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SYNTHESIS AND EVALUATION OF ZINC–QUERCETIN COMPLEX: *IN VITRO* ANTI-GLYCATION AND DNA METHYLATION ANALYSIS WITH MOLECULAR DOCKING STUDIES

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Type 2 diabetes mellitus (T2DM) is a growing global health concern, associated with complications driven by molecular alterations such as excessive protein glycation and abnormal DNA methylation. These processes contribute to the progression of metabolic and epigenetic dysfunctions characteristic of T2DM.

The aim. *This study aimed to synthesize a zinc–quercetin complex (ZQC) and evaluate its in vitro biological activities, particularly its potential to inhibit the formation of advanced glycation end products (AGEs) and modulate DNA methylation levels.*

Materials and methods. *ZQC was synthesized and tested for antiglycation activity using BSA-methylglyoxal and BSA-glucose model systems. DNA methylation levels were assessed via cell imaging in HEK293T and C2C12 cells using an oxazole yellow-based fluorescent probe. Molecular docking was performed to assess the interaction of ZQC with DNA methyltransferase 1 (DNMT1).*

Results. *ZQC exhibited dose-dependent antiglycation effects, with significantly reduced fluorescence intensity compared to untreated and quercetin-treated groups, suggesting potent inhibition of AGE formation. In DNA methylation assays, ZQC more effectively reduced methylation levels than free quercetin. Molecular docking showed a stronger binding affinity of ZQC (–11 kcal/mol) to DNMT1 compared to quercetin alone (–8.1 kcal/mol), indicating the potential for enhanced inhibitory activity.*

Conclusion. *The zinc–quercetin complex demonstrated superior antiglycation and epigenetic-modulating effects relative to free quercetin. These findings support the potential of ZQC as a candidate for therapeutic intervention in glycation-associated and epigenetically driven complications of T2DM*

Keywords: *zinc-quercetin complex, type 2 diabetes, glycation, DNA methylation*

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1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by insulin resistance and elevated blood glucose levels. If poorly managed, the condition could lead to various complications, including vascular damage, neuropathy, and kidney failure [1]. Two key processes, protein glycation and DNA methylation, play significant roles in the development and progression of T2DM [2–4].

Protein glycation occurs when excess glucose reacts non-enzymatically with proteins, forming advanced glycation end-products (AGEs). These AGEs accumulate in tissues and contribute to diabetic complications by promoting inflammation, oxidative stress, and impairing insulin signaling. The accumulation of AGEs further disrupts glucose metabolism and exacerbates insulin resistance, creating a vicious cycle in the disease progression [2, 5]. DNA methylation, an epigenetic modification, also influences the pathogenesis of T2DM. Abnormal DNA methylation patterns can alter the expression of genes involved in insulin secretion, glucose metabolism, and inflammation. Changes in the methylation status of key genes may impair pancreatic β-cell function and exacerbate insulin resistance. Additionally, AGEs can affect DNA methylation, further influencing gene expression related to glucose homeostasis and creating a feedback loop that worsens the diabetic condition [4, 6].

Despite the growing understanding of these mechanisms, therapeutic approaches targeting both glycation and DNA methylation remain underexplored. Natural compounds like quercetin, a flavonoid with antioxidant and anti-inflammatory properties, have shown potential in modulating these pathways [7–9]. Recent studies on zinc–quercetin complexes suggest that such complexes may offer enhanced bioactivity in scavenging reactive oxygen species, potentially providing a novel therapeutic strategy for managing T2D [10, 11].

This study investigates the effects of a zinc–quercetin complex on AGE inhibition and DNA methylation as a potential therapeutic approach to mitigate the molecular contributors to T2D.

2. Planning (methodology) of research

The methodology includes the following in experiments to evaluate the bioactivity of zinc-quercetin complex (ZQC):

1. Evaluate the antiglycation potential of ZQC by utilizing BSA-MGO and glucose model systems.

2. Investigate the ability of the complex to reduce the levels of DNA methylation in HEK293T and C2C12 cells using an oxazole-yellow fluorogenic probe.

3. Assess the ability of ZQC to inhibit DNMT1 enzyme by molecular docking using AutoDock.

3. Materials and methods

3. 1. General information

All chemicals and reagents were purchased from commercial suppliers and used without further purification. Solvents and reagents were of analytical grade. The formation of the synthesized zinc-quercetin complex (ZQC) was confirmed through various characterization techniques, including UV-visible spectroscopy (JASCO V-730 spectrophotometer), infrared (IR) spectroscopy (JASCO FT/IR-4600 spectrometer), and powder X-ray diffraction (Bruker D2 Phaser, 2nd Generation). Thin-layer chromatography (TLC) analysis was also performed to verify the purity of the final product.

