

Nature meets Metal: enhancing chrysin's antidiabetic efficacy through Cu(II) and Co(II) coordination

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Abstract

Diabetes mellitus affects over 500 million people worldwide and remains one of the most pressing unmet medical challenges. Enhancing the efficacy of natural compounds with antidiabetic properties offers a promising path toward expanding therapeutic options. Chrysin, a flavonoid recognized for its metabolic benefits, suffers from poor aqueous solubility and low bioavailability, which restricts its clinical potential. Here, we report for the first time the coordination of chrysin with copper (Cu^{2+}) and cobalt (Co^{2+}) ions as an approach to overcome these limitations. The resulting metal–chrysin complexes were thoroughly characterized and evaluated in Caco-2, Hep-G2, and 3T3-L1 cell models. Both complexes significantly reduced intestinal glucose absorption ($\sim 20\%$) and increased hepatic glucose uptake ($10\text{--}23\%$), with a tendency to enhance adipose tissue glucose uptake - effects not observed with free chrysin. They also modulated the expression of key transcription factors, SREBP-1c and ChREBP, suggesting a role in metabolic regulation. Together, these results highlight metal coordination as a strategy to potentiate flavonoid bioactivity and identify Cu(II)- and Co(II)-chrysin complexes as promising platforms for the development of antidiabetic lead compounds.

1. Introduction

Diabetes mellitus (DM) comprises a group of metabolic disorders characterized by chronic hyperglycemia due to insulin deficiency, impaired insulin action, or both. The two main types are type 1 diabetes (T1DM), an autoimmune condition leading to β -cell destruction and absolute insulin deficiency (5–10% of cases), (American Diabetes Association 2013) and type 2 diabetes (T2DM), which results from insulin resistance combined with progressive β -cell dysfunction (90–95% of cases). (American Diabetes Association 2013; AL-Ishaq et al. 2019; Goyal et al. 2025) The global prevalence of diabetes has risen dramatically, with estimated 589 million adults affected in 2024, (International Diabetes Federation 2025) largely due to lifestyle-related risk factors such as obesity. (World Health Organization 2016) Diabetes is a leading cause of morbidity and mortality, contributing to cardiovascular disease, stroke, blindness, renal failure, and premature death, (American Diabetes Association 2013; Steinmetz et al. 2021; Alicic et al. 2017) and accounts for ~ 1.5 million deaths annually, (World Health Organization 2016) ranking as the 8th leading cause worldwide. (World Health Organization 2024) Although insulin therapy (for T1DM) and oral antidiabetic drugs such as metformin (for T2DM) remain the cornerstones of treatment, (Sapra and Bhandari 2021) current therapies often lack long-term efficacy and may cause undesirable side effects. This motivates the search for novel therapeutic agents with enhanced activity and safety profiles. (Antar et al. 2023)

Flavonoids, a large family of polyphenolic secondary metabolites, have attracted increasing interest for their potential antidiabetic effects. They are known to regulate carbohydrate digestion, intestinal glucose absorption, insulin sensitivity, and adipogenesis, as well as to support pancreatic β -cell proliferation and insulin secretion. Several flavonoids, including quercetin, rutin, kaempferol, fisetin, isorhamnetin, morin, apigenin, luteolin, chrysin, genistein, and cyanidin, have demonstrated activity in experimental models of diabetes. (AL-Ishaq et al. 2019)

Chrysin (Chr) (5,7-dihydroxyflavone) is a naturally occurring flavone found in fruits, honey, bee pollen, propolis, and plants such as *Passiflora caerulea* L. and *Tilia tomentosa*.(AL-Ishaq et al. 2019) It has been widely studied for its antioxidant, anti-inflammatory, anticancer, and hepatoprotective properties, among others,(Naz et al. 2019). Importantly, Chr has also been shown to improve several metabolic parameters associated with diabetes and metabolic syndrome, including hyperinsulinemia, hyperleptinemia, hypertriglyceridemia, hepatic fibrosis, and hypertension.(Andrade et al. 2020) Despite these promising effects, its clinical translation is severely limited by poor aqueous solubility and rapid metabolism, resulting in low oral bioavailability.(National Center for Biotechnology Information 2022; Stompor-Gorący et al. 2021)

Metal coordination has emerged as a strategy, to improve the physicochemical and biological properties of flavonoids, often enhancing solubility, stability, and membrane permeability, while potentiating or introducing novel biological activities.(Selvaraj et al. 2014a) Indeed, coordination of flavonoids with different metal ions has been explored for anticancer,(Uivarosi and Munteanu 2017) antioxidant, (Samsonowicz et al. 2017) neuroprotective,(Rodríguez-Arce and Saldías 2021) and, more recently, antidiabetic properties.(Lv et al. 2025) In the context of diabetes, Zn(II)-,(Lee et al. 2019; Refat et al. 2021; Gopalakrishnan et al. 2015) VO(IV)-,(Cazarolli et al. 2006; Pillai et al. 2013a, b) Mn(II)-,(Dong et al. 2017) and Co(II)- (Mohamed M. et al. 2020) complexes have demonstrated significant activity, and vanadium complexes (particularly V(IV/V)) have been extensively studied for their insulin-mimetic effects.(Levina and Lay 2011) However, studies on Cu(II)- and Co(II)-flavonoid complexes remain scarce, and most involve ligands other than chrysin.(Levina and Lay 2011; Rajeshwari et al. 2021) Only a few reports describe antidiabetic activity of Cu(II)- or Co(II)-flavonoid systems, typically based on quercetin, morin, or primulin.(Mohamed M. et al. 2020; Jabeen et al. 2017) To our knowledge, no studies have investigated Cu(II)- or Co(II)-chrysin complexes in the context of diabetes.

Given this gap, we aimed to investigate whether coordination of Chr with Cu(II) and Co(II) could enhance its biological activity. To this end, Cu(II)- and Co(II)-Chr complexes were synthesized using solvothermal (SVT) and microwave-assisted synthesis (MWAS), the latter being a relatively underexplored approach in coordination chemistry that offers advantages such as shorter reaction times, higher yields, and milder conditions.(Marinho et al. 2012; Gabano and Ravera 2022a) The complexes were thoroughly characterized to assess their structural properties, stability, and suitability for biological applications. Their effects on glucose and fructose uptake, lipid accumulation, and oxidative stress were evaluated in relevant cellular models (Caco-2, HepG2, and 3T3-L1), alongside cytotoxicity assays to define safe working concentrations. Furthermore, we investigated their impact on the expression of key metabolic regulators, including SREBP-1c and ChREBP, to gain insight into potential molecular mechanisms

Although the present study focuses on *in vitro* pharmacological characterization, the complexes are intended as proof-of-concept chemical entities and early-stage leads rather than immediate drug candidates. Given that diabetes requires chronic treatment, the development of metal-based agents must consider safety and regulatory aspects. Copper and cobalt are essential trace elements with tightly regulated homeostasis but may exert toxicity at elevated exposure levels.(EFSA Scientific Committee et

al. 2023; Finley et al. 2012) Importantly, metal coordination can modulate metal ion reactivity, bioavailability, and toxicity relative to free ions,(Selvaraj et al. 2014b), and several metal complexes have been successfully explored as therapeutic agents.(Barry and Sadler 2013) Future *in vivo* studies on pharmacokinetics, biodistribution, and chronic toxicity will therefore be essential to define therapeutic windows and guide structural optimization aimed at minimizing metal exposure while preserving efficacy.

Altogether, this work provides the first evidence that Cu(II)- and Co(II)-Chr complexes potentiate the antidiabetic activity of Chr, offering valuable insights into their action and contributing to the growing field of bioactive metal–flavonoid complexes. By joining coordination chemistry with metabolic biology, these findings highlight the potential of metal coordination as a promising strategy for the development of next-generation antidiabetic agents.

2. Experimental section

2.1. Synthetic procedures

2.1.1. Reagents

All reagents and solvents were commercially purchased and used without any additional purification. Copper(II) acetate monohydrate ($\text{Cu}(\text{CO}_2\text{CH}_3)_2 \cdot \text{H}_2\text{O}$; > 98%) was sourced from Fluka (Seelze, Germany). Cobalt(II) acetate tetrahydrate ($\text{Co}(\text{CO}_2\text{CH}_3)_2 \cdot 4\text{H}_2\text{O}$; $\geq 98\%$) was purchased from Panreac (Barcelona, Spain). Chr ($\text{C}_{15}\text{H}_{10}\text{O}_4$; $\geq 96.5\%$), triethylamine ($(\text{C}_2\text{H}_5)_3\text{N}$; $\geq 99.5\%$), and potassium bromide (KBr; $\geq 85\%$) were purchased from Sigma-Aldrich (Burlington, MA, USA). Dimethylsulfoxide (DMSO; $(\text{CH}_3)_2\text{SO}$; $\geq 99.8\%$) was acquired from CARLO ERBA (Cornaredo, MI, Italia). Absolute ethanol ($\text{CH}_3\text{CH}_2\text{OH}$; > 99.8%) was obtained from Chem-Lab (Zedelgem, Belgium).

2.1.2. Synthesis of $[\text{Cu}(\text{Chr})_2]$ (1)

The SVT and MWAS procedures for compounds 1 and 2 are schematically illustrated in Fig. 1 and were carried out as described below.

Solvothermal method

Complex 1 was synthesized by combining Chr (254 mg, 1 mmol) with copper acetate monohydrate (100 mg, 0.5 mmol) in 6 mL of ethanol. The mixture was stirred briefly at room temperature, followed by the addition of triethylamine (70 μL , 0.5 mmol). The resulting mixture was then transferred to a Teflon-lined autoclave and maintained under static conditions at 100°C for 24 hours. The green precipitate formed was collected by filtration, washed with ethanol and dried at room temperature overnight. The product was obtained in a yield of $90.9 \pm 8.1\%$. Elem. Anal. Calc. for $[\text{C}_{30}\text{H}_{28}\text{O}_8\text{Cu}]$: C, 63.23; H, 3.18; Found: C, 61.78; H, 3.53%.

Microwave-assisted method

The MWAS followed a procedure similar to that of the solvothermal method. Briefly, Chr (127 mg, 0.5 mmol) and copper acetate monohydrate (50 mg, 0.25 mmol) were placed in a microwave reaction vessel, followed by the addition of ethanol (6 mL) and triethylamine (70 μ L, 0.5 mmol). The vessel was then inserted into a microwave reactor (CEM Discover SP, CEM Corporation, Matthews, NC, USA) and heated at 100°C for 5 minutes at a power of 25 W, under continuous stirring. The resulting solid was collected by filtration, washed with ethanol, and dried at room temperature, as described for the solvothermal method. The product was obtained in a yield of $91.5 \pm 3.8\%$. Elem. Anal. Calc. for $[C_{30}H_{28}O_8Cu]$: C, 63.23; H, 3.18; Found: C, 63.91; H, 3.21%.

