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Design, Synthesis, Characterization, ADME Profiling, and Protective Role of Bis-Chalcones against Chlorpyrifos-induced Hepatorenal Damage in Male Albino Rats

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ABSTRACT

Background: Pesticides, particularly organophosphates (OPs) such as chlorpyrifos, pose major public health risks through environmental contamination, dietary residues, and occupational exposure, with metabolites widely detected in human biomonitoring. Chlorpyrifos induces hepatorenal toxicity primarily through hepatic cytochrome P450-mediated bioactivation to chlorpyrifos-oxon, leading to reactive oxygen species overproduction, oxidative stress, and organ injury.
Objectives: To evaluate the protective potential of synthesized bis-chalcone derivatives against chlorpyrifos-induced oxidative stress and hepatorenal damage in male albino rats.
Materials and methods: Three bis-chalcone derivatives (A1-A3) were synthesized by environmentally friendly Claisen-Schmidt condensation, characterized by proton nuclear magnetic resonance and Fourier-transform infrared spectroscopy, and assessed for purity by elemental microanalysis (CHNO). Oxidative stress, liver, renal, and lipid biomarkers were measured in 56 male albino rats assigned to eight groups (7 rats for each group), comprising one control group, three groups receiving bis-chalcones (A1-A3) alone, one chlorpyrifos group, and three groups pretreated with A1, A2, or A3 followed by chlorpyrifos (oral gavage, 14 days).
Results: Chlorpyrifos markedly increased thiobarbituric acid reactive substances (TBARS), liver enzymes, total bilirubin, urea, total cholesterol, and triglycerides, while decreasing reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), total protein (TP), and albumin (ALB). A1 pretreatment most effectively normalized these indices (e.g., TBARS 28.76 ± 2.18 nmol/L, GSH 14.92 ± 0.56 µmol/L, aspartate aminotransferase (AST) 78.4 ± 4.1 U/L, and alanine aminotransferase (ALT) 58.7 ± 3.2 U/L; all P-values < 0.05 vs. chlorpyrifos).
Conclusion: Bis-chalcone derivatives, particularly A1, exert strong protective effects against OPs toxicity in rats by restoring oxidative balance and preserving hepatocellular, renal, and metabolic functions.
Keywords: Bis-chalcones; Chlorpyrifos; Hepatorenal damage; Male albino rats (Rattus norvegicus); Oxidative stress.

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INTRODUCTION

Pesticides enhance agricultural productivity and crop storage but exert profound adverse effects on ecosystems, human health, and economies. Organophosphates (OPs), such as chlorpyrifos (O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) phosphoroth-

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ioate), remain one of the most widely detected OP pesticides in environmental samples, food residues, and human biomatrices, with metabolites detected in the urine of more than 90% of the US population, posing chronic low-dose exposure risks through dietary, occupational, and indoor contamination pathways. Its selection enables direct translation to real-world public health concerns, unlike less prevalent OPs [1–3].

Chlorpyrifos exerts toxicity through dual mechanisms: cytochrome P450, primarily cytochrome P450 3A4/2B6-mediated oxidative desulfuration to chlorpyrifos-oxon, a potent acetylcholine-shape inhibitor causing a cholinergic crisis; and oxon-driven reactive oxygen species (ROS) overproduction via redox cycling and lipid peroxidation, independent of cholinesterase inhibition. Polymorphics-oxon undergoes phase II detoxification via glutathione conjugation by glutathione S-transferases (GSTs) and hydrolysis by paraoxonase 1 (PON1), where polymorphic cytochrome P450 (CYP)/GST/PON1 variants explain interindividual toxicity susceptibility observed in human cohorts. These hepatic metabolic dynamics amplify hepatorenal oxidative stress with elevated levels of thiobarbituric acid-reactive substances (TBARS) and reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT), validating chlorpyrifos as the optimal model for bis-chalcone antioxidant intervention [4, 5].