For ZQC and quercetin treatments preparation, the stock solution ($7.5x10^{-4}$M) was prepared by dissolving 5 mg ZQC and quercetin in 10mL pure DMSO and an aliquot of 66.67 uL was diluted to a final volume of 5mL with PBS of pH 7.4 obtaining the 10uM solution (0.013% DMSO in PBS).

3. 2. Synthesis of zinc-quercetin complex

The synthesis protocol was adapted from the method described by [12]. Quercetin was dissolved in 10 mL of methanol, while the zinc acetate salt was dissolved separately in 10 mL of distilled water, maintaining a 2:1 molar ratio of quercetin to zinc as described by Da Silva, et.al. The two solutions were combined in a 50-mL flask and stirred at room temperature. After 20 minutes of stirring, three drops of triethylamine were added, which immediately induced a noticeable colour change in the solution. The reaction mixture was then stirred continuously for 3 hours. Following this, the solution was placed in a refrigerator and kept at a low temperature for 2 days to allow complete precipitation of the synthesized complex. The resulting precipitate was collected by filtration using a porous plate funnel and subsequently stored in a desiccator under vacuum until further use.

3. 3. Cell culture for live cell imaging

HEK293T, a human embryonic kidney cell line known for its high transfection efficiency, and C2C12 cells, a mouse myoblast cell line capable of differentiating into myotubes, were cultured in Dulbecco's Modified Eagle Medium with Penicillin–streptomycin (DMEM (+)) under standard conditions. After incubation, the culture medium was aspirated, and cells were washed three times with 2 mL of PBS (except for HEK293T cells, which did not require washing). One millilitre of PET solution was added to each dish, along with an additional 1 mL of PBS for HEK293T cells, followed by incubation in a CO_2 incubator for 3 minutes. Cells were then detached by adding 2 mL of DMEM (–) medium and gentle pipetting. The cell suspensions were collected into 15 mL tubes and centrifuged at 1000 rpm for 3 minutes. After centrifugation, the supernatant was removed, and the cell pellets were resuspended in 4 mL of DMEM (–) medium. Cell counting was performed by loading 20 µL of the suspension onto an Invitrogen cytometer slide, using the "20 fields" option for increased accuracy.

3. 4. BSA-glucose model system

Bovine serum albumin (BSA, 10 mg/mL) and glucose (90 mg/mL) were each dissolved in phosphate buffer (pH 7.4). Test solutions were prepared by mixing 0.5 mL of quercetin and zinc–quercetin complex (10, 5, and 1 µM) with 0.5 mL of BSA solution and 0.5 mL of glucose solution in polypropylene tubes. Negative controls consisted of phosphate buffer, BSA, and glucose, while positive controls contained 10 µM aminoguanidine instead of test compounds. All mixtures contained 0.3 mL of 0.01% sodium azide to prevent microbial growth. Samples were capped and incubated at 37°C in the dark for 18 days.

3. 5. BSA-MGO model system

Bovine serum albumin (BSA, 2 mg/mL) and methylglyoxal (MGO, 40 mg/mL) were each dissolved separately in phosphate buffer (pH 7.4). Test and control solutions were prepared following the same procedure as the BSA–glucose model system. Samples were then incubated at 37°C in the dark for 5 days.

AGE fluorescence measurement.

The fluorescence of AGEs was measured after completing the appropriate incubation procedure, using excitation and emission wavelengths of 360 nm and 420 nm for BSA-glucose, and 340 nm and 420 nm for BSA-MGO. Measurements were taken with an Infinite M1000 PRO microplate reader. Each set included triplicate samples, and the percentage inhibition of AGE formation was calculated using the formula

$$\%\ Inhibition = 1 - \frac{Intensity\ of\ sample}{Intensity\ of\ blank} \times 100. \quad (1)$$

3. 6. Transfection, pre-imaging preparation, and confocal microscopy

Cells were transfected using Lipofectamine 3000 following the manufacturer's protocol. Briefly, 0.45 µL of Lipofectamine 3000 in OPTI-MEM (Optimized Minimal Essential Medium) was prepared (Tube 1), while a mixture of 12.61 µL OPTI-MEM, 0.55 µL Plus reagent, and 0.592 µL HA-PYP3R-MBD112 plasmid was prepared separately (Tube 2). Solutions were combined, and a total volume of 27.5 µL was added to each well. Plates were incubated for 24 hours post-transfection.

For pre-imaging preparation, the culture medium was removed, and wells were washed with Hank's Balanced Salt Solution (HBSS). Cells were incubated for 1 hour in clear DMEM containing 1 µL of 2 mM PYP-ligand-conjugated Oxazole Yellow (YOCNB), washed three times with HBSS, and then maintained in clear

DMEM supplemented with 10% FBS. Cell viability and adherence were verified prior to imaging. Confocal microscopy was performed using a Zeiss LSM 880 microscope with a Plan Apochromat 63x/1.4 oil objective. Imaging was carried out with excitation at 463 nm and emission at 551 nm, and images were analyzed using Zeiss Microscopy Labscope software.