2.1.3. Synthesis of $[Co(Chr)_2] \cdot 4H_2O$ (2)

Solvothermal method

Complex 2 was synthesized by mixing Chr (254 mg, 1 mmol) with cobalt acetate tetrahydrate (124 mg, 0.5 mmol) in 4 mL of ethanol. After brief stirring at room temperature, 2 mL of a 0.2 M potassium hydroxide solution were added. The resulting mixture was then transferred to a Teflon-lined autoclave under static conditions at 150°C for 7 days. The yellow solid obtained was collected by filtration, washed with ethanol and dried. The product was obtained in a yield of $87.30 \pm 5.2\%$. Elem. Anal. Calc. for $[C_{30}H_{26}O_{12}Co]$: C, 56.52%; H, 4.11%; Found: C, 53.40%; H, 4.40%.

Microwave-assisted method

Chr (127 mg, 0.5 mmol) and cobalt acetate tetrahydrate (62 mg, 0.25 mmol) were introduced into a microwave reaction vessel, followed by the addition of ethanol (4 mL) and 2 mL of a 0.2 M potassium hydroxide solution. The vessel was then placed in a microwave reactor (CEM Discover SP, CEM Corporation, Matthews, NC, USA) and heated at 100°C for 5 minutes at a power of 50 W, under continuous stirring. The resulting solid was collected by filtration, washed, and dried as previously described. The yield was $94.0 \pm 1.5\%$. Elem. Anal. Calc. for $[C_{30}H_{26}O_{12}Co]$: C, 56.52%; H, 4.11%; Found: C, 56.67; H, 4.36%.

2.2. Physicochemical characterization of the materials

The X-ray diffraction (XRD) patterns of the samples were recorded in a PANalytical-X'pert-PRO diffractometer (Malvern PANalytical, Malvern, UK) with $K\alpha(Cu)$ radiation, curved graphite monochromator. The analysis of the complexes was performed in the range $3.5^\circ < 2\theta < 50^\circ$, with a step size of 0.0263° , at room temperature. The patterns of the metal oxides were acquired in the range $10^\circ < 2\theta < 80^\circ$, with a step size of 0.0525° , also at room temperature. Elemental analysis (EA) was performed to determine carbon (C) and hydrogen (H) contents, using a Leco TruSpec-Micro CHNS 630-200-200 instrument (LECO, Saint Joseph, MI, USA). Scanning Electron Microscopy (SEM) was performed in a Field Emission Gun (FEG) – SEM SU70 microscope (Hitachi, Tokyo, Japan) operated at 15 keV. A

spectrometer Jasco FT/IR-4X (JASCO Corporation, Tokyo, Japan) was used to acquire FTIR spectra, samples were prepared using the KBr technique. For the analysis 128 scans were collected in the range of 4000 to 350 cm^{-1} , with 2.0 cm^{-1} of resolution. The thermogravimetric analysis (TGA) was performed in a Hitachi STA300 instrument (Hitachi, Tokyo, Japan) with a heating rate of 5°C/min, from 25 to 800°C, under a nitrogen/oxygen atmosphere. UV–VIS spectra were obtained in a Jasco V-780 (JASCO Corporation, Tokyo, Japan), in the range of 250 to 1000 nm. Electron paramagnetic resonance spectroscopy (EPR) spectra were recorded using a Bruker ELEXSYS E500 spectrometer, available at Laboratory of Electron Paramagnetic Resonance (CEMUP – Centro de Materiais da Universidade do Porto, Portugal), operating at 9 GHz (X-band) using the following general experimental conditions: 100 $\pm$ 5 K, modulation frequency of 100 kHz, microwave power 10 mW, modulation amplitude 8 Gauss (G), 60 dB of receiver gain, acquisition time of 60 seconds and 5 scans. Xepr software (Bruker, Billerica, MA, USA) was used for spectra acquisition. Graphical representations were obtained using OriginPro 8.5 software. For sample preparation, a solution of each complex was freshly prepared before analysis at 5 mM in DMSO and when necessary diluted to 0.5 mM in order to obtain hyperfine interaction resolution. The mixture was vortexed and an aliquot was collected and placed in the quartz EPR tube for spectra acquisition. The spectra were immediately acquired. Copper quantification was performed by inductively coupled plasma mass spectrometry (ICP-MS) using a Thermo X Series instrument (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a Burgener nebulizer. Samples were digested and diluted according to standard trace-metal analysis procedures to ensure complete solubilization of the metal content. Electrospray ionization high-resolution mass spectra (ESI-HRMS) were acquired on an Orbitrap Exploris 120 mass spectrometer (Thermo Scientific, Waltham, MA, USA) operating in positive-ion mode, with an ion source voltage of 3.5 kV and resolution was set to 90 000. Samples were prepared to a nominal concentration of 20 $\mu\text{g mL}^{-1}$ in dichloromethane, methanol, or $\text{H}_2\text{O}/\text{MeOH}$ (20:80) and centrifuged to remove undissolved material. Sample analysis was carried out by direct infusion.

2.3. Stability assay

Samples used in the stability tests were prepared as for the biological assays. Concentrated solutions of complexes 1 and 2, as well as Chr, were first prepared in DMSO and subsequently diluted in phenol red-free DMEM (Dulbecco's Modified Eagle Medium, from PAN-Biotech™, Aidenbach, Germany). The spectrum of these solutions was recorded immediately after dilution alongside the spectrum of Chr under the same conditions. To evaluate the stability over time, the solutions were then incubated at 37°C for 24 hours, after which new spectra were recorded.

2.4. *In vitro* cell-based studies

2.4.1. Cell culture and maintenance

The Caco-2 cell line was obtained from ATCC (Manassas, VA, USA) and used between passage numbers 15–41. Cells were cultured in Minimum Essential Medium (MEM), containing 5.55 mM glucose, 25 mM HEPES (Sigma, St. Louis, MO, USA) supplemented with 15% (v/v) fetal bovine serum (FBS) and 1% penicillin/ streptomycin/ amphotericin B solution (both from PAN-Biotech™, Aidenbach, Germany), and

maintained in a humidified atmosphere (5% CO₂, 95% air) at 37°C. The culture medium was refreshed every 3–4 days, and cells were passaged every 12 days. For subculturing, cells were enzymatically detached using 0.25% trypsin-EDTA (5 min, 37°C), split at a 1:3 ratio, and seeded in culture dishes (21 cm², Ø 60 mm; TPP®, Trasadingen, Switzerland).

The HepG2 cell line was obtained from ATCC (Manassas, VA, USA) and used between passage numbers 55–62. Cells were cultured in DMEM containing 0.3 g/l L-glutamine, 4.5 g/L glucose, 3.7 g/L NaHCO₃, supplemented with 15% (v/v) FBS and 1% penicillin/ streptomycin/ amphotericin B solution (all from PAN-Biotech™) and maintained in a humidified atmosphere (5% CO₂, 95% air) at 37°C. The culture medium was refreshed 2–3 times per week. For subculturing, cells were enzymatically detached using 0.25% trypsin-EDTA (2 min, 37°C), split at a 1:3 ratio, and seeded in culture dishes (21 cm², Ø 60 mm, TPP®).

The 3T3-L1 cell line was obtained from ATCC and used between passage numbers 20–30. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 0.3 g/l L-glutamine, 4.5 g/L glucose, 3.7 g/L NaHCO₃, supplemented with 15% (v/v) FBS and 1% penicillin/ streptomycin/ amphotericin B solution (all from PAN-Biotech™) and maintained in a humidified atmosphere (5% CO₂, 95% air) at 37°C. The culture medium was refreshed 2–3 times per week. For subculturing, cells were enzymatically detached using 0.25% trypsin-EDTA (40 sec, 37°C), split at a 1:3 ratio, and seeded in culture dishes (21 cm², Ø 60 mm, TPP®). Prior to the experiments, adipogenic differentiation of 3T3-L1 cells was induced. For this, cells were seeded in 24-well or 96-well plates, depending on the assay, and cultured in DMEM with 10% (v/v) FBS until they reached 70–80% confluence. After 2 days of confluence, the medium was replaced with differentiation medium (DMEM supplemented with 10% (v/v) FBS and 1% penicillin/streptomycin/amphotericin B solution, 0.5 mM isobutyl-1-methylxanthine (IBMX), 1 µM dexamethasone and 10 µg/mL insulin (all from Sigma) and maintained for 2 days. The differentiation medium was then replaced with DMEM supplemented with 10% (v/v) FBS and 1% penicillin/streptomycin/amphotericin B solution and 10 µg/mL insulin for further 2 days and maintained in DMEM supplemented with 10% (v/v) FBS and 1% penicillin/streptomycin/amphotericin B solution until the experiments. Adipocytes were used for experiments on days 8–12 after differentiation.

2.4.2. Cell viability assay (MTT)

Cell viability was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay. Prior to the assay, Caco-2, HepG2 and 3T3-L1 cells were exposed for 24 h to Chr or its metal complexes in FBS-free culture medium. All compounds were dissolved in DMSO, which was used as the vehicle control, and the final DMSO concentration in the culture medium was maintained at 1% (v/v) to minimize solvent-related effects. For the assay, cells were seeded in 96-well plates (TPP®) and treatment was performed after the cell culture reached 70–80% confluence. At the end of the 24 h-treatment period, the culture medium was removed and replaced with PBS, followed by the addition of 10 µL of MTT reagent (Sigma) to each well. The plate was then incubated for 3 h (1h for HepG2) to allow for formazan crystal formation. After this period, the PBS was removed and 100 µL of DMSO was added to dissolve the formazan crystals. Absorbance was then measured at 550 nm and 650 nm using a

microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). Cell viability was determined by calculating the difference between absorbance at 550 nm and 650 nm and was expressed as a percentage of the control.

