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Type 2 diabetes mellitus–associated cancer risk: oxidative stress dysregulation and synthesis of antioxidant amide derivatives supported by molecular docking analysis: a case-control study

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Abstract

Introduction: Type 2 diabetes mellitus (T2DM) is increasingly associated with elevated cancer risk through interconnected biochemical disturbances, particularly oxidative stress dysregulation, chronic inflammation, and altered intracellular signaling. **Objective:** This study investigated cancer risk–related biochemical markers in women with T2DM and adopted an applied biological chemistry approach through the synthesis and evaluation of selected antioxidant amide derivatives supported by molecular docking analysis. **Methods:** Serum levels of Akt protein, C-reactive protein (CRP), malondialdehyde (MDA), and catalase (CAT) were assessed in diabetic patients and healthy controls. T2DM patients exhibited significant Akt hyperactivation, elevated MDA levels, reduced CAT activity, and increased CRP concentrations, indicating a pro-risk biochemical environment driven by redox imbalance and inflammation. **Results:** In parallel, antioxidant amide derivatives were synthesized, and two compounds were selected based on antioxidant screening. Their total antioxidant capacity was evaluated using the phosphomolybdate assay, and molecular docking analysis provided mechanistic insight into their interactions with oxidative stress–related protein targets. **Conclusion:** Overall, the findings highlight oxidative stress dysregulation as a central biochemical link between T2DM and increased cancer risk and support the applied evaluation of antioxidant amide derivatives as potential biochemical candidates for oxidative stress modulation.

Keywords: Type 2 diabetes mellitus. Cancer risk. Oxidative stress dysregulation. Akt signaling. Molecular docking. Applied biological chemistry.

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by persistent hyperglycemia, insulin resistance, and progressive biochemical dysregulation. Beyond its classical metabolic and cardiovascular complications, accumulating evidence indicates that T2DM is associated with an increased risk of cancer development through shared biochemical disturbances rather than direct oncogenic mechanisms [1–3]. This association has attracted increasing attention from an applied biological chemistry perspective, particularly in relation to oxidative stress imbalance and altered intracellular signaling. Oxidative stress represents a

central biochemical feature of T2DM. Chronic hyperglycemia enhances the generation of reactive oxygen species (ROS) through multiple pathways, including mitochondrial dysfunction, glucose autoxidation, and activation of NADPH oxidases **[4,5]**.

When antioxidant defense systems are insufficient, excessive ROS induce lipid peroxidation, protein oxidation, and DNA damage. Malondialdehyde (MDA), a stable end-product of lipid peroxidation, is widely used as a biochemical marker of oxidative injury and has been consistently reported to be elevated in diabetic patients **[6,7]**. In contrast, antioxidant enzymes such as catalase (CAT) play a crucial role in maintaining redox homeostasis by decomposing hydrogen peroxide, and reduced CAT activity has been linked to enhanced oxidative damage and disease susceptibility **[8]**.

In parallel, dysregulation of intracellular signaling pathways further contributes to diabetes-associated cancer risk. The phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) pathway is a key regulator of cellular metabolism, survival, and stress responses. Hyperactivation of Akt signaling has been reported in T2DM and is closely associated with insulin resistance, redox imbalance, and altered apoptotic regulation **[9,10]**. Importantly, Akt signaling is functionally interconnected with oxidative stress and inflammatory pathways, suggesting a biochemical environment that may favor carcinogenic susceptibility rather than overt malignancy **[11]**.

Chronic low-grade inflammation is another hallmark of T2DM that synergizes with oxidative stress. C-reactive protein (CRP), a sensitive biomarker of systemic inflammation, is frequently elevated in diabetic individuals and has been associated with increased cancer incidence and risk in epidemiological studies **[12,13]**. Together, oxidative stress dysregulation, Akt hyperactivation, and persistent inflammation form an integrated biochemical profile that may predispose diabetic patients to cancer development. From an applied biological chemistry standpoint, antioxidant-based strategies have emerged as promising approaches for modulating oxidative stress–related disease mechanisms. Several studies published in the *Journal of Applied Biological Chemistry* have highlighted the relevance of natural and synthetic antioxidant compounds in restoring redox balance and mitigating disease-associated biochemical alterations **[14–16]**.

Among these, amide-containing compounds represent an important class of bioactive molecules due to their structural stability, hydrogen-bonding capacity, and broad biological applicability **[17]**. The synthesis of antioxidant amide derivatives therefore constitutes a rational applied approach for investigating potential biochemical interventions targeting oxidative stress. In addition to experimental antioxidant evaluation, molecular docking analysis has become a valuable computational tool in applied biological chemistry, providing mechanistic insight into ligand–protein interactions and supporting structure–function relationships **[18]**.

Docking studies can complement biochemical assays by predicting binding affinity and interaction modes with oxidative stress–related protein targets, thereby strengthening the interpretation of antioxidant activity. Accordingly, the present study investigates cancer risk– associated biochemical markers in women with T2DM, with particular emphasis on oxidative stress dysregulation, Akt signaling, and inflammatory status. In parallel, selected antioxidant amide derivatives were synthesized and evaluated for their antioxidant capacity, supported by molecular docking analysis.

