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Effect of new biphenyl chalcone derivatives against gamma radiation induced oxidative stress markers in *E .coli* K 12 and evaluation of their antimicrobial activities

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ABSTRACT

*This work reports the synthesis, characterization of new (2E)-1-(biphenyl-4-yl)-3-aryl)-prop-2-en-1-one derivatives (**C1-C10**). The compounds exhibited moderate to good DPPH scavenging activity; amid those C5, C7 and C8 exhibited excellent scavenging activity. The chalcone derivatives were evaluated for their radioprotective activity in E.coli K12, in vitro. In C5 pretreated and irradiated E. coli K12 bacteria sample, the level of antioxidant enzymes SOD and CAT were restored to near basal level. It also showed significant demulating effect on the level of TBARS indicating good antioxidant activity. The chalcone derivatives were also evaluated for in vitro and in silico antibacterial and antifungal activities. All the compounds exhibited moderate to good antimicrobial activity at MIC 10-40 µg/mL. The compounds were docked at the active site of the antibacterial target methionyl-tRNA synthetase (metRS) (PDB ID: 1A8H) to predict their putative interactions. Among the tested compounds the compounds C3, C9 and C10 showed -4.7, -4.1 and -5.2 ΔG in Kcal/mol binding energies respectively.*

Key words: Biphenyl chalcone derivatives, radioprotection, antioxidant activity, antimicrobial activity, molecular docking.

INTRODUCTION

A perfunctory look at the literature cited in relation to chalcones in recent years indicates that there is a growing interest in evaluating the pharmaceutically important biological activities of chalcones and its derivatives, presuming their role in the prevention of various degenerative diseases and other human ailments. Chalcones (trans-1,3-diphenyl-2-propen-1-ones) are the biogenetic precursors of all known flavonoids and are abundant in edible plants [1] Scientific investigations on the bioavailability of chalcones from food sources are limited but variety of synthetic chalcones has been reported to possess a wide range of pharmaceutically important biological activities. Hesperidin methyl chalcone is a methylated derivative of the flavonoid hesperidin. Hesperidin methyl chalcone supports and protects the integrity of the vascular system with particular activity in the capillaries and veins. Plethora of literature has accumulated in the recent years suggesting the role of chalcone and its derivatives as an antiinflammatory [2], anticancer and antioxidant agents [3]. Hydroxy-methylated chalcone such as 2'-hydroxy- 4'-methoxychalcone has been described as an effective antiangiogenic and antitumor agent [4,5]. Nevertheless chalcones have also been

attributed with wide range of antimicrobial activity like antimalarial, antileishmanial and antibacterial [6]. It was reported that antioxidants may also be involved in regulating signaling pathways and cellular responses [7,8]. The increasing need for new antibiotics to overcome rapidly developing resistance mechanisms observed in clinical isolates of Gram-positive and Gram-negative bacteria has placed critical emphasis on the search for new antibacterial enzyme targets and the structural and mechanistic investigation of such targets. So an *in silico* docking of the newly synthesized compounds to methionyl-tRNA synthetase enzyme was attempted. Many tRNA synthetases can be considered as good targets for antibacterial discovery because they are broadly conserved, essential for growth, and distinct enough from their human orthologs to anticipate the discovery of selective inhibitors [9,10]. The development of radioprotective agents has been the subject of intense research in view of their potential for use within a radiation environment, such as space exploration, radiotherapy and even nuclear war. However, no ideal, safe synthetic radioprotectors are available to date, so the search for potent radioprotector from different sources has been ongoing for several decades. A systematic screening approach leads in identifying potential new candidate drugs from any sources, for mitigation of radiation injury.

Hence, this work is focused on protective efficacy of some synthetic chalcone derivative against radiation induced oxidative stress in *E.coli K12*. Bacteria reproduce asexually by binary cell fission (clonal replication), calculating the lifespan has proven elusive. Without the determination of a lifespan, age studies using bacteria have limited application. Further, it has been proposed that organisms whose somatic cell line is not distinct from its germ line such as single celled organisms like *E.coli K12* are immortal [11]. The cell wall of Gram-positive bacteria is composed of a thick layer of murein (a peptidoglycan), compared to gram negative bacteria that have a thin layer of murein, [12] Therefore, it would be deduced that a free radical scavenger has limitations in crossing the cellular wall of a Gram-positive bacteria. The outer cell wall of Gram-negative bacteria, such as *E. coli*, is fairly permeable to smaller molecules below a molecular weight of approximately 400Da. The optimum conditions for a scavenger to cross the cellular wall would occur when the cell is metabolically active, aerobic, and contains a gram-negative wall. The bacterial model selected for this study was *E. coli K12* is a gram-negative, aerobic, non-spore forming bacterial model that is safe and reliable [13].