3. 7. Molecular docking studies

Molecular docking studies were performed to assess the binding of Quercetin and the Zinc-Quercetin Complex (ZQC) with DNMT1. The ligands were initially drawn in ChemDraw, and their SMILES notations were generated. The 3D structures were then optimized in Avogadro using the steepest descent and conjugate gradient algorithms to obtain stable, low-energy conformations. Chimera X 1.9 was used to prepare the ligands by adding hydrogen atoms, assigning partial atomic charges, and applying the Universal Force Field (UFF) for ZQC and the MMFF94 force field for Quercetin. For protein preparation, the crystal structure of DNMT1 (PDB ID: 4WXX) was retrieved, and Chain B was removed to focus on the active site in Chain A. Hydrogen atoms were added, solvent molecules and non-essential ions were removed, and atomic charges were assigned using Chimera X. The structure was then converted to PDBQT format using AutoDock Tools (ADT) for docking.

Docking simulations were performed using Autodock in the PyRx platform, where the ligands were minimized and a grid box was positioned to target the CYS1226 residue of DNMT1. The docking models were evaluated based on binding affinities, and the model with the lowest affinity was selected for further analysis. Finally, the docking poses were analyzed in Biovia Discovery Studio to visualize interactions, including hydrogen bonds and π-interactions, providing insight into the potential binding modes and inhibitory effects of the ligands on DNMT1.

3. 8. Statistical analysis

All experiments and assays were performed in triplicates, and results were presented as the mean ± standard deviation (mean ± SD). Statistical analysis was conducted using GraphPad Prism version 9.3.1 (GraphPad Software, San Diego, California, USA, www.graphpad.com). Data were subjected to single-factor ANOVA, and if significant differences were observed ($p < 0.05$), appropriate post hoc tests, including Dunnett's and Tukey's tests, were applied.

4. Results

4. 1. Spectroscopic characterization of ZQC

The successful formation of the zinc–quercetin complex (ZQC) was initially indicated by a visible color change from the pale yellow of free quercetin to a deep orange hue, suggesting coordination between the ligand and zinc ions. This observation was supported by UV–Vis spectroscopic analysis, where free quercetin displayed two major absorption bands: Band I at 364 nm, corresponding to the cinnamoyl system (ring B and carbonyl group in ring C), and Band II at 256 nm, attributed to the benzoyl system (ring A and adjacent hydroxyl groups) (Fig. 1).

Upon complexation, Band I underwent a notable bathochromic shift to 432 nm, indicating significant electronic perturbation due to metal coordination, while Band II exhibited a slight shift to 258 nm. In addition, the emergence of a distinct absorption peak at 380 nm, assigned to ligand-to-metal charge transfer (LMCT), further confirmed the formation of a stable metal–ligand complex (Fig. 2).

Complementary FTIR spectral analysis revealed marked shifts in vibrational frequencies upon complexation (Table 1). The strong C=O stretching vibration observed at 1610 cm^{-1} in free quercetin shifted to 1590 cm^{-1} in ZQC, implying involvement of the carbonyl oxygen in coordination. Shifts were also detected in the C–O stretching (from 1375 to 1248 cm^{-1}) and C–O–C asymmetric stretching bands (from 1165 to 1090 cm^{-1}), indicating structural changes within the ligand framework. The appearance of new bands in the region of 1200–1330 cm^{-1}, corresponding to C–O–metal stretching vibrations, further supported chelation through oxygen donor atoms. A distinctive band around 610 cm^{-1}, absent in the free ligand, was assigned to Zn–O stretching, serving as a spectral signature of complex formation. Importantly, the retention of broad O–H bands (3200–3300 cm^{-1}) and C=C stretching bands (1480–1530 cm^{-1}) confirmed the preservation of hydroxyl groups and the conjugated system in the flavonoid backbone.

