zn2(H2O)4RutO4 OS]131, 2:1). The complex structure having the best agreement with experimental UV–VIS data from ref 12 (structure 17) is not the same as the one showing the best accordance with experimental 1H NMR data from ref 28 (structure 3). In addition, we showed that deprotonation at the OH−C5 position of rutin in DMSO solution, as proposed in ref 28, should be viewed with care on the basis of experimental deprotonation studies for other flavonoids and also theoretical 1H NMR results. Our predicted complex structure in methanol solution based on comparison of theoretical and experimental UV–VIS data, complex 17 deprotonated at the OH−C5 position, lead us to think that the metal ion should play an important role, somehow inducing deprotonation at the OH−C5 position of free rutin. We showed in this work that it does not take place in the absence of the Zn(II) ion. The determination of the rutin deprotonation state at the experimental conditions, pH = 6.712 and pH = 7.20, 28 is certainly of relevance since experimental access to the degree of deprotonation is difficult. We found that, in the two independent synthesis of metal complexes, distinct forms of rutin (OH−C5 and O(1−)−C5) are present. As it is discussed in detail in ref 25, deprotonation of the OHS is strongly facilitated in the presence of Zn(II) ions, and it is a phenomenon that is time-dependent and can be followed by the use of 1H NMR.

Complex 17 has the lowest Gibbs free energy of complex formation in solution (thermodynamic product); however, it is not predicted as the preferred structure on the basis of 1H NMR spectroscopic analysis in DMSO solution, where complex 3 exhibited the best agreement with the experimental NMR profile but has a disfavored positive energy of formation. Our results lead us to conclude that calculation of energy of formation may not be the best criterion to determine the observed structure in solution since it depends on the chosen reaction model. A comparative analysis of theoretical/experimental spectroscopic data seems more adequate. According to our 1H NMR/UV−VIS analysis, the two experimental synthesis of Zn(II)−Rutin complexes in DMSO and methanol solution ended up with distinct mode of complexation, 1:2 and 2:1, respectively, which may be seen as an intriguing experimental result strongly supported by our theoretical 1H NMR and UV−VIS spectra calculations in solution. In addition, our DFT-calculated solvation energies may be considered as an estimate of flavonoid solubility. Therefore, our results strongly indicate that complex 17 with a 2:1 stoichiometry ([Zn2(H2O)4RutO4 OS]131, having large stabilization energy due to solvent effect, will exhibit improved solubility as compared to free rutin, which has very low solubility in aqueous media, which may be related to the observed increased biological property.

**COMPUTATIONAL METHODS**

The predicted rutin geometry in DMSO solution reported previously28 was used as a starting point to optimize several trial Zn(II)–Rutin complex structures employing the DFT10 method with the B3LYP functional110,11 using the 6-31G(d,p) basis set12 for carbon, oxygen, and hydrogen atoms and effective core potential (ECP) LANL2DZ13 for the Zn(II) metal. The gas phase optimized geometries were used for DFT calculations of spectroscopic properties in solution. DFT-calculated harmonic frequencies were used to assess the main IR band, and UV−VIS data were computed according to the TD-DFT formalism.14 1H NMR magnetic shielding constant (δ) calculations with chemical shifts (δ) determined on a δ-scale relative to the TMS, taken as a reference, were done using the gauge-independent atomic orbital (GIAO) method.15 The polarizable continuum model (PCM)16 approach was used to describe solvent effects. As shown in previous works,17,18,19 the B3LYP/6-31G(d,p)-PCM level of theory can be considered sufficient for the evaluation of NMR chemical shifts for CHx protons. All calculations were performed with the Gaussian 09 package.19

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b04174.

The optimized Cartesian coordinates of all structures shown in this article and also additional figures and tables (PDF)

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**Notes**

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