Bis-chalcones represent symmetrical dimeric analogs of the core chalcone scaffold (1,3-diaryl-2-propen-1-one), featuring dual α,β-unsaturated carbonyl moieties linked through a central terephthalaldehyde-derived spacer that enhances their redox potential and biological activity. These structural modifications confer superior antioxidant, anti-inflammatory, antibacterial, and antiparasitic properties compared to monochalcones, making them particularly suitable for counteracting pesticide-induced oxidative hepatorenal damage [6].

The aim of this study was to synthesize three new bis-chalcone derivatives (A1-A3) via an eco-friendly Claisen-Schmidt condensation, to characterize them spectroscopically and confirm their purity, and to evaluate their protective efficacy and hypothesized attenuation of chlorpyrifos-induced oxidative stress, hepatorenal damage, protein disruption, and dyslipidemia biomarkers in male albino rats.

MATERIALS AND METHODS

Materials

P-Hydroxyacetophenone, p-aminoacetophenone, and terephthalaldehyde were obtained from HiMedia (India), and m-aminoacetophenone was obtained from Sigma-Aldrich (Germany). All reagents were of analytical grade. Melting points were determined using a Stuart Electrothermal Engineering Ltd. apparatus (England; up to 400 °C). Fourier-transform infrared (FT-IR) spectra were recorded on a Shimadzu FT-IR 8400 spectrophotometer (Japan; KBr discs). Elemental analysis (C, H, N, O) was performed on a Euro-vector EA3000A analyzer (Italy) at the Faculty of Pharmacy, University of Kufa, Iraq. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained on a Bruker 400 MHz spectrometer (DMSO-d_6, TMS) at the University of Basrah, Iraq. Thin-layer chromatography (TLC) was carried out on silica gel GF_{254} plates using petroleum spirit and ethyl acetate (7:3) as the mobile phase.

All biochemical assays utilized commercial diagnostic kits and standard laboratory apparatus sourced from established suppliers. Biochemical kits: TBARS/GSH (Sigma-Aldrich); SOD/CAT (Cayman); liver/renal/lipid parameters (Bio Systems S.A., Spectrum Diagnostics). Spectrophotometry: BioTek Synergy HTX.

ADMET properties analysis

SwissADME (http://www.swissadme.ch) assessed bis-chalcone ADME properties, blood-brain barrier permeability, P-glycoprotein interaction, and bioavailability to identify promising oral candidates [7]. Bis-chalcones A1-A3 showed excellent drug-like properties (Table 1), with full Lipinski's Rule of Five compliance (Ro5=0), optimal molecular weights/lipophilicity, high gastrointestinal absorption, and bioavailability scores (0.55). Derivatives A2 and A3 exhibited higher molar refractivity (suggesting stronger protein binding), while A1 showed parameters consistent with better membrane permeability, positioning all as viable oral candidates.

Chemical synthesis of new bis-chalcone derivatives (A1-A3)

Terephthalaldehyde (5 mmol, 0.67 g) and aromatic ketone [p-hydroxyacetophenone (1.36 g), p-aminoacetophenone (1.35 g), or m-aminoacetophenone (1.35 g); 10 mmol each] were dissolved in 20 mL of water with vigorous stirring in a preheated oil bath. Na_2CO_3 (2.5 mmol, 0.265 g) in 5 mL of water was added, and the mixture was refluxed at 100°C for 4 hours with magnetic stirring. Reaction progress was monitored by TLC [8]. The product was isolated by Büchner filtration after cooling, washed with H_2O (2 × 30 mL) and cold EtOH (2 × 10 mL), dried, and required no further purification (Figure 1).

The structures of the bis-chalcones were confirmed by ^{1}H NMR and FT-IR, and their purity was verified by CHNO microanalysis, with melting points supporting the characterization.