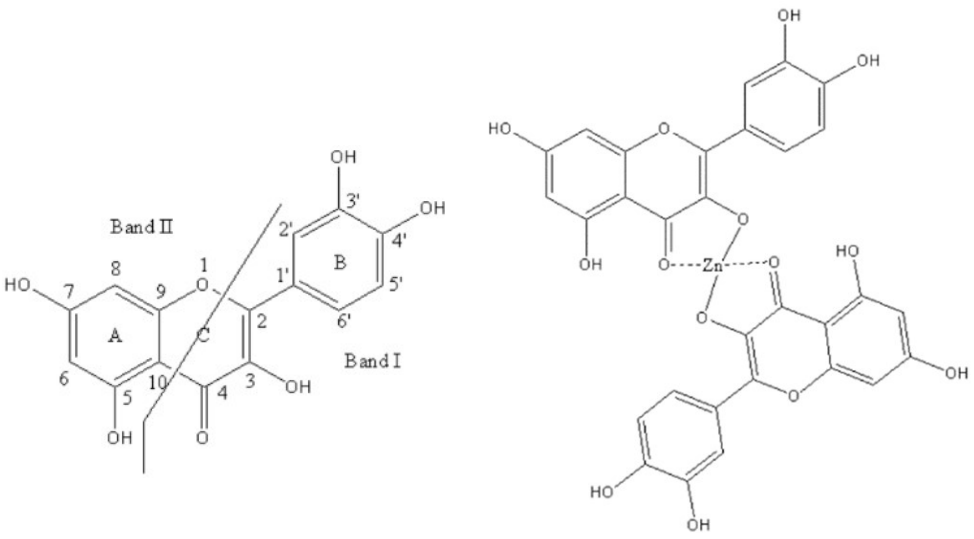

Fig. 1. Molecular Structure of Quercetin (left) and Zinc-Quercetin Structure [12] (right)

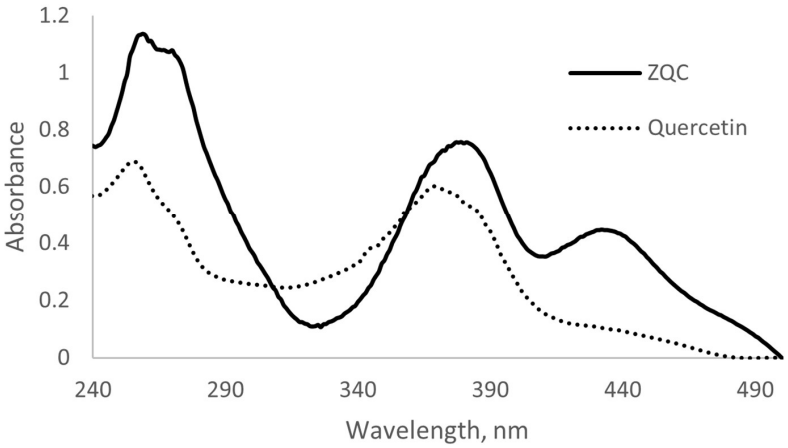

Fig. 2. UV-Vis Spectra of Quercetin and ZQC

Table 1

Infrared Spectroscopy vibrational data (in cm^{-1}) of ZQC and quercetin

Compound	ν(O-H)	ν(C=O)	ν(C=C)	ν(C-OH)	ν(C-OC)	σ(C-OH)	ν(M-O)
Quercetin	3200	1610	1520	1375	1165	1020	–
ZQC	3240	1590	1490	1248	1090	1000	559

positive control, showed 8.61 ± 1.37% inhibition. However, these differences were not statistically significant ($p > 0.05$), possibly due to the short 5-day incubation limiting AGE accumulation.

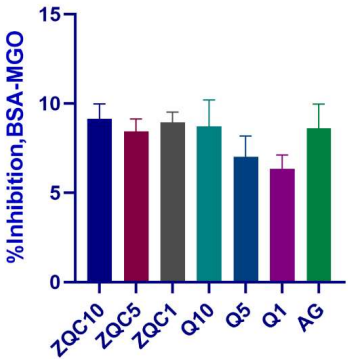

Fig. 4. Inhibitory activities of the different concentrations of ZQC and quercetin in BSA-MGO model system. (ns, Tukey's test)

4. 2. Powder XRD characterization

The PXRD pattern of pure quercetin, previously characterized by Wangsawangrung et al. (2022), displayed sharp peaks at 2θ values of 10.66°, 12.34°, and 27.26°, confirming its crystalline structure [13].

In contrast, the zinc–quercetin complex (ZQC) exhibited notable changes, including the disappearance of peaks at 10.66° and 12.34° and an overall reduction in peak intensity (Fig. 3). These alterations indicate a loss of crystallinity and suggest that zinc coordination disrupts the native molecular packing of quercetin, resulting in a more amorphous structure.