By integrating clinical biochemical assessment with applied synthetic and computational approaches, this work aimed to provide a coherent biochemical framework linking T2DM-associated oxidative stress to increased cancer risk and to explore antioxidant amide derivatives as potential biochemical candidates for oxidative stress modulation.

Materials and Methods

Study Design and Ethical Considerations

A case-control study was conducted to evaluate biochemical markers associated with cancer risk in women diagnosed with Type 2 diabetes mellitus (T2DM). The study protocol was approved by the relevant institutional and clinical authorities, and all participants provided informed consent prior to sample collection. The study was performed in accordance with ethical standards for research involving human subjects and followed STROBE rules.

Study Population

The study included a total of 40 female participants divided into two groups:

- **Control group (G1):** 20 apparently healthy females
- **T2DM group (G2):** 20 females diagnosed with Type 2 diabetes mellitus

Participants were recruited from outpatient clinics and Al-Diwaniyah Teaching Hospital. Subjects with acute infections, chronic inflammatory diseases other than diabetes, or known malignancies were excluded to avoid confounding effects.

Sample Collection and Serum Preparation

Venous blood samples (5 mL) were collected aseptically from each participant using gel tubes. Samples were allowed to clot and then centrifuged at 3000 × g for 10 minutes to separate serum. The obtained serum samples were aliquoted into sterile Eppendorf tubes and stored at −20 °C until biochemical analysis.

Determination of Serum Akt Protein

Serum Akt levels were quantified using a sandwich enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions. Briefly, serum samples were added to microplate wells pre-coated with anti-Akt antibodies, followed by incubation with horseradish peroxidase–conjugated secondary antibodies. The enzymatic reaction with tetramethylbenzidine (TMB) substrate produced a colored product, which was measured at 450 nm using a microplate reader. Akt concentrations were calculated from a standard calibration curve.

Assessment of Oxidative Stress Markers

Serum Malondialdehyde (MDA)

Serum malondialdehyde levels were determined as an indicator of lipid peroxidation using the thiobarbituric acid reactive substances (TBARS) method. Serum samples were mixed with thiobarbituric acid and trichloroacetic acid reagents and heated at 95 °C for 15 minutes. After cooling and centrifugation, the absorbance of the resulting chromogen was measured at 532 nm. MDA concentrations were calculated using the appropriate molar extinction coefficient.

Serum Catalase (CAT) Activity

Catalase activity was determined spectrophotometrically by measuring the rate of hydrogen peroxide decomposition at 240 nm. The reaction mixture consisted of serum diluted in phosphate buffer (pH 7.0) and hydrogen peroxide solution. Enzyme activity was expressed in units per milliliter (U/mL) based on the decrease in absorbance per minute.

Determination of Serum C-Reactive Protein (CRP)

Serum CRP levels were measured using a fluorescence-based immunoassay according to the manufacturer's protocol. The assay is based on a sandwich immunodetection principle, and fluorescence intensity was measured using a dedicated reader. CRP concentrations were expressed in mg/L.

Synthesis of Antioxidant Amide Derivatives

A series of amide derivatives were synthesized by coupling carboxyl-containing non-steroidal anti-inflammatory drugs with an aniline derivative using dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) as coupling reagents. The reaction was carried out in dichloromethane under controlled temperature conditions. The reaction progress was monitored by thin-layer chromatography (TLC). The synthesized compounds were purified and characterized using Fourier-transform infrared spectroscopy (FT-IR), proton nuclear magnetic resonance (^{1}H-NMR), and carbon-13 nuclear magnetic resonance (^{13}C-NMR). Based on antioxidant screening results, Compounds 2 and 8 were selected for further evaluation.

Evaluation of Antioxidant Activity

The total antioxidant capacity of the selected amide derivatives (Compounds 3 and 4) was assessed using the phosphomolybdate assay. Briefly, the compounds were mixed with a reagent solution containing ammonium molybdate, sodium phosphate, and sulfuric acid, followed by incubation at 95 °C for 90 minutes. After cooling, absorbance was measured at 765 nm. Ascorbic acid was used as a reference standard, and antioxidant activity was expressed as percentage inhibition relative to the control.

Molecular Docking Analysis

Molecular docking studies were performed to investigate the interaction of the selected amide derivatives with oxidative stress–related protein targets. The three-dimensional crystal structure of the target protein (PDB ID: 2X08) was obtained from the Protein Data Bank. Ligand structures were optimized and docked into the active site of the protein using appropriate docking software. Binding energies and interaction modes, including hydrogen bonding and hydrophobic interactions, were analyzed to support the antioxidant relevance of the synthesized compounds.

Synthesis of Antioxidant Amide Derivatives

General Synthetic Strategy

In this study, a series of amide derivatives were synthesized by coupling selected nonsteroidal anti-inflammatory drug (NSAID) carboxylic acid precursors with an aniline derivative using dicyclohexylcarbodiimide (DCC) as a coupling agent and 4-dimethylaminopyridine (DMAP) as a catalytic activator. The reactions were carried out at room temperature under mild conditions to facilitate efficient amide bond formation. The progress of the coupling

reactions was monitored by thin-layer chromatography (TLC), and the developed chromatograms were visualized using iodine vapor and ultraviolet (UV) light. The general reaction pathway for amide formation is illustrated in Figure 1, which outlines the coupling mechanism between the carboxylic acid and aniline moieties.