A1[(2*E*,2'*E*)-3,3'-(1,4-phenylene)bis(1-(4-hydroxyphenyl)prop-2-en-1-one)]: purple-red solid; yield 81%; mp 227-229°C; Rf 0.39; anal. calcd for $C_{24}H_{18}O_4$ (370.40): C, 77.82; H, 4.90; found: C, 76.55; H, 4.81; IR (KBr, cm^{-1}): 3360 (O–H str.), 3107 (aromatic/vinylic C–H str.), 2894 and 2804 (C–H str.), 1639 (α,β-unsat. C=O str.), 1597, 1498 and 1436 (Ar C=C str.), 1377 (C–H bend), and 1294 (Ar–O str.); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 10.14 (s, 2H, phenolic OH), 6.82–8.11 (m, 16 H, Ar-H, and olefinic protons).

A2[(2*E*,2'*E*)-3,3'-(1,4-phenylene)bis(1-(4-aminophenyl)prop-2-en-1-one)]: yellow solid; yield 78%; mp 274-276°C; Rf 0.39; anal. calcd for $C_{24}H_{20}N_2O_2$ (368.44): C, 78.24; H, 5.47; N, 7.60; found: C, 79.12; H, 5.53; N, 7.69; IR (KBr, cm^{-1}): 3469 and 3375 (NH_2 str.), 3103 (Ar/=C–H str.), 1683 (conj. C=O str.), 1600-1450 (Ar C=C str.), and 1176 (C–N str.); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 5.5 (br s, 4H, 2×NH_2), 6.8–7.9 (m, Ar–H), 7.3–7.8 (d, CH=CH, trans).

A3[(2*E*,2'*E*)-3,3'-(1,4-phenylene)bis(1-(3-aminophenyl)prop-2-en-1-one)]: yellowish-white solid; yield 69%; mp 282-284°C; Rf 0.31; anal. calcd for $C_{24}H_{20}N_2O_2$ (368.44): C, 78.24; H, 5.47; N, 7.60; found: C, 79.35; H, 5.44; N, 7.56; IR (KBr, cm^{-1}): 3450 and 3300 (NH_2 str.), 3110 (Ar/=C–H str.), 1660-1625 (conj. C=O str.), 1600-1450 (Ar C=C str.), and 1290-1180 (C–N str.); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 5.8 (br s, 4H, 2×NH_2), 7.2–7.8 (d, CH=CH, trans), 6.7–8.0 (m, Ar–H; complex due to meta-substitution).

Table 1. The output parameters of drug-likeness and ADME for the bis-chalcone derivatives (A1-A3) in the SwissADME platform*.

Parameters / Comp.	MW (g/mol)	nRB	nHBA	nHBD	MR (m^3/mol)	TPSA (Å^2)	iLOGP	GI absorption	BBB permeant	P-gp	BS	Ro5
A1	370.4	6	4	2	110.1	74.6	2.75	High	No	No	0.55	0
A2	368.43	6	2	2	114.86	86.18	2.69	High	No	No	0.55	0
A3	368.43	6	2	2	114.86	86.18	2.71	High	No	No	0.55	0

* Abbreviations: BS, bioavailability score; Comp., compound; GI, gastrointestinal absorption; iLOGP, image-based lipophilicity; MR, molar refractivity; MW, molecular weight; nHBA, number of hydrogen bond acceptors; nHBD, number of hydrogen bond donors; nRB, number of rotatable bonds; P-gp, P-glycoprotein; Ro5, Lipinski's Rule of Five violations; TPSA, topological polar surface area.

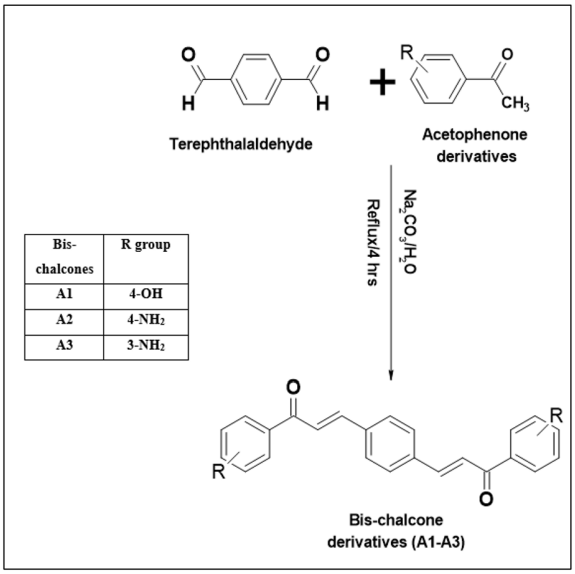

Figure 1. Scheme for synthesis of the bis-chalcone derivatives (A1-A3).