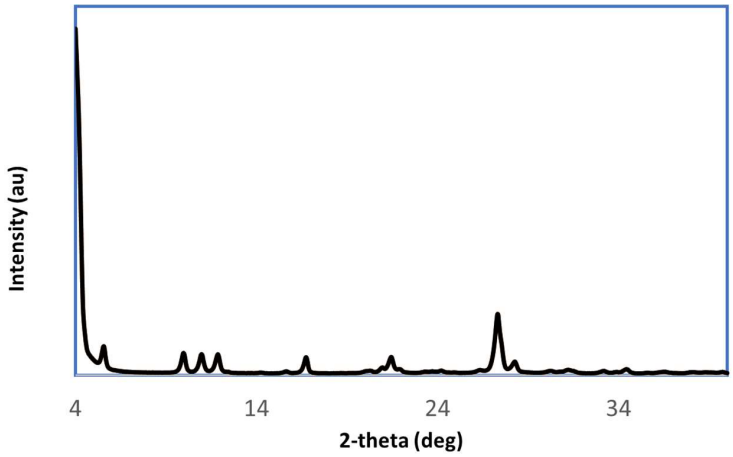

Fig. 3. PXRD Spectra of ZQC

4. 3. Antiglycation activity using BSA–MGO and glucose model systems

The antiglycation potential of ZQC was evaluated using BSA–MGO and BSA–glucose model systems. In the BSA–MGO system, all concentrations of ZQC (1, 5, and 10 µM) showed comparable inhibition, with the highest activity observed at 10 µM (9.14 ± 0.85%). Free quercetin exhibited slightly lower inhibition (8.70 ± 1.50%) at the same concentration, while aminoguanidine, used as a

In contrast, the BSA–glucose system incubated for 18 days revealed significant, dose-dependent antiglycation effects. ZQC displayed superior efficacy, with 53.45% and 43.04% inhibition at 10 µM and 5 µM, respectively, compared to 33.42% and 28.72% for free quercetin (*$p < 0.05$). Even at 1 µM, ZQC outperformed aminoguanidine, which only showed 16.61% inhibition. These results indicate that zinc complexation enhances quercetin's antiglycation activity, particularly in early-stage models like BSA–glucose.

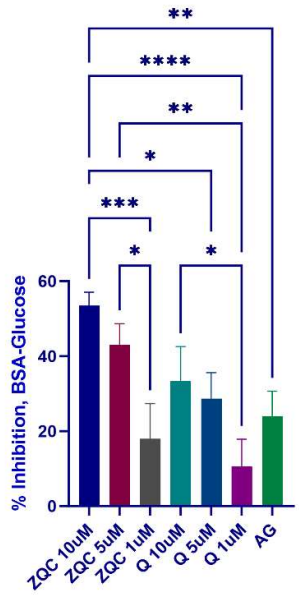

Fig. 5. Inhibitory activities of the different concentrations of ZQC and quercetin in BSA-glucose model system (*$p < 0.05$, Tukey's test)

4. 4. DNA methylation imaging

To assess the effects of ZQC and quercetin on DNA methylation, a fluorescence-based live-cell imaging system was employed using a methylation-specific

probe (YOCNB), developed by Dr. Yuichiro Hori [14]. This probe selectively binds to the HA-PYP3R-MBD module, which targets methylated CpG regions in genomic DNA.

In HEK293T cells (Fig. 5, left), no fluorescence was observed in the absence of the HA-PYP3R-MBD module (Fig. 7, *a*), confirming the specificity of the detection system. Cells transfected with the probe but left untreated (Fig. 5, *b*) showed strong nuclear fluorescence. Treatment with the zinc-quercetin complex (10 µM, Fig. 5, *c*) led to a noticeable reduction in fluorescence, suggesting potential DNMT1 inhibition and decreased methylation. In contrast, free quercetin (10 µM, Fig. 5, *d*) had little effect, with fluorescence intensity similar to the control. Azacytidine (10 µM, Fig. 5, *e*) showed the strongest signal reduction, validating the assay's sensitivity.

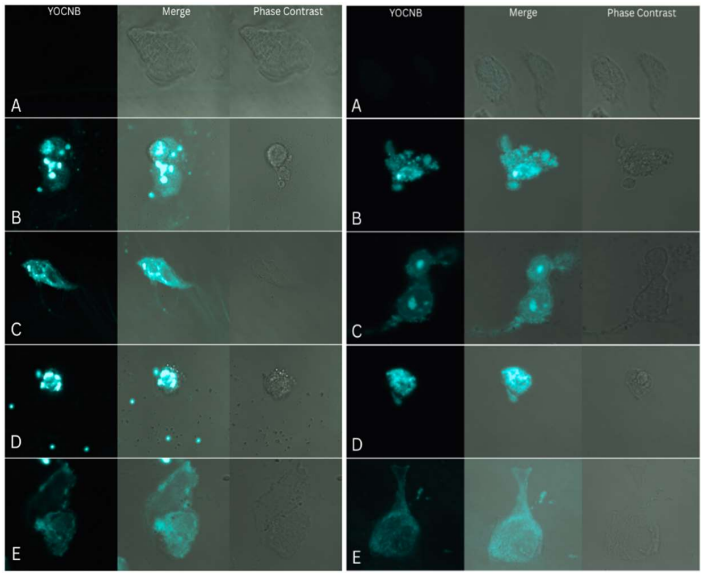

Fig. 5. Live cell imaging results on HEK293T Cells (left) and C2C12 cells (right) with different treatments:
A – without HA-PYP3R-MBD (112) module;
B – untreated (control); C – ZQC (10 uM);
D – quercetin (10 uM), e. azacytidine (10uM)

A similar trend was observed in C2C12 cells (Fig. 5, right),where ZQC treatment resulted in reduced fluorescence compared to quercetin. Although not as potent as azacytidine, ZQC consistently lowered methylation levels in both cell types. These results indicate that complexation with zinc enhances quercetin's epigenetic activity. The lack of effect from free quercetin may be due to insufficient concentration, as previous studies have reported that higher concentration is required to modulate epigenetic effects efficiently [15, 16]. Future studies with higher doses and extended exposure are warranted to explore ZQC's full potential.