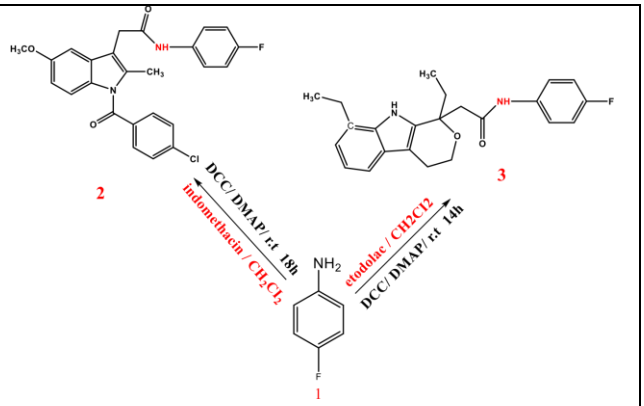

Figure 1. General synthetic route for amide derivative formation. Source: Own authorship.

Typical Synthetic Procedure

The carboxylic acid precursor (1.0 mmol) was dissolved in anhydrous DCM (15 mL) and cooled to 0 °C. DCC (1.1 mmol) and DMAP (0.1 mmol) were added, followed by dropwise addition of the aniline derivative (1.0 mmol). The reaction mixture was stirred initially at 0 °C and then allowed to reach room temperature, where it was maintained for 12–18 h. Reaction progress was monitored by thin-layer chromatography (TLC). After completion, the precipitated dicyclohexylurea (DCU) by-product was removed by filtration. The filtrate was concentrated under reduced pressure, and the crude product was purified by column chromatography to afford the desired amide derivative in solid form.

Phosphomolybdate Assay (Total Antioxidant Capacity)

The total antioxidant capacity of the selected amide derivatives was evaluated using the phosphomolybdate assay, with ascorbic acid employed as a reference standard. The assay is based on the reduction of molybdenum (VI) to molybdenum (V) by antioxidant compounds, resulting in the formation of a green phosphate/molybdenum (V) complex under acidic conditions. The reagent solution was prepared by mixing 28 mM sodium phosphate, 4 mM ammonium molybdate, and **0**.6 M sulfuric acid. An aliquot of 1 mL of the sample solution was combined with the reagent mixture in a test tube. The reaction mixture was incubated in a water bath at 95 °C for 90 minutes. After incubation, the tubes were allowed to cool to room temperature. The absorbance of each sample was measured at 765 nm against a blank using a UV–visible spectrophotometer. A control solution containing the reagent mixture and solvent without the test compound was prepared following the same procedure. Ascorbic acid (vitamin C) was used as a positive control under identical experimental conditions.

The total antioxidant capacity was expressed as percentage inhibition and calculated according to the following equation:

$$100 \times \frac{({}_{sample}Abs_{control} - Abs)}{{}_{control}Abs} = (\%)\ \text{Antioxidant effect}$$

Spectral Characterization

2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)-N-(4-fluorophenyl)acetamide2

Prepare derivative 2 according to the general method after stirring a mixture of one of the aniline derivatives with (indomethacin) for 21 hours. (Yield 83%) *M.P*: 222-224°C.*Rf* = 0.6. *IR (KBrcm^{-1})* of derivative (2): 3327 (NHamid) and 1699 (C=O)¸ and 1656 (C=O amid) *^{1}H.NMR (DMSO-d6, ppm)* δ 8.10 (s, 1H¸NH)¸2.57 (s¸3H¸CH_3)¸ 4.19 (sCH2-H1)¸ 4.21 (s,OCH3) 6.36-8.05(d¸14H¸H-arom) *^{13}C.NMR (DMSO-d6,ppm)*:δ161.79(C=O(Cabonyl))¸161.64(C=O amid) 25.50(CH_3- aliphatic) 31.91(CH_2- aliphatic) 51.42(OCH_3) 110.44-158.20(C- arom) (Figures 2-4).

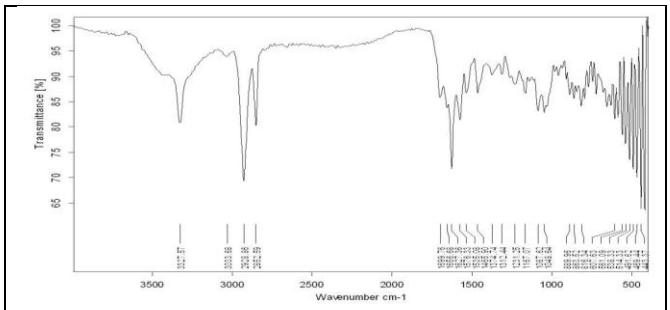

Figure 2. FT-IR spectrum of derivative 2. Source: Own authorship.

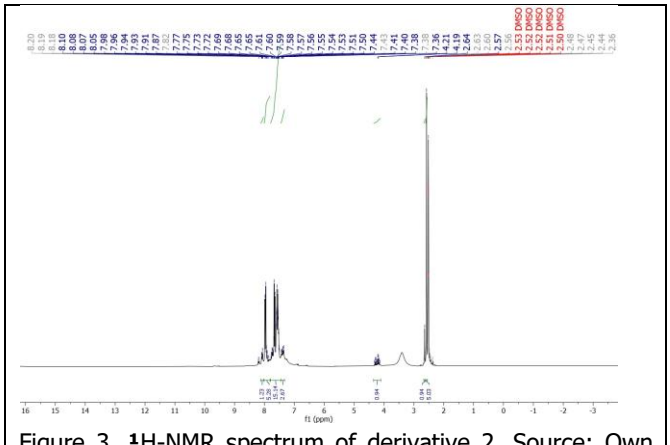

Figure 3. ^{1}H-NMR spectrum of derivative 2. Source: Own authorship.