2.4.3. ^3H -DG and ^{14}C -fruct uptake assay

For this assay, Caco-2, HepG2 and 3T3-L1 cells were seeded in 24-well plates (TPP®) and treatment was performed after the cell culture reached 90–100% confluence. After the 24h-treatment period, cells were washed with 300 μL of glucose-free Krebs buffer (composition in mM: 125 NaCl, 4.8 KCl, 1.2 MgSO_4 , 1.2 CaCl_2 , 25 NaHCO_3 , 1.6 KH_2PO_4 , 0.4 K_2HPO_4 , and 20 HEPES, pH 7.4) at 37°C. The cell monolayers were then preincubated at 37°C for 20 min in 300 μL of Krebs buffer containing the respective compounds (or DMSO). Uptake was then initiated by replacing the preincubation buffer with 200 μL of Krebs buffer (37°C) containing the test compounds (or DMSO) along with either 10 nM ^3H -2-deoxy-D-glucose (^3H -DG, specific activity: 60 Ci/mmol) or 100 nM ^{14}C -fructose (^{14}C -fruct, specific activity: 300 Ci/mmol) for 6 min (3 min for HepG2) (American Radiolabeled Chemicals, St. Louis, MO, USA). Uptake was terminated by aspirating the incubation medium and rinsing the cells with 500 μL of ice-cold buffer. Cells were subsequently solubilized in 300 μL of 0.1% (v/v) Triton X-100 (5 mM Tris-HCl, pH 7.4) and stored at 4°C overnight. Intracellular radioactivity was measured by liquid scintillation counting (LKB Wallac 1209 Rackbeta, Turku, Finland), and results were normalized to total protein content. For 3T3-L1 cells, only ^3H -DG uptake studies were performed.

2.4.4. Lipid peroxidation assay (TBARS)

Quantification of Thiobarbituric Acid Reactive Substance (TBARS) levels was measured in cells using a Lipid Peroxidation (MDA) assay kit (MAK085; Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) according to the manufacturer's instructions. Results were expressed as malondialdehyde (MDA) concentration (nmol/mL).

2.4.5. Real-time quantitative PCR

Total RNA was extracted from Caco-2 and HepG2 cells after treatment with the compounds (or DMSO) for 24 h, using NZYol reagent (NZYTech, Lisbon, Portugal) according to the manufacturer's instructions. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed as previously described. (Peixoto et al. 2022) The primer pairs and annealing temperatures (AT) used are shown in Table 1. The mRNA expression levels of these genes were normalized to the housekeeping gene human β -actin. Cycling conditions for gene amplification also followed the protocol previously described. (Peixoto et al. 2022) Results were analyzed using LightCycler® 96 SW 1.1 software (Roche, Mannheim, Germany) based on the Ct method. (Schmittgen and Livak 2008)

Table 1 - Genes used for qRT-PCR analysis, primer sequences and annealing temperatures (AT).

Gene	Forward	Reverse	AT(°C)
GK	5'-CAT ATG TGC TCC GCA GGA CTA G-3'	5'-CTT GTA CAC GGA GCC ATC CA -3'	60
ACC1	5'-CCT TGT CAA CGC ATG GGC G-3'	5'-GGC TTT GGG GTG GGG AGT CG -3'	60
SREBP-1c	5'-GTG GGT CTC CTC CGA AGC CG-3'	5'-AGC ATG TCT TCG ATG TCG GTC AAG -3'	60
ChREBP	5'- TGC GGG ATG AGA TTG AGG A -3'	5'- TCC AGT TGT GCA GCG TAC -3'	60
β-actin	5'-AGA GCC TCG CCT TTG CCG AT-3'	5'-CCA TCA CGC CCT GGT GCC T-3'	65

Abbreviations: GK, glycogen synthase; ACC1, acyl-CoA carboxylase 1; SREBP-1c, sterol regulatory element binding protein 1c; ChREBP, carbohydrate-responsive element-binding protein.

2.4.6 Oil Red O staining and lipid accumulation assay

Lipid accumulation in 3T3-L1 adipocytes was assessed using Oil Red O staining. Cells were first washed with PBS and fixed with 4% paraformaldehyde for 1 h at room temperature. After fixation, cells were rinsed twice with deionized water, followed by incubation with 60% isopropanol for 5 min. The isopropanol was then aspirated, and the cells were allowed to dry completely before staining with an Oil Red O solution (0.6%) for 10 min at room temperature. Cells were washed three times with deionized water to remove excess stains. For the quantification of lipid content, the Oil Red O stain was eluted with 100% isopropanol for 10 min using a plate shaker. An aliquot (200 µL) of each sample was transferred in duplicate to a 96-well clear plate, and absorbance was measured at 492 nm. Results were expressed as a percentage of the control.

2.4.7 Total protein determination

The protein content of cell monolayers was determined using the Bradford assay,(Bradford 1976) with human serum albumin as the standard.

2.4.8 Data presentation and statistical analysis

Results are expressed as means ± standard error of the mean (S.E.M.). The sample size (n) represents the number of replicates from at least two independent experiments. Statistical significance between two groups was assessed using Student's t-test, with $p < 0.05$ considered statistically significant. All analyses were performed using Excel for Microsoft 365 MSO (Version 2501) (Redmond, WA, USA). Graphical content was prepared using Excel for Microsoft 365 MSO (Version 2501), and GraphPad Prism 10 (San Diego, CA, USA).

3. Results and Discussion

3.1. Physicochemical characterization and solution behavior

3.1.1 Synthesis, crystallinity and solid-state morphology

The SVT synthesis protocols and reaction conditions were optimized for each Chr-metal complex to ensure the formation of crystalline and phase-pure materials. For complex 1, optimal synthesis was achieved at 100 °C after 24 hours, while complex 2 required more demanding conditions, 150 °C for 7 days. Attempts to grow single crystals suitable for XRD analysis were unsuccessful. To develop a more sustainable and time-efficient route, both complexes were also obtained using MWAS, reducing reaction times from days to minutes (5 min at 100 °C). The reproducibility of the synthetic outcome was therefore assessed across both methods.

Powder XRD patterns (Fig. S1) confirmed the formation of crystalline phases in all cases and, importantly, the diffractograms obtained by SVT and MWAS are essentially identical. This demonstrates that both methods lead to the same underlying crystalline framework, despite the markedly different reaction times and heating profiles.

The reaction yields were comparable across both methods (data available in the experimental section), with the microwave-assisted synthesis exhibiting slightly higher efficiency. EA reveals small but consistent differences in the degree of hydration between samples obtained by the two methods. These variations are fully compatible with variable hydration, a common feature of coordination compounds crystallized slowly, and reflect the distinct crystallization kinetics of SVT and MWAS. For complex 1, the SVT sample shows a slight carbon deficit and a modest excess of hydrogen relative to the anhydrous formulation obtained by microwave synthesis, corresponding approximately to the incorporation of one additional water molecule per formula unit. For complex 2, the larger deviation in carbon content for the SVT material indicates the presence of about two extra water molecules beyond the four lattice waters characteristic of the MWAS sample. In contrast, the microwave-assisted materials exhibit excellent agreement with the theoretical compositions, consistent with their faster crystallization and reduced solvent entrapment. These trends were reproducible across multiple independent batches and are consistent with the known influence of synthetic conditions on hydration in metal–organic systems (Phan et al. 2023; Gabano and Ravera 2022b)

Crystal morphology was observed by SEM analysis (Fig. S2). For complex 1 the observed small crystals exhibit a lamellar morphology, with thin sheets with significant anisotropy in thickness, stacked randomly to one another. Complex 2 also rendered small crystals but predominantly exhibits an acicular morphology; the anisotropic growth results in slender, filiform structures with a divergent arrangement. Regarding crystal morphology, no evident differences were observed between the two synthesis methodologies. Solubility tests revealed that both complexes are soluble in aprotic solvents such as DMSO and DMF, sparingly soluble in short-chain aliphatic alcohols (e.g., ethanol and methanol), and practically insoluble in water.

3.1.2 Spectroscopic and analytical characterization

3.1.2.1 FTIR and coordination mode

The FTIR spectra confirmed coordination between the metal centers and the Chr ligand (Fig. S3; Table S1). In free Chr, one of the characteristic carbonyl (C=O) stretching bands appears at 1650 cm^{-1} . Upon complexation, this band shifts to 1632 cm^{-1} in complex 1 and 1639 cm^{-1} in complex 2, consistent with coordination through the 4-keto group.(E. Halevas et al. 2021) The band at 1609 cm^{-1} in Chr, which contains contributions from both C=O and C–C stretching, also shifts to lower wavenumbers in both complexes, further supporting metal–ligand interaction involving the carbonyl functionality.(Muñoz et al. 2016) The $\nu(\text{C–O–C})$ stretching band at 1247 cm^{-1} in Chr shows only minimal shifts (2–3 cm^{-1}), indicating that the pyrone oxygen is unlikely to participate directly in coordination.(E. Halevas et al. 2021) Similarly, the C=C stretching vibrations of the γ -pyrone and benzene rings exhibit only minor changes upon complexation. The O–H and C–H stretching region (3472–2300 cm^{-1} in Chr) becomes narrower in both complexes, consistent with changes in hydrogen-bonding environments. Finally, the bands at 1119–1123 cm^{-1} were assigned to in-plane O–H bending vibrations.(E. Halevas et al. 2021; Naso et al. 2022)

3.1.2.2 TGA analysis

The TGA curves of Chr and its metal complexes (Fig. S4), provide insight into their thermal stability and decomposition profiles. Complex 1 shows high thermal stability, with major weight loss beginning only above 330 °C, approximately 100 °C higher than the onset of Chr degradation, supporting the formation of an anhydrous complex. In contrast, complex 2 exhibits an initial weight loss below 115 °C, consistent with the presence of non-coordinated water molecules.(Kaur Jassal 2025; Jiang et al. 2017) The absence of ethanol in the structure was confirmed by the lack of its characteristic peaks in the IR spectrum.(Muthukumar et al. 2023) After these initial events, both complexes undergo continuous mass loss up to approximately 435 °C, without well-defined plateaus, suggesting progressive decomposition of the organic ligands into unstable intermediates. Above 435 °C, the residual mass corresponds to the formation of CuO (complex 1) and CoO (complex 2), as confirmed by XRD (Fig. S5). Experimental and theoretical residue percentages are in good agreement (Table S2), further supporting the proposed formulations.