4. 5. Molecular docking

Molecular docking analysis was carried out using the crystal structure of human DNA methyltransferase 1 (DNMT1; PDB ID: 4WXX), with a particular focus on its catalytic core region. The zinc–quercetin complex (ZQC) exhibited a significantly lower binding energy of –11 kcal/mol, compared to –8.1 kcal/mol for free quercetin. This suggests that ZQC forms a more stable and energetically favorable complex with DNMT1, indicating stronger binding affinity.

Table 2

Binding Affinities of ZQC and Quercetin

Compound	Binding Affinity (kcal/mol)
ZQC	–11
Quercetin	–8.1

Free quercetin interacted primarily through hydrogen bonds with key residues such as ARG1310 (3.07 Å), GLN1266 (2.39 Å), and ASN1267 (2.73 Å). It also exhibited π-cation and π-alkyl interactions with ARG1310 (4.82 Å) and VAL1268 (5.16 Å), as well as a pi-sulfur interaction with CYS1266 (5.84 Å). These interactions, while stabilizing, appeared limited in number and spatial distribution.

In contrast, ZQC formed a broader and more diverse set of interactions within the enzyme's active site. Notably, it engaged in hydrogen bonding with THR523 (2.48 Å and 2.49Å with the two catechol hydroxyls) and ARG1311 (2.57 Å) and showed a critical interaction with CYS1226 (2.49 Å), the catalytic residue responsible for initiating the methylation reaction. This direct interaction suggests that ZQC may effectively inhibit DNMT1 by blocking or interfering with the active site during the catalytic process.

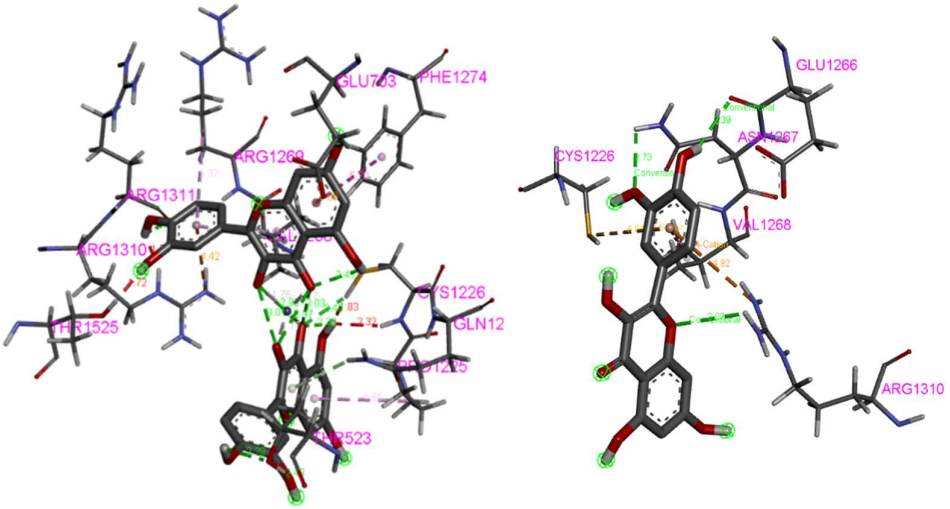

Fig. 6. Docking interactions of ZQC (left) and Quercetin (right) at the inhibition binding site of receptor protein (4WXX)

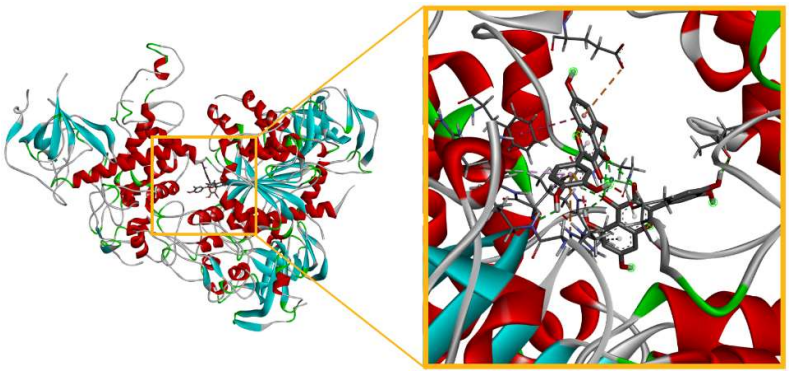

Fig. 7. ZQC – Protein binding complex at the catalytic site (hydrophobic core) of DNMT1 (PDB: 4WXX chain A)

Additionally, ZQC demonstrated enhanced binding stability through multiple π-alkyl interactions with VAL1268 (4.54 Å), PRO1225 (4.85 Å), and ARG1269 (5.37 Å), which likely contribute to the deeper insertion and stronger anchoring of the complex in the enzyme pocket. The incorporation of zinc may play a central role in modulating the electronic properties of the complex, further enhancing its structural rigidity and binding persistence.