Evaluation of the protective effects of bis-chalcone derivatives against chlorpyrifos-induced oxidative stress and organ damage

Experimental animals

Fifty-six adult male albino rats (Rattus norvegicus, 8–10 weeks, 145–175 g) from a licensed breeder were acclimated for 14 days in polycarbonate cages (22 ± 2 °C, 55 ± 10% RH, 12:12 h light/dark) with ad libitum access to chow and water. The study protocol was approved by the University of Karbala Ethics Committee (2024An.027, December 9, 2024).

Study design

Eight groups (7 rats for each group), comprising one control group (water), three groups receiving bis-chalcones A1-A3 alone (50 mg/kg each), one chlorpyrifos group (10 mg/kg), and three groups pretreated with A1, A2, or A3 (50 mg/kg each, 30 min prior) followed by chlorpyrifos (10 mg/kg); all via oral gavage for 14 days. Doses were selected according to the literature [9–11].

Blood collection

On day 14, animals were anesthetized with ether, and blood (4-5 mL/animal) was collected by aortic puncture into heparinized tubes kept on ice, then centrifuged at 860 × g for 20 minutes to obtain plasma, which was aliquoted and stored at -80 °C for analysis.

Evaluation of oxidative stress markers and antioxidant parameters

Assessment of TBARS and GSH

The method of Esterbauer and Cheeseman (1990) was used to determine plasma TBARS levels [12], whereas the method of Ellman (1959) was used to estimate plasma GSH levels [13].

Assessment of antioxidant enzymes' activities

The method of Misra and Fridovich (1972) was used to determine plasma SOD (EC 1.15.1.1) activity [14]. The method of Aebi (1984) was used to measure plasma CAT (EC 1.11.1.6) activity [15].

Evaluation of liver function parameters

Using diagnostic kits from Bio Systems S.A. (Barcelona, Spain), we determined the serum activities of aspartate aminotransferase (AST) and alanine aminotransferase(ALT) according to the laboratory method described by Reitman and Frankel [16].

The assay for serum ALP activity was conducted with a commercial diagnostic kit (Spectrum, Hannover, Germany) by the phenyl phosphate method, which had been developed by Principato et al. (1985) [17]. The serum level of total bilirubin (TB) was estimated by the Walters and Gerade method according to the procedure outlined in the commercial diagnostic kit (SCICO Diagnostics, Egypt) [18].

Plasma total protein (TP) and albumin (ALB) levels were determined using commercial diagnostic kits (Bio Systems S.A., Barcelona, Spain) according to the methods described by Armstrong and Carr (1964) [19] and Doumas et al. (1977) [20], respectively.

Evaluation of renal function parameters

Plasma urea concentrations were determined using commercial diagnostic kits (Bio Systems S.A., Barcelona, Spain) according to the Berthelot method described by Patton and Crouch (1977) [21].

Evaluation of lipid profile

The commercial diagnostic kits of total cholesterol (TC; cat. no. 11805), triglycerides (TG; cat. 11828), and high-density lipoprotein-cholesterol (HDL-c; cat. 11557) were used

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for the estimation of plasma levels, according to the manufacturer's instructions(Bio Systems S.A., Barcelona, Spain) [22–24]. Very low-density lipoprotein cholesterol (vLDL-c) concentration was calculated as TG/5. Serum low-density lipoprotein cholesterol (LDL-c) levels were calculated using the Friedewald equation: LDL-c = TC-(HDL-c + vLDL-c) [25].

Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS) for Windows, version 17 (IBM Corporation, Armonk, NY, USA). Following a one-way analysis of variance (ANOVA), Tukey's multiple-comparison test was employed to assess the significance of observed differences [26], and statistical significance was set at $P < 0.05$.