3.1.2.3 UV-Vis electronic features

The electronic absorption spectra of Chr and its metal complexes were recorded to investigate their electronic transitions and coordination environments (Fig. 2a). In free Chr, two characteristic absorption bands are observed: Band I at 269 nm, assigned to π – π , n – π , and n – σ^* transitions of the benzoyl system (A-ring), and Band II around 313 nm, associated with π – π^* transitions of the cinnamoyl system (B-ring). (Eleftherios Halevas et al. 2021) Upon metal coordination, both complexes exhibit spectral changes consistent with complex formation. Band I undergoes a bathochromic shift of 6 nm in complex 1 and 1 nm in complex 2, accompanied by a pronounced hyperchromic effect. Although Band II appears only as a shoulder, its intensity also increases. These modifications reflect changes in the electronic distribution of the ligand upon coordination, in agreement with previous reports for flavonoid–metal complexes.

(Eleftherios Halevas et al. 2021; Liu et al. 2023) A distinct absorption band at 292 nm is clearly observed in complex 1, whereas in complex 2 it appears only as a shoulder. After 24 h in DMSO (Fig. 2c), the intensity of this band increases in complex 1 while the band at 275 nm disappears, suggesting slow structural rearrangements upon dissolution. This behaviour is consistent with the known tendency of Cu(II) complexes to undergo ligand-field distortions. In contrast, the spectrum of complex 2 remains essentially unchanged over time, indicating greater solution stability. Both complexes display an additional absorption band at approximately 393 nm, attributed to ligand-to-metal charge transfer (LMCT) transitions, further supporting coordination through the conjugated 4-keto/5-hydroxyl system of Chr.(Eleftherios Halevas et al. 2020) To compare solid-state and solution behaviour, Fig. 2b shows the spectra recorded in the solid state and in 7 mM DMSO. In both complexes, new bands emerge in the visible region, consistent with d–d transitions characteristic of Cu(II) and Co(II). For complex 1, a broad d–d band appears at ~647 nm in the solid state and shifts to ~680 nm in solution, suggesting changes in ligand-field strength upon dissolution. Such shifts are common for Cu(II) complexes, which often adopt distorted coordination geometries. (N. N. Greenwood; A. Earnshaw 1997) In complex 2, the d–d features are less defined, making structural interpretation more challenging, as expected for Co(II) complexes with multiple accessible geometries. (Cotton and Cotton 1999) Overall, the UV–Vis data support the formation of metal–Chr complexes in both solid and solution phases, while also indicating that Cu(II) and Co(II) complexes exhibit distinct electronic behaviours consistent with their known coordination chemistry.

3.1.2.4 EPR studies

Information about the electronic structure, particularly the oxidation state of the Cu and Co ions in complexes 1 and 2, respectively, is essential for their proper characterization and for understanding the coordination environment around the metal centers. The EPR spectra of complex 1 exhibits the main characteristic features of an interaction of an unpaired electron ($S = 1/2$) with the copper nuclei ($^{65}\text{Cu}/^{63}\text{Cu}$, $I = 3/2$) (Fig. S6a, b). The characteristic g values were estimated and were found to be $g_{\parallel} = 2.30$ and $g_{\perp} = 2.06$. The obtained results are similar to those reported for copper(II) complexes with tetragonal distortions and an O_4 coordination sphere (Rangel, Leite, Silva, Moniz, et al. 2014) thus corroborating the proposed structure for complex 1. Regarding complex 2, no EPR signal characteristic of Co(II) ions was observed under the experimental conditions employed. A low-spin Co(II) species ($S = 1/2$) would be expected to display a well-defined signal at the measurement temperature; however, no such signal was detected. This observation could suggest the presence of an EPR-silent Co(III) species. Nevertheless, this possibility is not supported by the elemental analysis (EA) and thermogravimetric analysis (TGA), which are inconsistent with a Co(III) formulation. An alternative explanation is the presence of a high-spin Co(II) species. The characteristic EPR features of high-spin Co(II) ($S = 3/2$) are typically observable only at much lower temperatures (4–10 K), (Walpita et al. 2026; Rangel, Leite, Silva, De Castro, et al. 2014) which were not accessible in the present study. Therefore, considering the EA and TGA data, the most plausible interpretation is that complex 2 contains a Co(II) center in a high-spin configuration, whose EPR features could not be detected under the experimental conditions employed.

The very weak EPR signal observed for complex 2 (Fig. S6c,d) is attributed to trace Cu(II) impurities, as discussed in the Supporting Information. Although present only at very low levels, this residual copper is sufficient to account for the minor EPR features detected.

3.1.2.5 ESI-HRMS

Mass spectrometry provides complementary insight into the composition of metal-containing species in solution and the ions generated under electrospray conditions. Electrospray high-resolution mass spectrometry (ESI-HRMS) was therefore employed to investigate the ionic speciation of the Cu(II) and Co(II) chrysin systems. Spectra were acquired in dichloromethane and methanolic solutions, with additional measurements in H₂O/MeOH (20:80) to assess solvent effects (see Supporting Information).

Across all samples and solvent systems, the most intense signal corresponds to protonated chrysin ([Chr + H]⁺, m/z 255.06), Fig. S7 and S8. This behavior is fully consistent with the ionization of neutral metal–flavonoid complexes in positive-ion mode: protonation at the electrospray droplet surface favours ligand release, and the free ligand typically exhibits higher ionization efficiency and gas-phase stability than the intact complex. For compound 1, the ESI(+)-HRMS spectrum (Figure S7b) displays a clear peak at m/z 570.0364, assigned to the monocationic species [Cu(Chr)₂ + H]⁺, which constitutes the predominant Cu-containing ion. The associated isotopic pattern confirms the presence of copper, indicating that the [Cu(Chr)₂] unit is preserved under electrospray conditions. A higher-mass signal at m/z 885.0093 is attributed to an aggregated species formed during ionization, while a peak at m/z 348.0056 corresponds to a fragment resulting from partial ligand loss. In H₂O/MeOH mixtures (Figure S7e), the limited solubility of the complex leads to an increased contribution of background signals and ligand-derived ions, consistent with reduced availability of intact species for ionization.

For compound 2, the ESI(+)-HRMS spectrum (Figure S8) shows an analogous pattern. The main Co-containing ion appears at m/z 566.0400, assigned to [Co(Chr)₂ + H]⁺, supporting a 1:2 metal-to-ligand stoichiometry and demonstrating that the [Co(Chr)₂] unit is retained under the ionization conditions. A less intense peak at m/z 820.0978 is attributed to gas-phase aggregate adducts, while a fragment at m/z 353.0091 reflects partial ligand loss. As observed for the copper analogue, spectra acquired in H₂O/MeOH show increased background and ligand-derived signals, reflecting the reduced solubility of the complex in aqueous media.

It is important to note that both complexes exhibit limited solubility in aqueous mixtures, as also indicated by the solubility tests. Consequently, in MeOH/H₂O media, the lower concentration of intact complex leads to a relative increase in background and ligand-derived ions. Although some contribution from metal–ligand equilibria cannot be excluded, the predominance of ligand-based ions is most plausibly attributed to solubility limitations and to the intrinsic ionization behavior of neutral complexes in positive-ion ESI.

Overall, the ESI-HRMS data for both compounds consistently support the presence of mononuclear M(Chr)₂ species (M = Cu, Co), confirming that well-defined metal–chrysin coordination units are present

in solution and remain detectable under electrospray ionization, while undergoing partial ligand dissociation under the ionization conditions. These observations are fully consistent with the structural formulation established from elemental analysis, EPR spectroscopy and the broader coordination environment of the complexes.

3.2 Tentative structural assignment

The combined spectroscopic, thermal and mass spectrometric data support the formulation of the two complexes as bis(chrysinato) metal(II) species. Based on these results and on comparison with structurally related flavonoid–metal complexes reported in the literature, the tentative structures shown in Fig. 2 are proposed. In complex 1, two chrysin ligands coordinate to the Cu(II) centre in a bidentate fashion through the 5-hydroxyl and 4-keto groups, forming a distorted square-planar environment typical of Cu(II)–flavonoid systems (Fig. 3a). Complex 2 is proposed to adopt an analogous coordination mode around Co(II), with the additional presence of four lattice water molecules that contribute to the hydration of the solid but do not participate directly in metal coordination (Fig. 3b). These structural assignments are consistent with the FTIR shifts associated with chelation, the electronic absorption features indicative of ligand-to-metal interactions, the EPR signature of both complexes, and the predominance of $[M(\text{Chr})_2 + \text{H}]^+$ ions in the ESI-HRMS spectra. However, in the absence of single-crystal X-ray diffraction data, the proposed structures should be regarded as plausible models rather than definitive geometrical descriptions.

3.3 Stability in cell culture medium

To assess the integrity and stability of the complexes under cell culture conditions, UV–Vis spectra were recorded in phenol red-free DMEM (Fig. 4) for up to 24 hours at 37°C. The spectral profiles of both complexes in cell culture medium were consistent with those obtained in DMSO solution. A bathochromic shift of approximately 20 nm for complex 1 and 2 nm for complex 2 was observed, in line with the trends previously noted in DMSO. After 24 hours of incubation at 37°C, a further decrease in absorbance intensity occurred without changes in band shape or position, suggesting gradual aggregation or precipitation of the complexes over time. Although partial aggregation may occur, the absence of major spectral shifts indicates that the chromophoric core of the complexes remains largely intact.

This interpretation is consistent with the ESI-HRMS data, which confirm the presence of intact mononuclear ions $[M(\text{Chr})_2 + \text{H}]^+$ ($M = \text{Cu}, \text{Co}$) in solution, even though ligand-derived ions dominate the spectra due to the intrinsic ionization behaviour of neutral complexes in positive-ion electrospray. Together, these observations suggest that, in cell culture medium, the complexes likely coexist with partially dissociated species in dynamic equilibria rather than undergoing complete decomposition.

Speciation considerations. The characterization data collectively indicate that the metal–chrysin complexes retain their core $[M(\text{Chr})_2]$ structure while engaging in medium-dependent equilibria. UV–Vis measurements in cell culture medium show that the complexes remain spectroscopically identifiable

over time, despite partial aggregation or precipitation. In parallel, the ESI-HRMS spectra confirm the presence of intact mononuclear ions $[M(\text{Chr})_2 + \text{H}]^+$ ($M = \text{Cu}, \text{Co}$), together with ligand-derived fragments that arise from the intrinsic ionization behavior of neutral O,O-bound flavonoid complexes in positive-ion electrospray. These observations, supported by elemental analysis, FTIR, TGA and EPR data, indicate that the complexes exist as well-defined coordination units whose speciation can shift depending on the medium, without evidence for complete dissociation under the conditions examined. This behaviour is consistent with the dynamic equilibria typically observed for labile O,O-chelating metal–organic systems. (Levina et al. 2017; Kostova and Balkansky 2013; Walencik et al. 2024)

3.4 In vitro biological assays

Before analyzing the cellular responses, it is important to consider the chemical speciation under the assay conditions. The concentrations used in the biological experiments are far below those at which free Cu^{2+} or Co^{2+} ions typically induce cytotoxicity, and the two complexes display distinct biological profiles that do not resemble the behavior of unbound metal ions. These observations indicate that the cellular effects are unlikely to arise from nonspecific metal-ion toxicity, but rather from the coordination entities present in solution, in line with the speciation considerations discussed above.