Interestingly, a donor–donor interaction between ZQC and THR1525 (1.72 Å)/ARG1310 (1.74 Å) was also observed. While such interactions can introduce unfavourable electrostatic repulsion, the overall stability of the complex appeared to be preserved due to compensatory interactions with other residues, especially the catalytic CYS1226 and surrounding hydrophobic contacts. These results suggest that the complexation of quercetin with zinc not only improves its binding affinity to DNMT1 but may also enhance its potential as a catalytic inhibitor. The ability of ZQC to interact directly with the enzyme's active site, particularly the catalytic cysteine residue, supports its potential to modulate DNA methylation processes.

5. Discussion

The successful synthesis and characterization of the zinc–quercetin complex (ZQC) were validated through a combination of electronic, structural, and biochemical analyses, each contributing to a comprehensive understanding of its coordination behaviour and potential bioactivity. Visually, the shift in colour from light yellow (free quercetin) to dark orange upon zinc complexation served as a preliminary indication of a modified electronic environment, a result of ligand–metal interactions.

UV–Vis spectral data provided further insights into these interactions. The significant bathochromic shift of Band I from 364 nm to 432 nm in the ZQC, with minimal changes to Band II, suggests that the primary coordination site is the cinnamoyl system, particularly involving the 3-OH and 4-carbonyl groups on ring C. This is supported by the appearance of a new absorption peak at 380 nm, attributed to ligand-to-metal charge transfer (LMCT), indicating stable complex formation [12, 17–18]. These findings align with the computational results from density functional theory (DFT), which identified the 3-hydroxyl and 4-carbonyl groups as energetically favourable coordination sites for zinc, further reinforcing the suggested geometry [19]. The acidity of the 3-OH group, enhanced by the adjacent carbonyl, allows for deprotonation and efficient chelation, a mechanism supported by both LDA and GGA theoretical models.

FTIR spectra provided complementary structural confirmation. A shift in the C=O stretching frequency from 1610 cm^{-1} to 1590 cm^{-1} in the ZQC spectrum reflects electron redistribution due to coordination, while additional shifts in the C–O and C–O–C bands (1375 → 1248 cm^{-1} and 1165 → 1090 cm^{-1}, respectively) indicate structural changes in the flavonoid framework [12]. Moreover, the emergence of asymmetric C–O–metal stretching vibrations in the 1200–1330 cm^{-1} region, consistent with the findings of [20], and the appearance of a Co–O band around 604 cm^{-1}, provided direct spectroscopic evidence of complexation.

X-ray diffraction (XRD) further demonstrated that complexation altered the solid-state structure of quercetin. The disappearance of sharp crystalline peaks (e.g., at 10.66° and 12.34°) and the overall broadening of the diffraction pattern in the ZQC suggest a transition to a more amorphous or less ordered state [13, 21]. This structural transformation upon metal binding is common in coordination compounds and reflects disruptions in molecular packing and intermolecular forces [27, 28].

ZQC demonstrated promising antiglycation effects, outperforming free quercetin and the positive control, aminoguanidine, in both BSA–MGO and BSA–glucose models. Although the inhibition observed in the MGO model was modest (9.14% at 10 μM ZQC), the glucose model showed substantial inhibition (53.45% at 10 μM ZQC), underscoring the complex's superior efficacy during early glycation stages. The consistent performance of ZQC across concentrations and models suggests that complexation enhances quercetin's bioactivity by improving stability or increasing reactive site availability [22, 23].

Mechanistically, quercetin is known to trap dicarbonyl intermediates like methylglyoxal (MGO) via hydroxyl groups at positions C-6 and C-8 on ring A, preventing the formation of advanced glycation end-products (AGEs) [24]. Furthermore, quercetin's ability to stabilize serum albumin via hydrogen bonding interactions, as shown by [22], may also contribute to its antiglycation function. The improved performance of ZQC likely stems from its structure, which provides two quercetin moieties per zinc ion, thereby amplifying the number of active sites and enhancing protein protection.