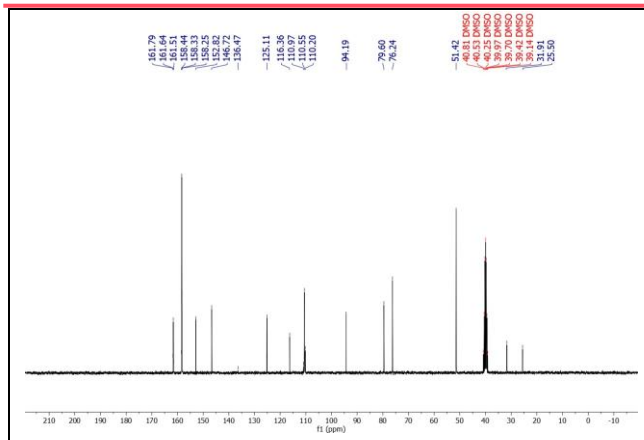

Figure 4. ^{13}C-NMR spectrum of derivative 2. Source: Own authorship.

Synthesis

2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)-N-(4-fluorophenyl)acetamide3

Prepare derivative 3 according to the general method after stirring a mixture of one of the aniline derivatives with (etodolac) for 21 hours (Yield 75%) *M .P*: 211-213°C *Rf* = 0.56: *IR (KBr¸ cm^{-1})* of derivative(2) : 3322(NH) and 1676(C=O" amid) *^{1}H.NMR (DMSO-d6, ppm)* 10.37 (s, 1H¸NHamid)¸ 8.40(s, 1H¸NH) (t,CH2-CH$_3$)¸ 2.38¸ (t,CH$_3$) 2.40, (q,CH$_2$)2.54 (s,CH2) 2.96, (t,CH2) 2.99 6.58-8.39(d¸9H¸H-arom) *^{13}C .NMR (DMSO-d6,ppm)*: δ 165.34 (C=O(amid))¸ 116.98-156.26(C- arom) (Figures 5 and 6).

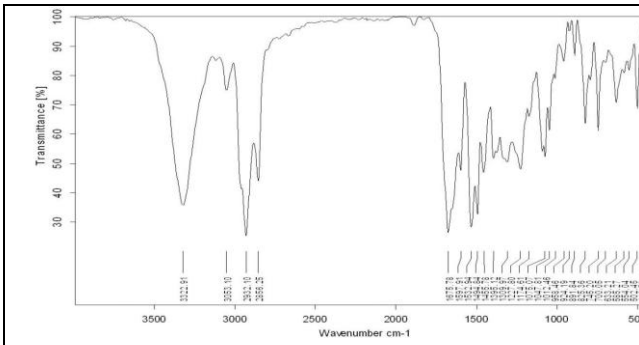

Figure 5. FT-IR spectrum of derivative 3. Source: Own authorship.

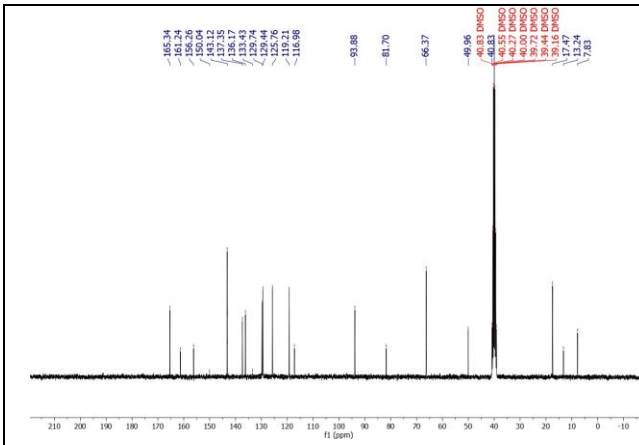

Figure 6. ^{13}C-NMR spectrum of derivative 3. Source: Own authorship.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism software. Data were expressed as mean ± standard error of the mean (SEM). Differences between groups were evaluated using an unpaired Student's t-test. A p-value ≤ 0.05 was considered statistically significant.

Ethical Approval and Safety Considerations

The study protocol was reviewed and approved by the Institutional Ethics Committee of Al-Mustaqbal University, Hilla City, Babylon Governorate, Iraq. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki (revised on 2024). All biochemical analyses involving human serum samples were conducted in accordance with institutional biosafety guidelines. Laboratory procedures involving chemical synthesis of antioxidant amide derivatives were performed under controlled conditions, following standard chemical safety protocols. Appropriate personal protective equipment (PPE) was used at all times, and chemical and biological waste was disposed of according to approved safety regulations. Molecular docking analysis was carried out using validated computational tools and did not involve any additional ethical or safety concerns. The datasets generated and analyzed during this study, including biochemical marker measurements (Akt, CRP, MDA, and CAT), antioxidant assay results, and molecular docking data, are available from the corresponding author upon reasonable request. Data will be shared for research purposes in accordance with institutional ethical guidelines and data protection regulations.