3.4.1 Caco-2

The cytotoxicity of the two Chr metal complexes, as well as of pure Chr, was evaluated using the MTT assay. Assessment of the impact of different concentrations of these compounds on cell viability was crucial for selecting the appropriate concentrations for subsequent biological assays. Solutions of the Cu(II) and Co(II) complexes were prepared as previously described, and the final tested concentrations were 0.7, 7 and 70 μM for both complexes. For comparison, Chr solutions were also prepared in the same concentration range (1, 10 and 100 μM).

We found that only the lower concentrations of the complexes were non-cytotoxic to Caco-2 cells, as higher concentrations of both complex 1 and 2 (7 and 70 μM) significantly reduced cell viability (Fig. 5a). Based on these results, in subsequent studies with Caco-2 cells, complexes 1 and 2 were tested at 0.7 μM . In contrast, Chr was cytotoxic at all tested concentrations, and the lowest concentration (1 μM) was used for comparison in further assays.

It is important to note that the comparison between free chrysin and its metal complexes is not strictly equimolar. Because each complex ($[\text{Cu}(\text{Chr})_2]$ and $[\text{Co}(\text{Chr})_2] \cdot 4\text{H}_2\text{O}$) contains two coordinated chrysin ligands, the working concentration of 0.7 μM corresponds to approximately 1.4 μM chrysin equivalents, whereas free chrysin was tested at 1 μM . Although not equimolar, these concentrations are of the same order of magnitude, ensuring that the comparison remains biologically meaningful. The concentrations selected were constrained by the limited aqueous solubility of the metal complexes, which prevented testing them at higher levels to achieve exact equimolarity. Importantly, the biological effects of the complexes cannot be attributed solely to their higher nominal chrysin content, as metal coordination

substantially modifies the physicochemical and biological properties of chrysin, giving rise to distinct chemical entities with their own biological profiles.

To assess the potential impact of the compounds on intestinal sugar absorption, evaluation of their effects on glucose and fructose luminal uptake by Caco-2 cells, a well-established *in vitro* cell model of human enterocytes,(Ni et al. 2020) was performed. The results, presented in Fig. 5b, indicate that treatment with both Chr metal complexes led to a reduction of approximately 20% in ^3H -DG uptake, when compared with the control and Chr itself, which had no significant effect at the tested concentration. In contrast, no significant impact of any of the three tested compounds was observed on ^{14}C -fruct uptake (Fig. 5b).

In relation to Chr, a previous study reported that this compound reduced ^3H -DG and ^{14}C -fruct uptake by this cell line, but a much higher concentration of Chr (100 μM) was tested.(Andrade et al. 2018) The present findings support the idea that complexation of Chr with Cu(II) and Co(II) ions may positively influence its inhibitory effect upon intestinal glucose absorption when compared to pure Chr. By limiting intestinal glucose absorption, this mechanism could support better glycemic control in diabetic patients.

We further aimed to understand the possible mechanisms involved in the modulatory effect of the complexes on glucose uptake. Because both ^3H -DG and ^{14}C -fruct uptake by Caco-2 cells is sensitive to oxidative stress levels,(Andrade et al. 2018) we decided to evaluate the effect of the compounds on cellular redox levels, by determining their effects on lipid peroxidation levels. The TBARS assay revealed that the complexes had no effect on lipid peroxidation levels (Fig. 5c). These findings are consistent with the DPPH and FRAP assays, in which the complexes exhibited neither antioxidant nor pro-oxidant properties (data not shown). Thus, their inhibitory effect on intestinal glucose uptake does not appear to be mediated by redox modulation.

To further understand the possible mechanisms involved in the modulatory effect of the complexes on glucose uptake, RT-qPCR was performed to evaluate expression of the following genes: SREBP-1C and ChREBP. The results obtained allowed us to conclude that no differences were observed in relation to SREBP-1c, but both complexes drastically increase ChREBP expression (Fig. 5d). ChREBP is a transcription factor activated by high glucose that generally promotes the expression of genes involved in carbohydrate (glycolysis) and lipid (lipogenesis) metabolism.(Iizuka 2017b) Accordingly, at the intestinal level, ChREBP is known to induce GLUT2 and GLUT5 expression levels.(Iizuka 2017a; Kato et al. 2018) However, in the present report, a decrease in glucose cellular uptake was observed. It is possible that elevated ChREBP expression may signal a feedback response aimed at maintaining glucose homeostasis, leading to downregulation of glucose transporter activity, thereby reducing glucose intestinal absorption and preventing excessive glucose uptake during states of nutrient surplus. Alternatively, the observed effect may result from a direct effect of the complexes on glucose transporters' activity or expression. Interestingly, an opposite increase in the expression of ChREBP (increase) and GLUT2 (decrease) was found in hepatocellular carcinoma cells, in relation to non-

cancerous tissue.(Lei et al. 2020) So, the relationship between ChREBP and glucose transporters is not clear and should be further investigated.

3.4.2 HepG2

In HepG2 cells, significant reductions in cell viability were observed only at the highest concentrations of complex 1, complex 2 and Chr (Fig. 6a). Therefore, the selected concentrations for further experiments were 0.7 and 7 μ M for the complexes and 1 μ M for Chr.

Because the liver plays an important role in removing excess glucose and fructose from the bloodstream,(Roden and Shulman 2019) we decided to investigate the potential effect of these compounds on hepatic glucose and fructose uptake. Interestingly, both metal complexes significantly increased ^{3} H-DG uptake (by 10-23%) by HepG2 cells (Fig. 6b). These increases may correlate with a more efficient hepatic removal of blood glucose, which is beneficial in the diabetic context. The increase is even higher compared with Chr that, although did not significantly affect ^{3} H-DG uptake, tended to slightly reduce it. In a previous study, it was reported that Chr (at higher concentrations (10-15 μ M) than the ones used in the present study) stimulates glucose uptake in insulin-resistant HepG2 cells.(Zhou et al. 2021) This supports the evidence that complexation of Chr with copper or cobalt ions enhances its ability to modulate hepatic glucose uptake. In relation to ^{14} C-fruct uptake, it was significantly reduced by complex 2, although the decrease was lower than 20%, and no significant differences were found between the complexes and Chr.

Similar to the studies conducted on Caco-2 cells, we aimed to understand the possible mechanisms involved in the modulatory effect of the complexes on glucose uptake in HepG2 cells. So, RT-qPCR was performed to evaluate their effect on the gene expression of SREBP-1c, ChREBP, ACC1, and GK. The results demonstrated that complex 1 significantly downregulated SREBP-1c and ChREBP (Fig. 6c) expression, with reductions of approximately 81% and 66%, respectively. Additionally, none of the complexes interfered with the expression of ACC1 and GK (results not shown).

SREBP-1c (Sterol Regulatory Element-Binding Protein 1c) is a key transcription factor involved in lipid metabolism, particularly fatty acid and triglyceride synthesis. It is primarily activated by insulin and acts in coordination with ChREBP to regulate lipogenesis.(Ortega-Prieto and Postic 2019) SREBP-1c downregulation may be beneficial, as elevated SREBP-1c expression has been linked to insulin resistance and increased *de novo* lipogenesis and triglyceride accumulation,(Zhou et al. 2021)(Pai et al. 2019) and SREBP-1c inhibitors improve insulin resistance and have an overall beneficial effect on the main components of metabolic syndrome.(Wang et al. 2024) Previous studies conducted in other experimental models (animal liver tissue) demonstrated a negative effect of Chr on SREBP-1c expression levels,(Zhou et al. 2021; Pai et al. 2019; Oriquat et al. 2023; Campanher et al. 2025) although another study concluded that Chr can reduce lipid accumulation in the liver of rats,(Attia et al. 2022; Campanher et al. 2025) without interfering with SREBP-1c, ACC1 or GLUT2 expression.(Campanher et al. 2025) We verified that the Cu(II)-Chr complex (but not the Co(II)-Chr complex) downregulated SREBP-1c

and ChREBP expression in HepG2 cells. Although the effect of Chr on ChREBP has not been, to our knowledge, yet reported, our results suggest that the effect of Chr on these hepatic transcription factors is maintained after complexation with Cu(II), but not with Co(II).

Moreover, similar to what we observed in Caco-2 cells, an opposite trend in the expression of SREBP-1c and ChREBP (decrease) and on cellular glucose uptake (increase) was induced by complex 1 in HepG2 cells. GLUT2 accounts for over 97% of glucose transporters in hepatocytes,(Sun et al. 2023) and liver cells are freely permeable to glucose via this transporter.(Kennelly et al. 2023) So, the increase in glucose uptake may result from a direct effect of the complexes on GLUT2 activity or expression, again pointing to the need of further investigation on the relationship between SREBP-1c, ChREBP and GLUT2.

3.4.3 3T3-L1

In 3T3-L1 mature adipocytes, complex 1 exhibited significant cytotoxicity at all tested concentrations (Fig. 7a). However, at 0.7 and 7 μ M, cell viability was reduced by only approximately 20%. So, these concentrations of complex 1 were chosen for further studies. In contrast, complex 2 and Chr were not cytotoxic at any concentration, and so the two lower concentrations (0.7 and 7 μ M for complex 2 and 1 and 10 μ M for Chr) were selected for further assays.

Then, the effect of these compounds on glucose uptake was assessed. As shown in Fig. 7b, complex 2 at 7 μ M significantly increased ^{3} H-DG uptake (by about 50%) compared to the control and Chr. No other significant differences were observed, but a tendency to increase ^{3} H-DG uptake is also observed for complex 1 at 7 μ M, and complex 2 at 0.7 μ M. The stimulating effect of complex 2 on glucose removal by adipocytes is interesting in the context of insulin resistance and type 2 diabetes mellitus, as it may contribute to a glycemia-lowering effect.(Merz and Thurmond 2020) Indeed, insulin resistance is known to reduce adipose tissue GLUT4 expression, as well as glucose uptake.(Dubon et al. 2015) A previous study reported the insulin-mimetic properties of two complexes of Zn(II) with other plant-derived natural compounds (bis(maltolato)-Zn(II) complex and bis(allixinato)-Zn(II) complex), as these complexes successfully increased glucose uptake by rat adipocyte cells.(Adachi et al. 2004) In their study, a similar effect to the one observed for complex 2 at 7 μ M, but for a considerable higher concentration, was observed.