RESULTS

Chemical characteristics of the bis-chalcone derivatives (A1-A3)

Bis-chalcone derivatives were synthesized via modified Claisen-Schmidt condensation of terephthalaldehyde with substituted acetophenones under aqueous basic conditions (Na_2CO_3, 100°C, 4 hours of reflux). Yields were 81% (A1), 78% (A2), and 69% (A3), with products precipitating cleanly upon cooling, no recrystallization needed. The progress of reactions was monitored by TLC ($R_f = 0.31$–0.39) using a mobile phase (petroleum spirit: ethyl acetate 7:3) and spectroscopic data consistent with symmetrical 1,4-phenylene structures.

A1 formed as a purple-red solid (mp 227–229°C); amino analogs A2 (p-NH_2, yellow, mp 274–276°C) and A3 (m-NH_2, yellowish-white, mp 282–284°C) showed increased thermal stability due to enhanced π–π stacking, H-bonding, and lattice interactions, with meta-substitution in A3 adding conformational flexibility.

FT-IR confirmed structures: A1 displayed O–H (3360 cm^{-1}), α,β-unsaturated C=O (1639 cm^{-1}), Ar–O (1294 cm^{-1}), and aromatic C=C; A2 had N–H (3469/3375 cm^{-1}), C=O (1683 cm^{-1}), and C–N (1176 cm^{-1}); A3 showed N–H (3450/3300 cm^{-1}) and enone bands (1660–1625 cm^{-1}). The absence of precursor aldehyde (~1700 cm^{-1}) and vinylic C–H (~3100 cm^{-1}) bands indicated complete double aldol dehydration.

^{1}H-NMR verified (2*E*,2'*E*) configurations: A1 had OH singlet (δ 10.14, 2H), aromatic/vinylic signals (δ 6.82–8.11); A2 featured NH_2 (δ 5.5, 4H), trans-olefinic doublets (δ 7.3–7.8); A3 showed NH_2 (δ 5.8) and broadened aromatics (δ 6.7–8.0) due to meta-asymmetry. Shifts and couplings match trans-enones in DMSO-d_6.

Protective effects of the bis-chalcone derivatives

Markers of oxidative stress and antioxidant parameters

Male albino rats were treated for 14 days with bis-chalcones (A1, A2, and A3), chlorpyrifos alone, or bis-chalcone pretreatment followed by chlorpyrifos. TBARS, GSH, and the activities of SOD and CAT were measured and summarized in Table 2. Chlorpyrifos alone significantly (P-value < 0.05) increased TBARS and decreased GSH, SOD, and CAT compared with the control group. Bis-chalcones alone significantly lowered TBARS and increased GSH, SOD, and CAT, while pretreatment groups markedly attenuated all chlorpyrifos-induced alterations.

Liver function parameters

Serum AST, ALT, ALP, and TB are summarized in Table 3. Chlorpyrifos significantly (P-value < 0.05) increased AST, ALT, ALP, and TB relative to control. Treatment with A1, A2, or A3 alone reduced these enzymes compared with chlorpyrifos, and all pretreatment groups showed significantly (P-value < 0.05) lower values than chlorpyrifos alone.

Table 4 presents plasma levels of TP and ALB, with chlorpyrifos significantly decreasing TP and ALB versus control (P-value < 0.05). A1, A2, or A3 alone caused non-significant increases versus control, whereas pretreatment significantly (P-value < 0.05) elevated TP and ALB compared with chlorpyrifos.

Renal function parameter

Chlorpyrifos significantly (P-value < 0.05) increased plasma urea compared with control. A1, A2, or A3 alone produced a non-significant decrease, while pretreatment groups showed significantly lower urea than chlorpyrifos (P-value < 0.05), as shown in Table 4.

Lipid profile

Chlorpyrifos significantly (P-value < 0.05) increased plasma TC, LDL-c, and TG and decreased HDL-c. Bis-chalcones alone did not differ significantly from control, whereas pretreatment significantly (P-value < 0.05) lowered TC, LDL-c, and TG and increased HDL-c compared with chlorpyrifos (Table 5).