Result and Discussion

Alteration of Akt Signaling in T2DM Patients

Evaluation of serum Akt protein levels revealed a marked alteration in intracellular signaling associated with Type 2 diabetes mellitus. Women with T2DM showed a pronounced elevation in Akt levels compared to healthy controls, indicating hyperactivation of Akt signaling linked to metabolic and redox imbalance. Quantitatively, serum Akt concentrations increased from 46.74 ± 11.42 ng/mL in the control group to 98.40 ± 11.42 ng/mL in the T2DM group, and this difference was statistically highly significant (p = 0.0002) (Figure 7).

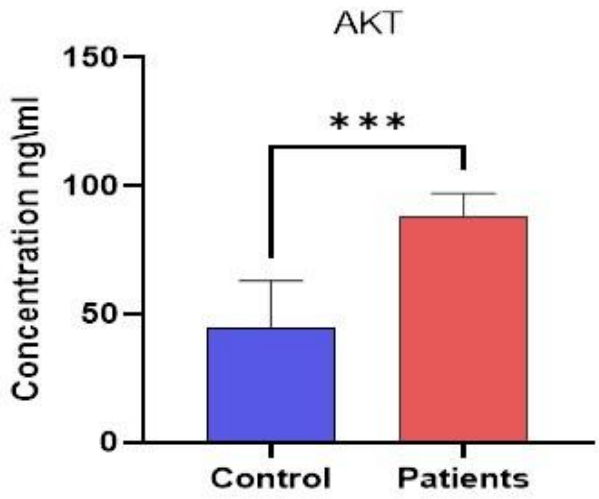

Figure 7. AKT expression levels in patients with diabetes and control groups. AKT levels was measured by ELIZA kit. Data are expressed as means ±SEM,*indicates significant differences compared to the control, p≤0.05. Source: Own authorship.

The significant elevation of serum Akt levels observed in women with T2DM indicates a marked dysregulation of intracellular signaling pathways associated with metabolic and redox imbalance. Akt (protein kinase B) plays a central role in regulating glucose metabolism, cell survival, and oxidative stress responses. Previous studies have demonstrated that chronic hyperglycemia and insulin resistance can induce sustained activation of the PI3K/Akt pathway, partly through oxidative stress–mediated mechanisms **[19,20]**. Reactive oxygen species (ROS) are known to activate Akt either directly or via upstream kinases, resulting in prolonged pro-survival signaling **[21]**. Importantly, persistent Akt hyperactivation has been widely implicated in cancer-related processes, including enhanced cell survival, resistance to apoptosis, and metabolic reprogramming **[22]**. In breast cancer, aberrant Akt signaling is considered a key molecular event that promotes tumor initiation and progression **[23]**. Therefore, the elevated Akt levels detected in T2DM patients in this study may represent an early molecular alteration linking metabolic dysregulation to increased cancer susceptibility rather than overt malignancy.

Oxidative Stress Status in T2DM Patients

Lipid Peroxidation Assessed by Serum MDA

To assess oxidative damage, serum malondialdehyde levels were measured as an indicator of lipid peroxidation. The results demonstrated a clear elevation of MDA levels in diabetic patients compared with controls, reflecting enhanced oxidative stress in T2DM. Mean serum MDA concentrations increased from 0.03669 ± 0.00504 mM in controls to 0.07352 ± 0.00504 mM in T2DM patients, representing a highly significant difference ($p < 0.0001$) (Figure 8).

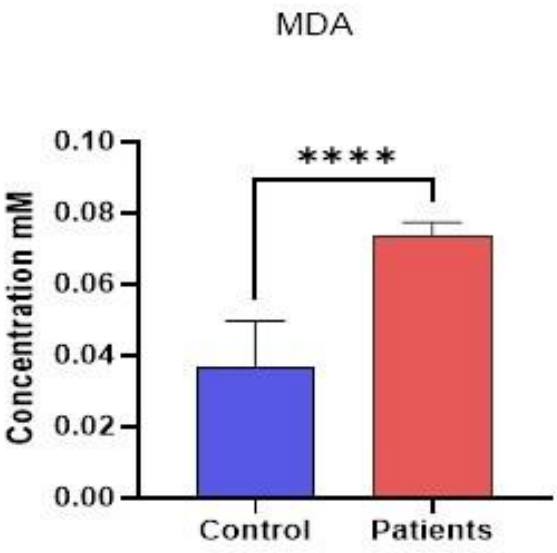

Figure 8. Serum MDA levels in patients with diabetes and control group. Serum samples were isolated from the blood of patients and control group. MDA activity was measured by colorimetric method. Data are expressed as means ± SEM. Source: Own authorship.

The marked increase in serum malondialdehyde (MDA) levels observed in T2DM patients confirms the presence of enhanced lipid peroxidation and systemic oxidative stress. MDA is a stable end-product of polyunsaturated fatty acid oxidation and is widely used as a reliable biomarker of oxidative damage. Hyperglycemia-induced ROS overproduction through mitochondrial dysfunction, glucose autoxidation, and activation of NADPH oxidases has been well documented in T2DM **[24,25]**. Elevated MDA levels have been associated with oxidative DNA damage and mutagenic processes that may contribute to carcinogenic susceptibility **[26]**. Lipid peroxidation products such as MDA can form adducts with DNA and proteins, thereby amplifying cellular injury and genomic instability **[27]**. The significant elevation of MDA detected in the present study supports the concept that T2DM is accompanied by a pro-oxidant environment capable of promoting long-term pathological consequences.