Finally, to evaluate the impact of these compounds on lipid accumulation, 3T3-L1 cells were stained with Oil Red O, a dye that specifically binds neutral lipids such as triglyceride and cholesteryl esters.(Pacifici et al. 2023) This assay is particularly relevant in the context of diabetes, since adipocyte dysfunction, often developing as a result of insulin resistance, is closely associated with T2DM,(Dubon et al. 2015) and 3T3-L1 cells are a well-established model for studying adipogenesis and lipid accumulation.(Cave and Crowther 2019) As shown in Fig. 7c, both the complexes and Chr significantly increased lipid accumulation in 3T3-L1 cells at all tested concentrations. No significant differences were observed between the Cu(II) and Co(II) complexes in this effect. However, complex 1 at 0.7 μ M resulted in significantly lower lipid accumulation compared to Chr, suggesting a potential difference in their impact

on adipogenesis. For complex 2, this increased lipid accumulation might be related to the increase in cellular glucose uptake, which may act as the precursor of triglycerides.

4. Conclusions

This work adopts a multidisciplinary approach, bridging inorganic chemistry, biochemistry, and cellular biology, to develop and evaluate novel metal–flavonoid complexes with antidiabetic potential. Cu(II) and Co(II)-chrysin complexes were successfully synthesized using solvothermal and microwave-assisted methods, the latter providing a greener, and faster (5 min) alternative without compromising product quality. Spectroscopic and thermal analyses confirmed their structural integrity and stability.

Biological evaluation in intestinal, hepatic, and adipocyte cell models revealed that complexation with Cu(II) and Co(II) markedly enhances the bioactivity of chrysin. Both complexes reduced intestinal glucose absorption and increased hepatic glucose uptake, effects not observed with free chrysin. In adipocytes, the Co(II) complex significantly stimulated glucose uptake. These biological outcomes were accompanied by modulation of key metabolic regulators, including ChREBP and SREBP-1c, suggesting distinct and tissue-specific mechanisms of action. Importantly, both complexes displayed low cytotoxicity at active concentrations.

To the best of our knowledge, this is the first demonstration of antidiabetic activity for Cu(II)- and Co(II)-chrysin complexes. These findings support the hypothesis that metal coordination can overcome pharmacokinetic limitations of flavonoids and potentiate their therapeutic action. Moreover, they position these metal–chrysin complexes as promising proof-of-concept systems for the development of metal-based antidiabetic leads. Nevertheless, further studies addressing *in vivo* efficacy, metal biodistribution, and long-term toxicity will be essential to assess their translational potential.

Declarations

Author Contribution

Diogo Marinheiro: Writing – original draft, Methodology, Investigation. Cláudia Silva: Writing – review & editing, Methodology, Investigation. Ricardo F. Mendes: Writing – review & editing, Methodology. Tânia Moniz: Writing – review & editing, Investigation, Methodology. André Silva: Investigation, Methodology. Maria Rangel: Writing – review & editing, Methodology. João Rocha: Writing – review & editing, Funding acquisition. Ana Luísa Daniel-da-Silva: Writing – review & editing, Supervision, Funding acquisition. Fátima Martel: Writing – review & editing, Supervision, Funding acquisition. Bárbara J. M. Leite Ferreira: Writing – review & editing, Validation, Supervision, Conceptualization.

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Data Availability

All data supporting the findings of this study are included in the article and its supplementary information files. Additional raw data are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no competing financial interest.

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Figures

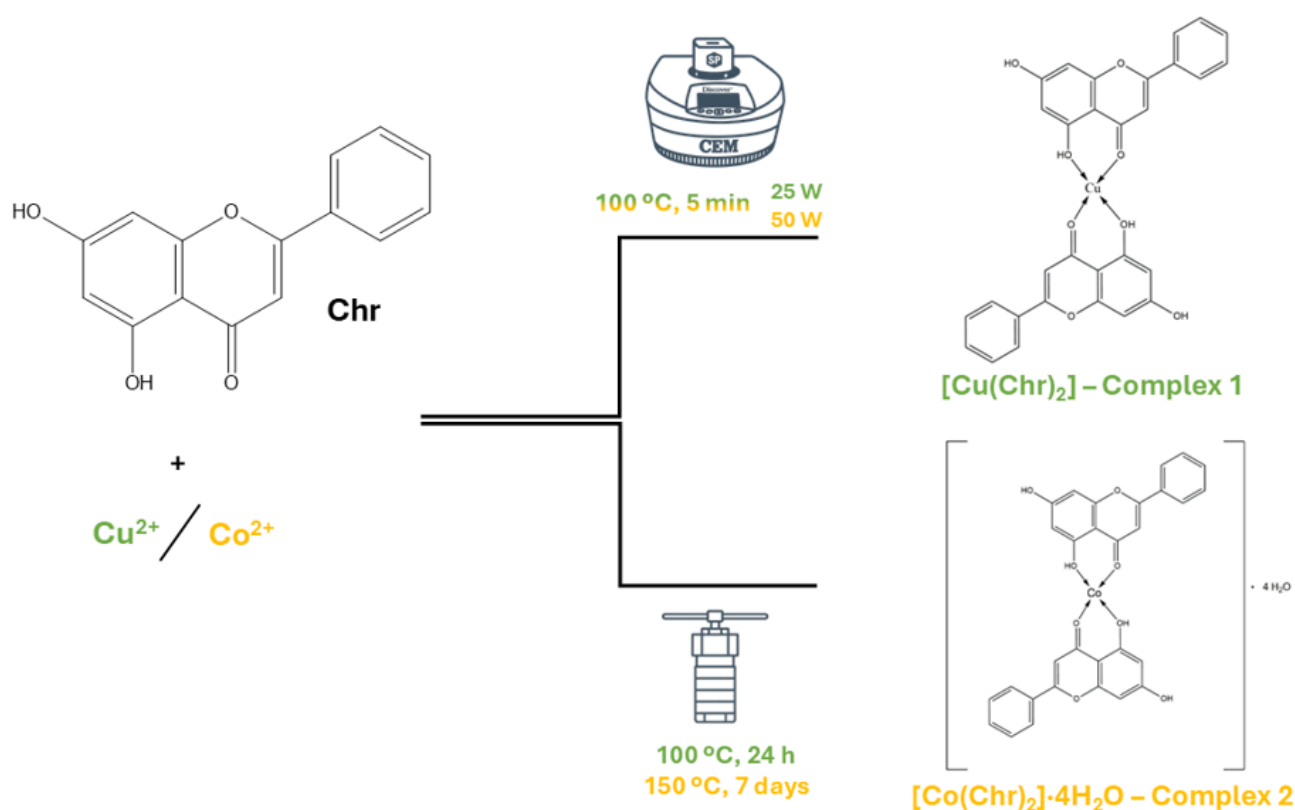


Figure 1

Schematic representation of the solvothermal and MWAS routes used for the preparation of complexes 1 and 2.

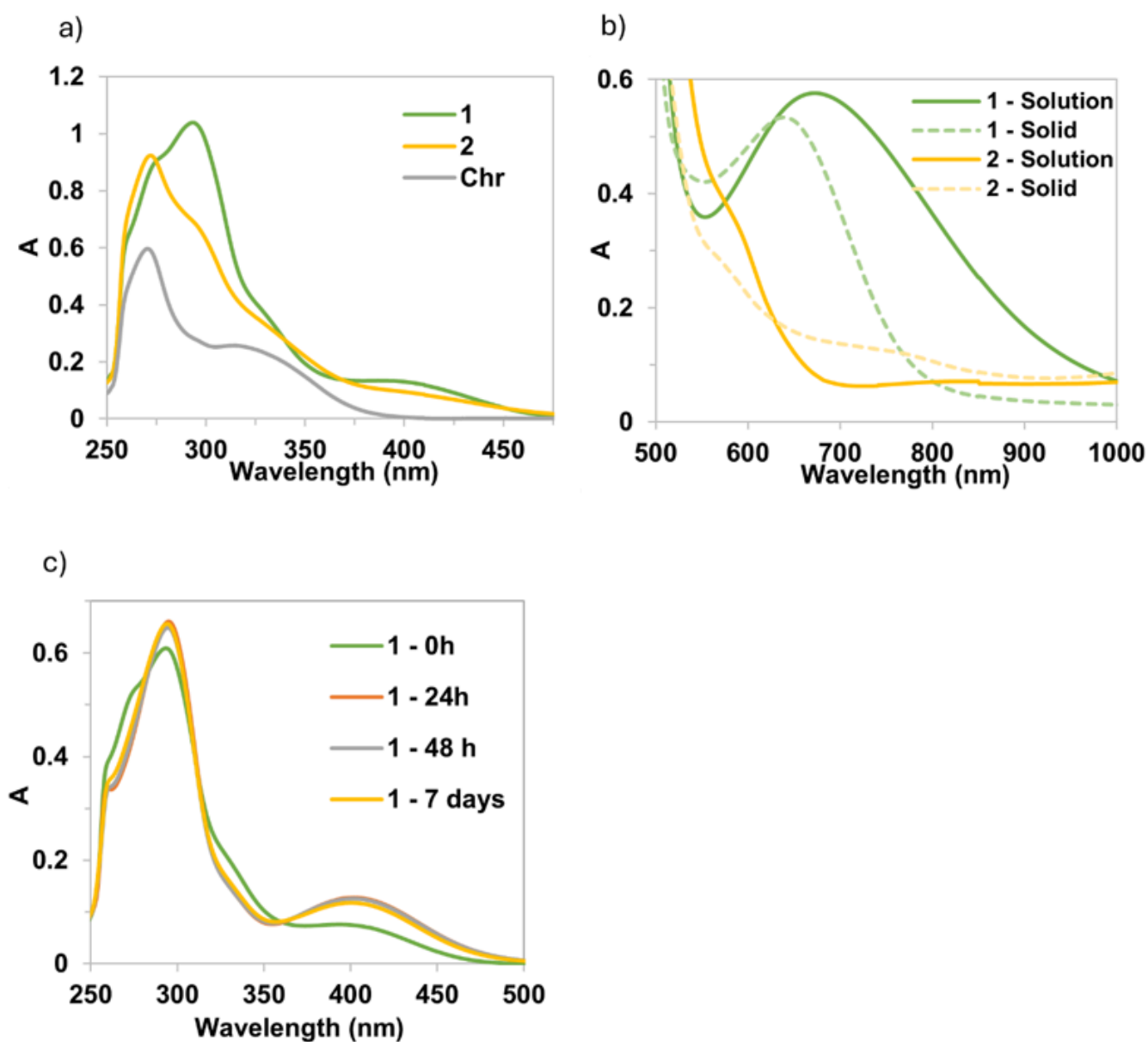


Figure 2

(a) UV-Vis absorption spectra of Chr, complex 1, and complex 2 recorded in 20 μM DMSO solutions; (b) Comparison of solid-state and solution-phase UV-Vis spectra for both complexes, in the visible region; (c) Time-dependent spectral changes of complex 1 in DMSO showing rearrangement and stabilization after 24 hours.