In the present study, the biphenyl substituted chalcone derivatives were synthesized, characterized and evaluated for *in vitro* and *in silico* antimicrobial and antioxidant activity. Their modulatory effect on oxidative stress markers in *E. coli K12* strain against gamma radiation induced oxidative stress was also studied. Results of such studies embody the content of this paper.

METHODS AND MATERIALS

Chemistry

Melting points were determined in an open capillary tube and are uncorrected. The mass spectra were recorded on SHIMADZU – LCMS 2010 Spectrometer. Elemental analysis was carried out on a FLASH EA 1112 SERIES CHN REPORT THERMO FINNIGAN. ^{1}H NMR spectra were recorded on a Bruker 400 MHz spectrometer in DMSO-d_6 using tetramethyl silane as internal standard and chemical shifts are reported in δ units and the coupling constants (J) are reported in Hertz. All chemicals were purchased from Sigma-Aldrich Co., U.S.A., and the solvents used for column chromatography were of reagent grade, and were purchased from commercial sources. The compounds were purified and monitored by thin layer chromatography on pre-coated sheets of silica gel-G (Merck, Germany) using iodine vapour for detection. Preparative flash column chromatography was carried out on silica gel (70 -240 mesh, G60 Merck). Analytical quantities of samples were weighed on a SARTORIUS/BP211D balance.

General Procedure for the synthesis chalcones (C)

Chalcones were synthesized by Clasien- Schmidt condensation using MeOH/EtOH/DMF as reaction solvent. A mixture of 4- phenyl acetophenone (0.01 mol) and substituted benzaldehyde (0.01 mol) in ethanol (25 mL) was cooled for 10-15˚C in ice bath. To a cooled solution was added 50 % (8 mL) NaOH drop by drop with continuous stirring for 5 hours using magnetic stirrer and left for overnight. The reaction mixture was poured onto crushed ice and acidified using dilute HCl. The solid obtained was filtered, washed with ice cold water, dried and recrystallized from ethanol and % yield and meting point were noted (Nakamura *et al*., 2002)

C1: (2E)-1-(Biphenyl-4-yl)-3-phenyl)prop-2-en-1-one

^{1}H NMR (400 MHz, d_6-DMSO): δ 7.42-7.48 (m, 6H, Ar-H), 7.51-7 .53(d, J= 8 Hz ,1H, =C-H), 7.72-7.74 (d, J=8Hz, 1H, =C-H), 7.76 (s, 1H, Ar-H), 7.79 (s, 1H, Ar-H), 7.82 (s, 1H, Ar-H), 7.85 (s, 1H, Ar-H), 7.89-7.91(t, J=4 Hz,1H, Ar-H), 7.96 (s, 1H, Ar-H), 8.02 (s, 1H, Ar-H),8.23-8.26 (d, J=12 Hz, 1H, Ar-H).

C2 :(2E)-1-(Biphenyl-4-yl)-3-(4-chlorophenyl)prop-2-en-1-one
^{1}H NMR (400 MHz, d_6-DMSO): δ 7.36 (s, 1H, Ar-H), 7.42-7.54(m, 5H, Ar-H), 7.65-7.68 (d, J=12Hz, 1H, Ar-H), 7.72 (s, 1H, Ar-H), 7.76-7.78 (t, 1H, J=4 Hz, Ar-H), 7.81-7.83 (d, 1H, J=8Hz, =C-H), 7.85(s, 1H, Ar-H), 7.88 (s, 1H, Ar-H), 7.93-7.95 (d, J=8 Hz, 1H, =CH) 7.99-8.08 (m, 1H, Ar-H) 8.23-8.26(d, J=12 Hz,1H,Ar-H).