Antioxidant Defense Reflected by Catalase Activity

In parallel with increased lipid peroxidation, antioxidant defense capacity was markedly impaired in the diabetic group. Serum catalase activity was significantly reduced in T2DM patients compared to healthy subjects, indicating compromised enzymatic

antioxidant protection. Catalase activity decreased from 0.0719 ± 0.0041 mM in the control group to 0.0371 ± 0.0041 mM in diabetic patients, with a statistically significant difference ($p = 0.0001$) (Figure 9).

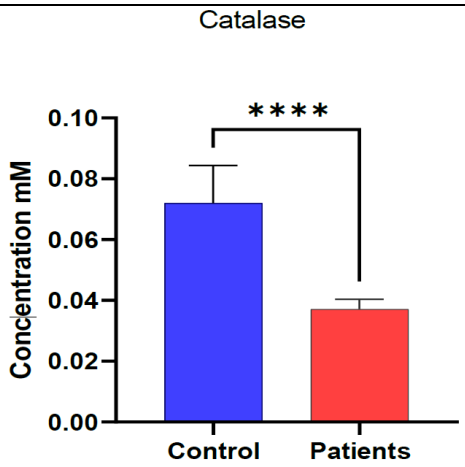

Figure 9. Serum CAT activity in patients with diabetes and control group. Serum samples were isolated from the blood of patients and control group. CAT activity was measured by colorimetric method. Data are expressed as means ± SEM. *indicates significant differences compared to the control, p≤0.05. Source: Own authorship.

In parallel with increased lipid peroxidation, a significant reduction in serum catalase activity was observed in T2DM patients, indicating compromised antioxidant defense capacity. Catalase plays a critical role in detoxifying hydrogen peroxide and maintaining redox homeostasis. Reduced catalase activity facilitates hydrogen peroxide accumulation, which can further propagate oxidative damage through the generation of highly reactive hydroxyl radicals **[28]**. Previous studies have reported depletion or inactivation of antioxidant enzymes in T2DM as a consequence of chronic oxidative stress **[29]**. The coexistence of elevated MDA and reduced catalase activity observed in this study reflects a severe redox imbalance characterized by excessive oxidant production and insufficient antioxidant protection. Such conditions are particularly relevant to carcinogenesis, as persistent oxidative stress promotes DNA damage, epigenetic alterations, and activation of redox-sensitive oncogenic pathways **[30]**.

Inflammatory Status Assessed by Serum CRP

Systemic inflammatory status was evaluated by measuring serum C-reactive protein levels. T2DM patients exhibited a substantial increase in CRP concentrations compared to controls, consistent with chronic low-grade inflammation associated with metabolic dysregulation. Mean CRP levels rose from 1.9 ± 1.17 mg/L in controls to 7.2 ± 1.17 mg/L in T2DM patients, and this increase was statistically significant ($p < 0.0001$) (Figure 10).

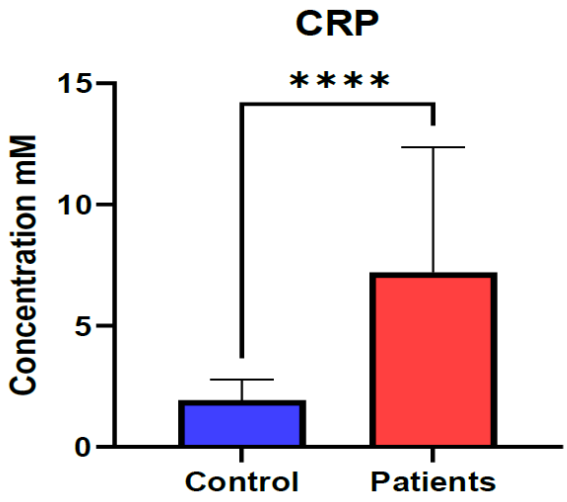

Figure 10. CRP in patients with diabetes and control group. Serum samples were isolated from the blood of patients and control group. CRP was measured by colorimetric method. Data are expressed as means ± SEM. ****indicates significant differences compared to the control, $P < 0.0001$. Source: Own authorship.

The significant elevation of serum C-reactive protein (CRP) levels in T2DM patients indicates the presence of chronic low-grade systemic inflammation. CRP is not only a marker of inflammation but also an active mediator linking inflammatory signaling with oxidative stress. In T2DM, hyperglycemia and oxidative stress activate inflammatory pathways that stimulate hepatic CRP production **[31]**. Chronic inflammation and oxidative stress are tightly interconnected; inflammatory mediators enhance ROS generation, while oxidative stress further amplifies inflammatory responses **[32]**. Sustained low-grade inflammation has been implicated in cancer development through its effects on genomic instability, angiogenesis, and tumor-promoting microenvironments **[33]**. Therefore, elevated CRP levels observed in this study further support the presence of a biochemical milieu that may favor cancer susceptibility in T2DM patients.