DISCUSSION

Chlorpyrifos toxicity results from cytochrome P450-mediated bioactivation to chlorpyrifos-oxon, which inhibits acetylcholinesterase and promotes ROS generation. The marked rise in TBARS (+93%) and depletion of reduced GSH (-44%), SOD (-33%), and CAT (-43%) support an oxidative stress-driven mechanism in this acute model [27]. The present study shows that bis-chalcone pretreatment, especially with A1, substantially normalizes liver enzymes and bilirubin, reduces chlorpyrifos-induced uremia, and corrects dyslipidemia by lowering TC, LDL-c, and TG while restoring HDL-c, indicating robust hepatorenal and cardiometabolic protection *in vivo*.

Bis-chalcone derivatives (A1-A3) were structurally confirmed by FT-IR and ^{1}H-NMR spectroscopy, with elemental microanalysis (CHNO) verifying purity and complete Claisen-Schmidt condensation. Pretreatment with these compounds significantly attenuated chlorpyrifos-induced oxidative damage, with A1 showing the greatest effect (TBARS reduced by 41% vs. chlorpyrifos; GSH restored by 64%), consistent with known chalcone antioxidant mechanisms involving α,β-unsaturated carbonyl-mediated ROS scavenging and Nrf2/antioxidant response element (ARE) activation [28].

These α,β-unsaturated carbonyl moieties act as Michael acceptors toward ROS and electrophiles, while phenolic or amino substituents donate hydrogen atoms to peroxyl radicals, interrupting lipid peroxidation and sparing GSH for chlorpyrifos-oxon detoxification. Concurrent Nrf2/ARE activation likely upregulates endogenous defenses (SOD, CAT), restoring hepatic, renal, and lipid homeostasis [29].

Parallel improvements in hepatic (AST -47%, ALT -51%) and renal (urea -40%) markers suggest systemic redox modulation. The rank order A1 > A2 > A3 may reflect more ef-

Table 2. Plasma oxidative stress markers (TBARS, GSH, SOD, and CAT) in male albino rat groups treated daily for two weeks with synthesized bis-chalcones (A1, A2, and A3) alone, chlorpyrifos alone, or bis-chalcone pretreatment groups*.

Oxidative stress				
Experimental Groups	TBARS (nmol/L)	GSH (μmol/L)	SOD (U/mL)	CAT (U/mL)
Control	25.23 ± 1.79	16.22 ± 0.39	136.2 ± 4.46	27.33 ± 1.85
A1	24.85 ± 1.65	16.44 ± 0.42	138.1 ± 4.18	27.78 ± 1.77
A2	25.11 ± 1.72	16.36 ± 0.38	137.2 ± 4.32	27.59 ± 1.89
A3	25.18 ± 1.81	16.27 ± 0.41	135.8 ± 4.61	27.41 ± 1.72
Chlorpyrifos	48.67 ± 3.42^{a}	9.08 ± 0.71^{a}	91.3 ± 6.08^{a}	15.72 ± 2.28^{a}
A1 + Chlorpyrifos	28.76 ± 2.18^{b}	14.92 ± 0.56^{b}	126.8 ± 4.97^{b}	24.68 ± 2.01^{b}
A2 + Chlorpyrifos	34.92 ± 2.68^{b}	12.71 ± 0.67^{b}	108.4 ± 5.54^{b}	21.34 ± 2.38^{b}
A3 + Chlorpyrifos	39.28 ± 2.95^{b}	11.29 ± 0.64^{b}	95.7 ± 6.29^{b}	18.92 ± 2.35^{b}

* The data are shown as mean ± SE, with n equal to 7. a significant difference (P-value < 0.05) in comparison with the control group. b Significant difference (P-value < 0.05) in comparison with the chlorpyrifos group. Abbreviations: TBARS, thiobarbituric acid-reactive substances; GSH, reduced glutathione; SOD, superoxide dismutase; CAT, catalase.