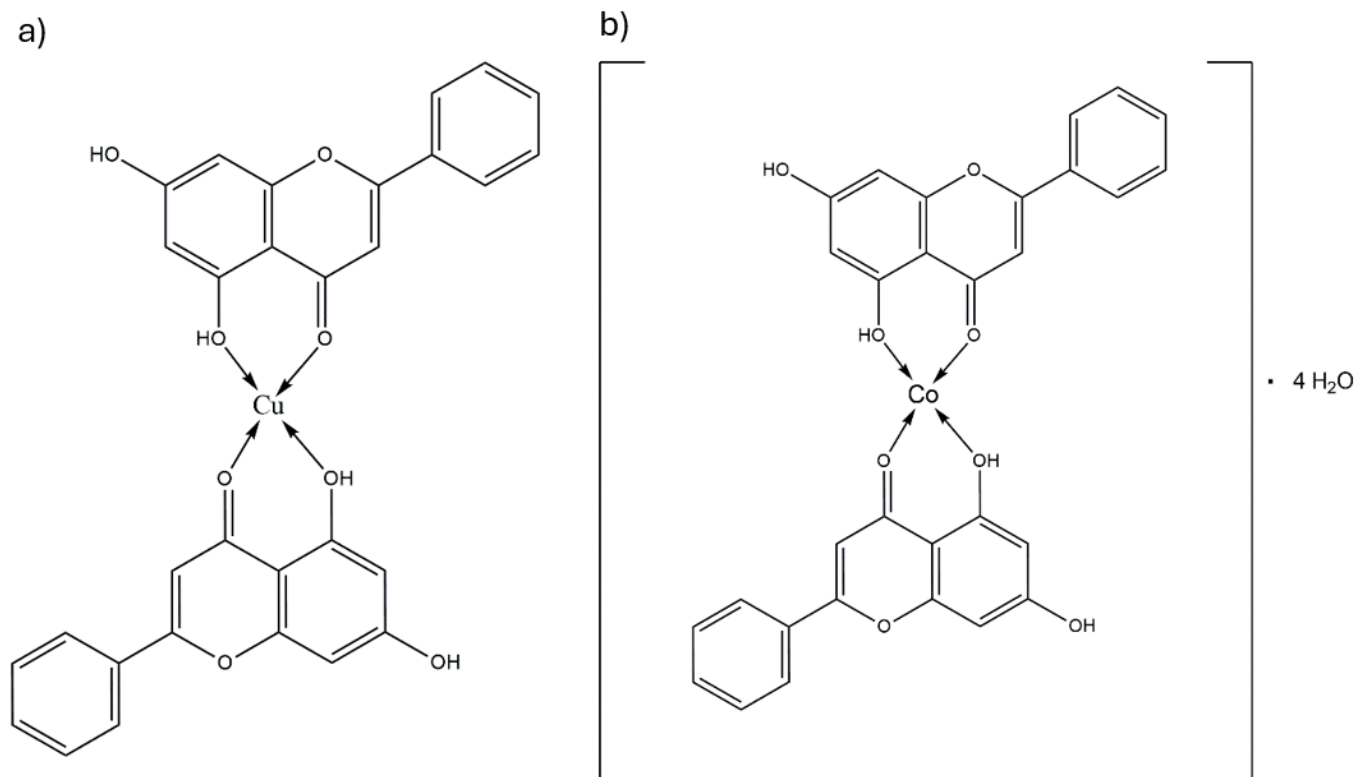


Figure 3

Tentative structures proposed for the Cu(II)- and Co(II)-Chr complexes. (a) Complex 1: $[\text{Cu}(\text{Chr})_2]$; (b) Complex 2: $[\text{Co}(\text{Chr})_2] \cdot 4 \text{H}_2\text{O}$, containing four non-coordinated hydration water molecules.

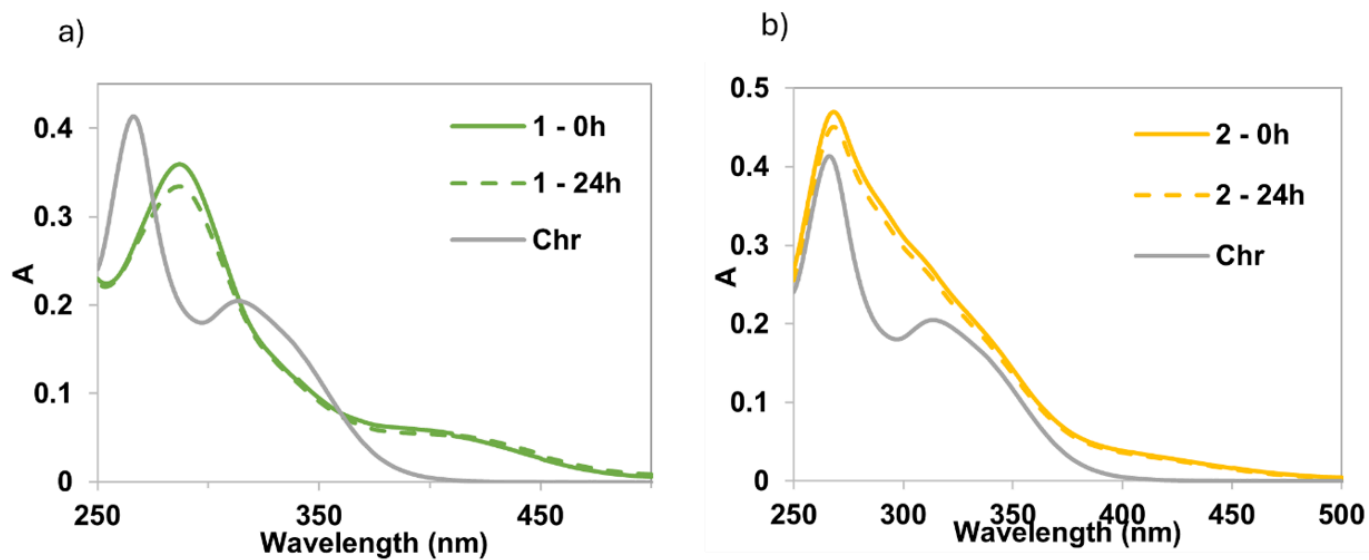


Figure 4

UV-Vis spectra of complex 1 (a) and complex 2 (b) in phenol red-free DMEM immediately after dilution (0h) and after 24 hours of incubation at 37°C, compared with Chr under the same conditions.

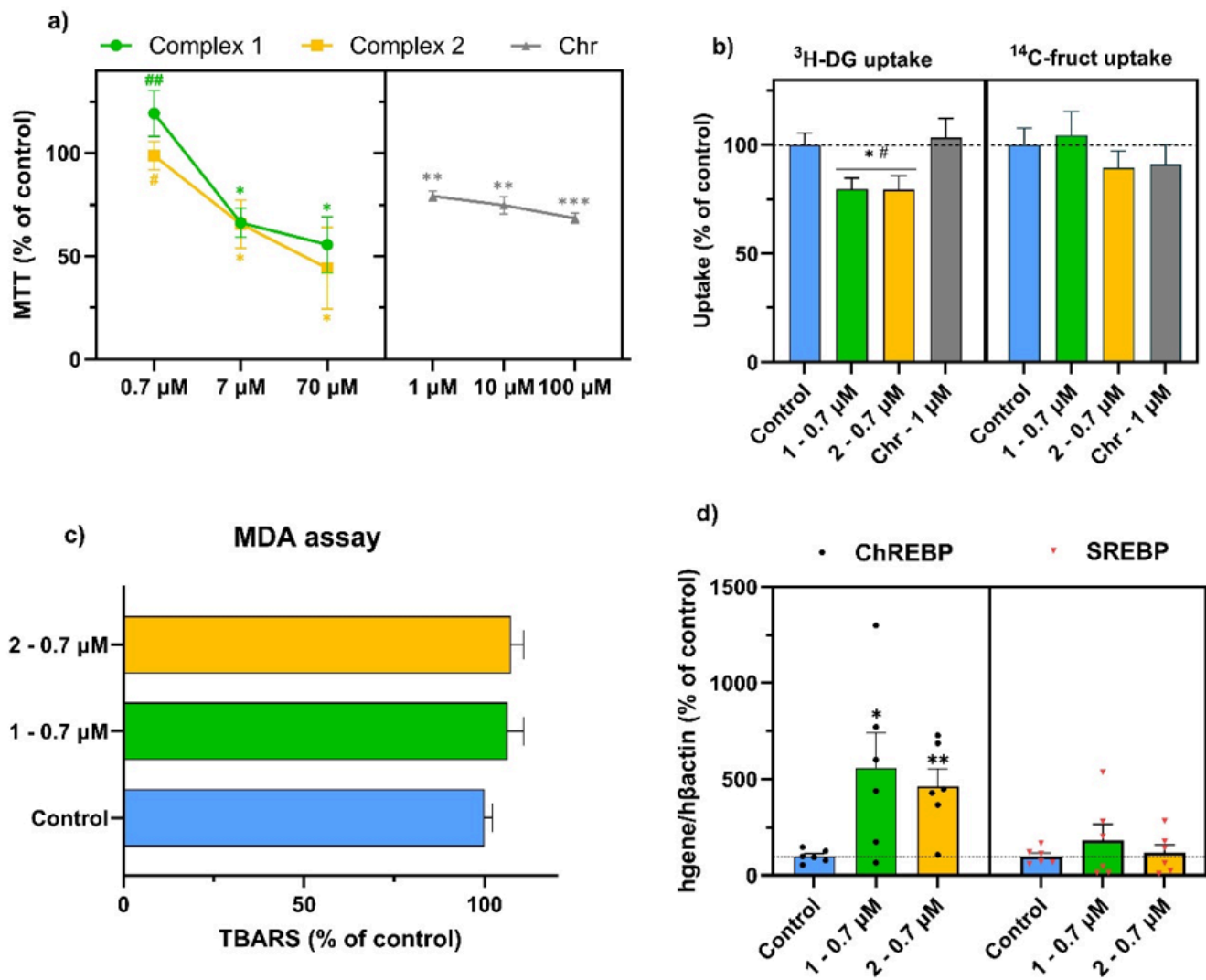


Figure 5

Impact of the Chr metal complexes (complex 1 (1) and complex 2 (2)) in Caco-2 cells. (a) Effect of different concentrations of Chr and its Cu(II) and Co(II) complexes on cell viability, assessed by the MTT assay (n=7). (b) Effect of Chr and its Cu(II) and Co(II) complexes on ^{3} H-DG uptake (n=12) and ^{14} C-fructose (^{14} C-fruct) uptake (n=12-16). (c) Effect of Chr Cu(II) and Co(II) complexes (0.7 μ M) on lipid peroxidation levels measured by the TBARS assay (n=6). (d) Effect of Chr Cu(II) and Co(II) complexes (0.7 μ M) on relative ChREBP and SREBP gene expression levels (n=6). Data are presented as mean $\pm$ SEM. * p < 0.05, ** p <0.01, *** p <0.001 compared to control; # p < 0.05, ## p <0.01 compared to Chr 1 μ M (Student's t test).