The epigenetic potential of ZQC was also explored through live-cell fluorescence imaging. In both HEK293T and C2C12 cells, ZQC treatment resulted in a visible reduction in fluorescence intensity, suggesting interference with DNA methylation. The probe system used, developed by Dr. Yuichiro Horis, allowed for specific detection of methylated CpG sites [14]. The decrease in fluorescence following ZQC treatment implies a demethylating effect, potentially through inhibition of DNA methyltransfer-

ase 1 (DNMT1). In contrast, free quercetin did not significantly alter fluorescence, indicating that it lacks the capacity to modulate DNA methylation at the tested concentration (10 μM). This is consistent with studies by [15, 16], which reported epigenetic modulation by quercetin only at higher concentrations (25–250 μM), through mechanisms involving the downregulation of IL15 cytokines and modulating p53 and Bcl2 gene expression. The present findings suggest that zinc coordination enhances quercetin's epigenetic impact, potentially making ZQC a candidate for mild demethylating therapy.

Molecular docking studies further supported these results. Using the DNMT1 catalytic structure (PDB ID: 4WXX), the ZQC displayed a lower binding energy than free quercetin, suggesting stronger and more stable interactions with the enzyme's active site, particularly around the catalytic residue Cys1226 [25–28]. These computational results, combined with the imaging and in vitro assays, point to a plausible mechanism by which ZQC may inhibit DNA methylation – either directly through DNMT1 binding or indirectly by stabilizing DNA or interfering with methyl group donors.

Overall, the zinc-quercetin complex exhibits enhanced bioactivity over free quercetin in terms of both antiglycation and epigenetic modulation. This enhancement is attributed to structural changes induced by zinc coordination, improved molecular stability, and increased functional group availability. The findings suggest that ZQC holds promise for therapeutic application in AGE-related diseases and epigenetically dysregulated conditions. However, future studies involving longer incubation times, dose-response profiling, and mechanistic evaluations are essential to fully elucidate its therapeutic potential.

Practical relevance. Considering the improved bioactivity of the zinc–quercetin complex, our results indicate that ZQC may serve as a promising candidate for managing glycation-associated disorders and epigenetic conditions such as diabetes. Nonetheless, further investigation – particularly cytotoxicity assessments – is necessary to better understand its chemical properties and ensure its safety for biological applications.

Research limitations. This study acknowledges several limitations and encourages further research to build upon the current findings. One key recommendation is the assessment of the long-term stability of the zinc-quercetin complex under various physiological conditions to ensure its reliability as a therapeutic agent. Additional anti-glycation assays such as fructosamine determination, carbonyl content analysis, and mass spectrometry-based AGE detection could provide a more comprehensive understanding of the complex's inhibitory mechanisms. Prolonged incubation periods are strongly recommended to mimic chronic glycation processes that occur in vivo, thereby allowing a more accurate evaluation of the complex's protective effects. Testing higher concentrations is also essential to determine the threshold between therapeutic efficacy and potential cytotoxicity, especially for eventual clinical applications. Furthermore, this study was conducted in vitro, which may not fully capture the complexity of biological systems. Hence, in vivo studies and pharmacokinetic profiling are crucial to confirm the bioavailability, metabolism, and systemic safety of the complex.

Prospects for further research. The findings of this study open opportunities for further exploration using cell models with well-characterized epigenetic markers. Future research should also focus on detailed epigenetic profiling, particularly analyzing DNA methylation and histone modifications, to assess how the zinc–quercetin complex influences gene expression and its potential therapeutic role in metabolic disorders. Additionally, this study lays the groundwork for exploring complexes formed with other flavonoids or quercetin derivatives. These future directions aim to validate and expand upon the current results and may ultimately contribute to the development of a novel, safe, and effective therapeutic agent for conditions associated with glycation and epigenetic dysregulation.

6. Conclusion

The zinc–quercetin complex (ZQC) was successfully synthesized and thoroughly characterized using spectroscopic techniques and PXRD analysis. Antiglycation assays revealed that zinc complexation enhanced the bioactivity of quercetin, with ZQC exhibiting a greater inhibitory effect on AGE formation compared to the free ligand. In addition, experiments in HEK293T and C2C12 cell lines demonstrated that ZQC more effectively reduced DNA methylation levels than free quercetin. Supporting these biological findings, molecular docking studies indicated that ZQC interacts more strongly with DNMT1, as evidenced by a lower docking score (−11 kcal/mol) relative to quercetin (−8.1 kcal/mol), suggesting a more stable and potent binding affinity. Overall, these results indicate that zinc complexation not only improves the functional properties of quercetin but also enhances its therapeutic potential for addressing glycation- and epigenetics-related diseases. However, further studies are warranted to fully validate the complex's efficacy and safety within biological systems.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

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Data availability

Data will be made available on reasonable request.

Use of artificial intelligence

The authors affirm that artificial intelligence tools were utilized for proofreading and improving the manuscript's structure. All data collection and analyses were independently performed and verified by the authors.

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