Integrated Biochemical Profile of Cancer Risk–Related Markers

When all measured parameters were evaluated collectively, a consistent biochemical profile emerged in the T2DM group, characterized by Akt hyperactivation, elevated oxidative stress (increased MDA), weakened antioxidant defense (reduced catalase activity), and enhanced inflammatory status (elevated CRP). This integrated pattern highlights a coordinated disturbance of redox balance and signaling pathways associated with increased cancer risk in diabetic patients (Figure 11).

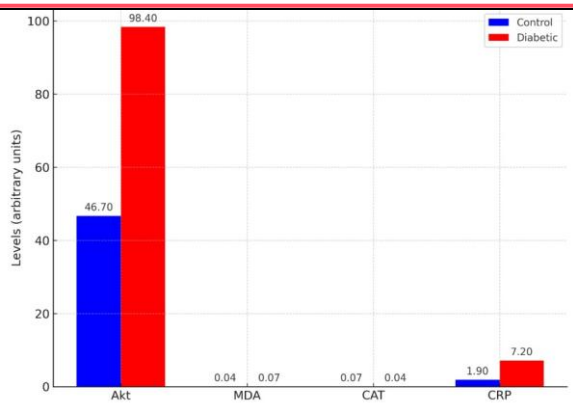

Figure 11. Comparison for all markers between patients and control group. Source: Own authorship.

When evaluated collectively, the simultaneous presence of Akt hyperactivation, elevated MDA levels, reduced catalase activity, and increased CRP concentrations outlines a coherent biochemical profile in T2DM patients. This integrated pattern reflects a coordinated disturbance involving redox imbalance, inflammatory activation, and pro-survival signaling. The convergence of oxidative stress and Akt signaling is particularly important, as ROS can activate Akt, while Akt activation promotes resistance to apoptosis and supports survival of oxidatively damaged cells **[34]**. This redox–signaling interplay may facilitate the accumulation of genetic and epigenetic alterations over time, thereby increasing susceptibility to cancer development. Epidemiological studies have reported an association between T2DM and increased risk of several cancers, including breast cancer, supporting the biological plausibility of this integrated biochemical profile **[35]**.

Antioxidant Activity of Selected Amide Derivatives

The antioxidant potential of the synthesized amide derivatives was evaluated using the phosphomolybdate assay, and Compounds 3 and 4 were selected based on their pronounced activity. Both compounds demonstrated strong total antioxidant capacity at the tested concentration (250 µM). Compound 3 exhibited a slightly higher antioxidant inhibition percentage (~82%) compared to Compound 4 (~78%), indicating robust antioxidant performance for both derivatives (Table 1).

Table 1. The antioxidant findings for the produced compounds 2a-d were calculated using the phosphomolybdate technique.

Concentration 250 µM		
Compounds	Absorbance of Samples	Conic of Samples
3	0.201± 0.003	30.452
4	0.200± 0.004	30.300
Ascorbic - acid	0.598	--------

Source: Own authorship.

The synthesized amide derivatives (Compounds 3 and 4) demonstrated strong total antioxidant capacity as determined by the phosphomolybdate assay. This assay evaluates the overall reducing capacity of compounds and is widely used as an indicator of antioxidant potential. The high inhibition percentages observed for both compounds indicate effective electron-donating ability and redox-modulating potential **[36]**.

From a structural perspective, the amide functional group contributes to molecular stability and hydrogen-bonding capacity, while aromatic moieties may facilitate electron delocalization, enhancing antioxidant behavior **[37]**. The slightly higher antioxidant activity observed for Compound 3 may be attributed to substituent effects influencing redox reactivity and molecular interactions. These findings support the rationale of designing amide-based scaffolds as antioxidant candidates targeting oxidative stress-related disorders.

Molecular Docking Analysis

To provide mechanistic support for the antioxidant activity, molecular docking analysis was performed against the oxidative stress–related protein target (PDB ID: 2X08). Both Compounds 3 and 4 showed favorable binding orientations within the active site of the protein. Compound 3 displayed a stronger predicted binding affinity (S-score = −8.11 kcal/mol) compared to Compound 4 (S-score ≈ −7.6 kcal/mol), forming stable hydrogen bonding and hydrophobic interactions. These interactions suggest a plausible molecular basis for the observed antioxidant activity (Table 2 and Figure 12) **[38,39]**.

Table 2. Remaining binding sites are utilized as an input to generate the receptor grid during Induced Fit Docking.

Receptors	**Sites**	Residues
2x08	**Sites 1**	(ALA36 ASP37 ILE40 GLY41 TYR42 PRO44 VAL45 VAL47 ARG48 TRP51 HIS52 PRO80 SER81 ALA83 GLY84 LEU85 ASN87 LEU144 PRO145 ASP146 ALA147 ASP148 LYS149 ASP150 TYR153 VAL154 PHE158 LEU171 MET172 ALA174 HIS175 LEU177 GLY178 LYS179 THR180 HIS181 LEU182 LYS183 ARG184 SER185 GLY186 TYR187 PHE191 LEU213 LYS215 ASN220 GLU221 TRP223 LEU232 PRO233 THR234 ASP235 TYR236 SER237 GLN240 PHE262 PHE266 LEU269)1:(PRO129¸ ARG130¸ ASP131¸ ARG250¸ GLY253¸ LYS254¸ HIS255¸ ASP256¸ ARG258¸ TRP260¸ GLN310¸ GLY313¸ ALA495¸ THR498¸ HIS499¸ VAL500¸ GLN502)
	Sites 2	(ALA27 LEU30 ARG31 ASP33 ASP34 TYR39 ILE40 GLY41 TYR42 GLY43 PRO44 GLN117 GLU118 MET119 GLN120 GLY121 PRO122 LYS123 ASN196 THR288 LEU289 GLU290 GLU291 LEU294)

Source: Own authorship.