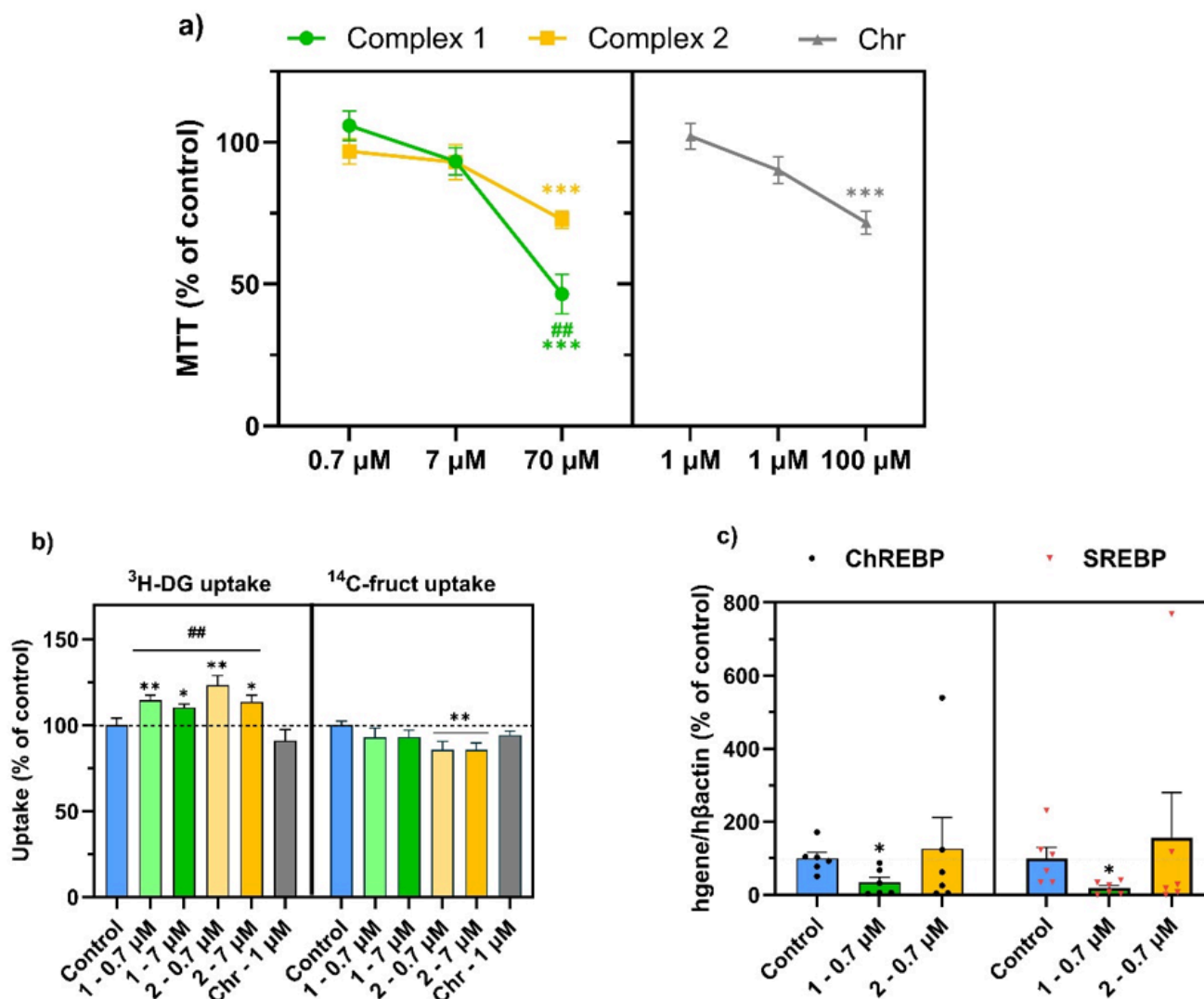


Figure 6

Impact of the Chr metal complexes (complex 1 (1) and complex 2 (2)) in HepG2 cells. (a) Effect of different concentrations of Chr and its Cu(II) and Co(II) complexes on cell viability, assessed by the MTT assay (n=9-12). (b) Effect of different concentrations of Chr and its Cu(II) and Co(II) complexes on ³H-DG and ¹⁴C-fruct uptake (n=8-16). (d) Effect of Chr Cu(II) and Co(II) complexes on relative SREBP-1C gene expression levels (n=6). (c) Effect of Chr Cu(II) and Co(II) complexes on relative ChREBP and SREBP gene expression levels (n=6). Data are presented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to control; ## $p < 0.01$ compared to Chr 1 μM (Student's t test).

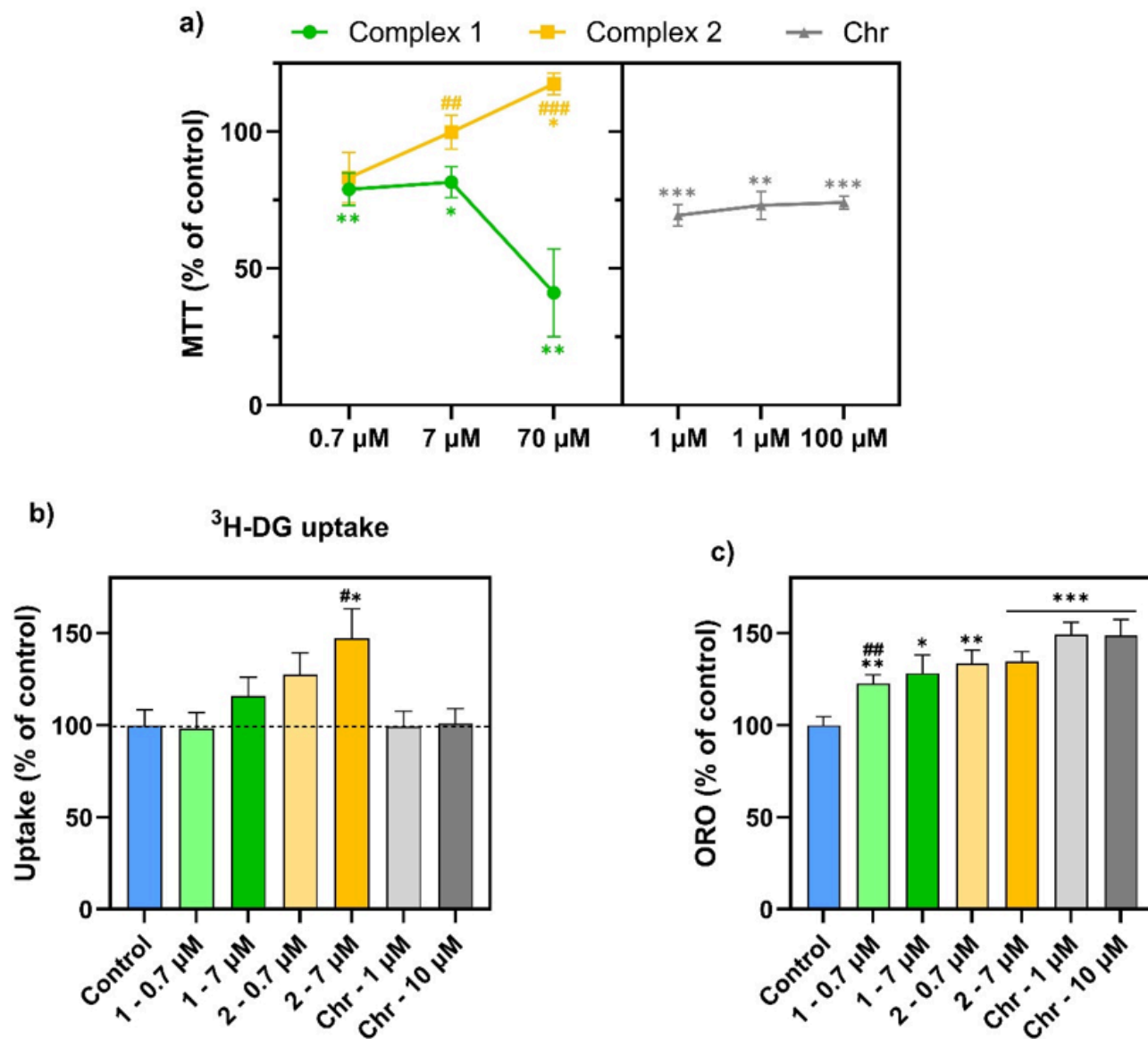


Figure 7

Impact of the Chr metal complexes (complex 1 (1) and complex 2 (2) in 3T3-L1 adipocytes. (a) Effect of different concentrations of Chr and its Cu(II) and Co(II) complexes on cell viability, assessed by the MTT assay (n=6). (b) Effect of different concentrations of Chr and its Cu(II) and Co(II) complexes on ^{3} H-DG glucose uptake (n=8-12). (c) Effect of Chr Cu(II) and Co(II) complexes on lipid accumulation (ORO staining) (n=12). Data are presented as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to control; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ compared to Chr (Student's t test).

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