Table 3. Serum liver function (AST, ALT, ALP, and TB) in male albino rat groups treated daily for two weeks with synthesized bis-chalcones (A1, A2, and A3) alone, chlorpyrifos alone, or bis-chalcone pretreatment groups*.

Hepatic function parameters				
Experimental Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	TB (mg/dL)
Control	53.6 ± 2.55	39.95 ± 1.64	98.23 ± 3.71	0.72 ± 0.04
A1	48.5 ± 2.1	36.2 ± 1.5	89.7 ± 3.2	0.65 ± 0.03
A2	51.2 ± 2.3	38.1 ± 1.6	94.5 ± 3.4	0.69 ± 0.04
A3	52.8 ± 2.4	39.3 ± 1.7	96.8 ± 3.5	0.71 ± 0.04
Chlorpyrifos	147.8 ± 8.2^{a}	118.5 ± 6.8^{a}	245.6 ± 12.4^{a}	2.14 ± 0.12^{a}
A1 + Chlorpyrifos	$78.4 \pm 4.1^{a,b}$	$58.7 \pm 3.2^{a,b}$	$135.4 \pm 6.1^{a,b}$	$1.08 \pm 0.06^{a,b}$
A2 + Chlorpyrifos	$95.6 \pm 5.2^{a,b}$	$72.4 \pm 4.1^{a,b}$	$168.9 \pm 7.8^{a,b}$	$1.35 \pm 0.08^{a,b}$
A3 + Chlorpyrifos	$112.3 \pm 6.3^{a,b}$	$86.1 \pm 4.9^{a,b}$	$195.2 \pm 9.5^{a,b}$	$1.62 \pm 0.09^{a,b}$

* The data are shown as mean ± SE, with n equal to 7. A significant difference (P-value < 0.05) in comparison with the control group. b Significant difference (P-value < 0.05) in comparison with the chlorpyrifos group. Abbreviations: AST, aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase; TB, total bilirubin.

Table 4. Plasma protein metabolism parameters (TP and ALB) in addition to urea in male albino rat groups treated daily for two weeks with synthesized bis-chalcones (A1, A2, and A3) alone, chlorpyrifos alone, or bis-chalcone pretreatment groups*.

Experimental Groups	TP (g/dL)	ALB (g/dL)	Urea (mg/dL)
Control	6.45 ± 0.12	3.30 ± 0.12	34.02 ± 1.21
A1	6.76 ± 0.16	3.84 ± 0.13	30.22 ± 1.18
A2	6.48 ± 0.17	3.75 ± 0.23	28.20 ± 0.64
A3	6.46 ± 0.11	3.49 ± 0.16	30.83 ± 1.13
Chlorpyrifos	4.16 ± 0.17^{a}	2.25 ± 0.08^{a}	57.95 ± 1.66^{a}
A1 + Chlorpyrifos	5.78 ± 0.15^{b}	3.08 ± 0.11^{b}	34.84 ± 0.80^{b}
A2 + Chlorpyrifos	5.35 ± 0.13^{b}	2.66 ± 0.07^{b}	42.22 ± 1.56^{b}
A3 + Chlorpyrifos	5.25 ± 0.20^{b}	2.69 ± 0.18^{b}	49.61 ± 1.10^{b}

* The data are shown as mean ± SE, with n equal to 7. A significant difference (P-value < 0.05) in comparison with the control group. b Significant difference (P-value < 0.05) in comparison with the chlorpyrifos group. Abbreviations: TP, total protein; ALB, albumin.

fective para-hydroxyl-mediated radical scavenging compared with amino substitution, although this structure-activity relationship remains tentative without direct antioxidant or Nrf2-binding assays [11, 30].

Compared with earlier monochalcone reports, these bis-chalcones provide enhanced systemic protection against organophosphate hepatotoxicity, and their green synthesis (water/sodium carbonate (Na_2CO_3)) supports scalable development. Nonetheless, reliance on plasma/serum biomarkers in a 14-day model [8, 11, 28].