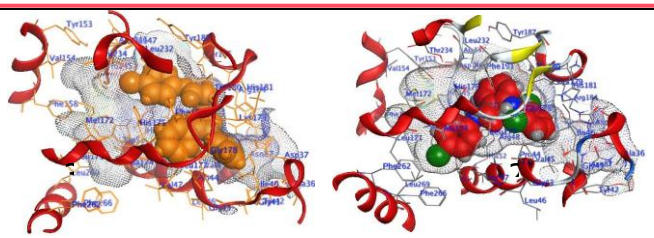

Figure 12. Display the structural forms for the 3,2 simulations involving the active site of cytochrome c peroxidase enzyme. Source: Own authorship.

The docking scores and key interactions of the selected compounds with the target protein are summarized in Table 3.

Table 3. The information derived from the prepared with 2X08 docking at site 1.

Compound	S score (Kcal/mol)	RMSD (Å)	Atom of compound	Atom of receptor	Involved Receptor residues	Type of Interaction bond	Distance (Å)	E (kcal/mol)
2	-7.11	1.80	Cl-8 N-17 C-13 N-19	O NE2 CA 6-ring	LEU177 HIS 175 PHE191 TRP 51	H-donor H-donor H-acceptor pi-pi	2.82	-1.8
3	-8.11	1.51	6-ring C-20 O-10 6-ring	NE2 NE2 CZ 5-ring	HIS 175 HIS 175 THR180 ARG48	H-donor H-donor pi-H pi-pi	3.66	-2.44

Source: Own authorship.

Molecular docking analysis provided mechanistic insight into the antioxidant activity of Compounds 3 and 4 by revealing favorable binding orientations and interactions with an oxidative stress–related protein target (PDB ID: 2X08). Compound 3 exhibited a stronger predicted binding affinity than Compound 4, which is consistent with its superior antioxidant performance observed experimentally. The formation of stable hydrogen bonding and hydrophobic interactions suggests that these compounds may effectively interact with redox-related protein sites, potentially modulating oxidative stress pathways. Although docking studies do not directly confirm biological efficacy **[40]**, they provide valuable structural support for experimental findings and strengthen structure–activity relationships in applied biological chemistry research.

Novelty and significance of the present study

The novelty of this work lies in integrating clinical biochemical evidence of oxidative stress, inflammation, and Akt signaling dysregulation in T2DM patients with an applied synthetic chemistry approach focused on the development of antioxidant amide derivatives. By demonstrating a consistent biochemical profile characterized by Akt hyperactivation, oxidative stress, and inflammatory activation, this study provides mechanistic insight into how T2DM may increase breast cancer susceptibility.

Furthermore, the synthesis and evaluation of antioxidant amide derivatives, supported by molecular docking analysis, introduce a rational chemical strategy aimed at attenuating oxidative stress–driven risk factors. Rather than claiming direct cancer prevention, this work supports the concept that antioxidant amide scaffolds may contribute to reducing oxidative stress and Akt-associated pro-oncogenic signaling, thereby lowering cancer susceptibility in high-risk metabolic conditions.

Conclusion

Overall, the findings highlight oxidative stress dysregulation as a central biochemical link between T2DM and increased cancer risk and support the applied evaluation of antioxidant amide derivatives as potential biochemical candidates for oxidative stress modulation.

CRediT

Conceptualization; Data Curating; Formal Analysis; and Investigation: Hamzah, Al-Abadi, Al-Silaykhee, Project Administration; Supervision; Writing - Original Draft: Hassan, Youssef , Alebady

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Ethical Approval

The study protocol was reviewed and approved by the Institutional Ethics Committee of Al-Mustaqbal University, Hilla City, Babylon Governorate, Iraq. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki (revised on 2024). All biochemical analyses involving human serum samples were conducted in accordance with institutional biosafety guidelines. Laboratory procedures involving chemical synthesis of antioxidant amide derivatives were performed under controlled conditions, following standard chemical safety protocols. Appropriate personal protective equipment (PPE) was used at all times, and chemical and biological waste was disposed of according to approved safety regulations. Molecular docking analysis was carried out using validated computational tools and did not involve any additional ethical or safety concerns.

Informed Consent

Informed consent was obtained from all participants involved in the study, with all procedures explained in detail before participation

Funding

Not applicable.

Data Sharing Statement

The datasets generated and analyzed during this study, including biochemical marker measurements (Akt, CRP, MDA, and CAT), antioxidant assay results, and molecular docking data, are available from the corresponding author upon reasonable request. Data will be shared for research purposes in accordance with institutional ethical guidelines and data protection regulations.

Conflict of Interest

The authors declare no competing interests.

Similarity Check

It was applied by Ithenticate[@].

Application of Artificial Intelligence (AI)

Not applicable.

Peer Review Process

It was performed.

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