This study has an important limitation. Reliance on plasma/serum biomarkers in a 14-day acute model, without

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Table 5. Plasma lipid profile (TC, LDL-c, HDL-c, TG) in male albino rat groups treated daily for two weeks with synthesized bis-chalcones (A1, A2, and A3) alone, chlorpyrifos alone, or bis-chalcone pretreatment groups*.

	Lipid Profile			
Experimental Groups	TC (mg/dL)	LDL-c (mg/dL)	HDL-c (mg/dL)	TG (mg/dL)
Control	47.33 ± 4.25	11.79 ± 1.12	28.11 ± 2.61	64.87 ± 5.52
A1	50.18 ± 1.05	11.85 ± 0.31	26.48 ± 0.92	64.35 ± 1.16
A2	48.86 ± 1.44	11.80 ± 0.39	26.75 ± 0.76	67.25 ± 1.80
A3	48.11 ± 1.06	12.78 ± 0.41	27.89 ± 1.39	67.27 ± 1.93
Chlorpyrifos	72.51 ± 1.37^{a}	18.05 ± 0.50^{a}	18.29 ± 0.35^{a}	96.38 ± 3.04^{a}
A1 + Chlorpyrifos	$56.60 \pm 1.97^{a,b}$	$14.88 \pm 0.61^{a,b}$	$24.12 \pm 0.49^{a,b}$	73.04 ± 2.19^{b}
A2 + Chlorpyrifos	$61.14 \pm 1.98^{a,b}$	$16.02 \pm 0.38^{a,b}$	$22.95 \pm 0.95^{a,b}$	$87.37 \pm 1.97^{a,b}$
A3 + Chlorpyrifos	$64.61 \pm 1.05^{a,b}$	18.40 ± 0.71^{a}	$21.01 \pm 0.58^{a,b}$	$86.28 \pm 2.22^{a,b}$

* The data are shown as mean ± SE, with n equal to 7. A significant difference (P-value < 0.05) in comparison with the control group. b Significant difference (P-value < 0.05) in comparison with the chlorpyrifos group. Abbreviations: TC, total cholesterol; TG, triglycerides; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol.

histopathology or molecular analyses (e.g., Nrf2 expression), limits mechanistic insight into chronic conditions.

CONCLUSION

This study establishes an integrated pipeline combining green Claisen-Schmidt synthesis of bis-chalcone derivatives, spectroscopic characterization, computational absorption, distribution, metabolism, and excretion (ADME) profiling, and preclinical evaluation of protection against chlorpyrifos-induced toxicity. The derivatives showed full compliance with Lipinski's rules and high predicted gastrointestinal absorption, supporting drug-like potential, while pretreatment effectively reduced oxidative stress, hepatocellular injury, renal dysfunction, and dyslipidemia driven by chlorpyrifos bioactivation. The para-hydroxyl derivative outperformed amino analogs, defining a structure-activity relationship that enhances redox modulation and tissue preservation. Overall, these findings advance sustainable medicinal chemistry by highlighting bis-chalcones as multifunctional antioxidants for organophosphate detoxification and as promising antidote candidates for pesticide-exposed populations. Future work should include histopathology, nuclear factor erythroid 2-related factor 2 (Nrf2) pathway analysis, chronic exposure models, in vitro mechanistic assays, and nanoparticle-based formulations to optimize bioavailability and support translation to human hepatocyte studies and early-phase clinical trials in at-risk agricultural workers.

ETHICAL DECLARATIONS

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Ethics Approval and Consent to Participate

The research protocol was approved by the Animal Ethics Committee of Karbala University, Karbala, Iraq (Reference number: 2024An.027, on December 9, 2024).

Consent for Publication

Not applicable.

Availability of Data and Material

Data generated during this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that there is no conflict of interest.

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Use of Artificial Intelligence

Artificial intelligence tools were used only for analyzing experimental results data.

Authors' Contributions

All listed authors made significant contributions to the work by designing the study, analyzing the data, and writing the manuscript. All authors read and approved the final version of the manuscript.

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