C3 :(2E)-1-(Biphenyl-4-yl)-3-(4-bromophenyl)prop-2-en-1-one
^{1}H NMR (400 MHz, d_6-DMSO): δ 7.39 (s, 1H, Ar-H), 7.41-7.54(m, 5H, Ar-H), 7.67-7.67 (d, J=8Hz, 1H, Ar-H), 7.73 (s, 1H, Ar-H), 7.76-7.78 (t, 1H, J=4 Hz, Ar-H), 7.80-7.82 (d, 1H, J=8Hz, =C-H), 7.86(s, 1H, Ar-H), 7.89 (s, 1H, Ar-H), 7.94-7.96 (d, J=8 Hz, 1H, =CH) 7.99-8.09 (m, 1H, Ar-H) 8.23-8.26(d, J=12 Hz, 1H,Ar-H).

C4: (2E)-1-(Biphenyl-4-yl)-3-(4-fluorophenyl)prop-2-en-1-one
^{1}H NMR (400 MHz, d_6-DMSO): δ 7.29-7.33 (t, 2H, J=4 Hz Ar-H), 7.42-7.43(dd, 1H, J=4 Hz Ar-H), 7.45-7.53 (m, 2H, Ar-H), 7.73-7.86 (m, 5H, Ar-H), 7.83(s, 1H, Ar-H), 7.86-7.88 (d, 1H, J=8Hz, =C-H), 7.96(s, 1H, Ar-H), 7.98-8.04 (m, 1H, Ar-H), 8.24-8.26 (d, J=8 Hz, 1H, =CH) .

C5:(2E)-1-(Biphenyl-4-yl)-3-(4-methoxyphenyl)prop-2-en-1-one
^{1}H NMR (400 MHz, d_6-DMSO): δ 7.02-7.05 (d, 1H, J=12 Hz Ar-H), 7.42-7.49(m, 6H, Ar-H), 7.51-7.54 (t, 1H, Ar-H), 7.51-7.54 (m, 5H, Ar-H), 7.83(s, 1H, Ar-H), 7.86-7.88 (d, 1H, J=8Hz, =C-H), 7.96(s, 1H, Ar-H), 7.98-8.04 (m, 1H, Ar-H), 8.24-8.26 (d, J=8 Hz, 1H, =CH) δ 3.8 (s, 3H, OCH_3)

C6: (2E)-1-(Biphenyl-4-yl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one
^{1}H NMR (400 MHz, d_6-DMSO): δ 7.27 (s, 1H, Ar-H), 7.42-7.47(m, 3H, Ar-H), 7.49-7.55 (m, 2H, Ar-H), 7.74-7.76 (t, 1H, J=4 Hz Ar-H), 7.78-7.80 (d, 1H, J=8 Hz =C-H)), 7.82-7.84 (m, 1H, Ar-H), 7.88-7.90 (t, 1H, J=4 Hz Ar-H), 7.94-8.26 (m, 1H, Ar-H), 8.24-8.26 (d, J=8 Hz, 1H, =C-H) 3.8 (s, 3H, OCH_3), 3.7 (s, 3H, OCH_3), 3.3 (s, 3H, OCH_3).

C7: (2E)-1-(Biphenyl-4-yl)-3-(2,5-dimethoxyphenyl)prop-2-en-1-one
^{1}H NMR (400 MHz, d_6-DMSO): δ 7.05 (s, 1H, Ar-H), 7.17-7.19(d, 1H, J=8 Hz, Ar-H), 7.40-7.48 (m, 4H, Ar-H), 7.49-7.50 (m, 4H, Ar-H), 7.78-7.80 (d, 1H, J=8 Hz =C-H), 7.88-7.90 (d, 1H, J=8 Hz =C H), 7.94-8.26 (m, 2H, Ar-H), 3.74-3.86 (m, 6H, OCH_3), LCMS (m/z, %), 345 (M+1, 50), 346 (M+2, 30), 197 (C_{14} H_{13} O, 100)

C8: (2E)-1-(Biphenyl-4-yl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one
^{1}H NMR (400 MHz, d6-DMSO): δ 7.40-7.44 (t, 2H, J=8Hz. Ar-H), δ 7.48-7.52 (m, 5H,. Ar-H), 7.73-7.75 (d, 1H, J=8 Hz =C-H), 7.80-7.83(m, 5H,Ar-H),) , 8.02-8.04 (d, 1H, J=8 Hz =C-H), 3.33-3.86(m, 6H, OCH_3). LCMS (m/z, %), 345 (M+1, 50), 346 (M+2, 30), 197 (C_{14} H_{13} O, 100)

C9:(2E)-1-(Biphenyl-4-yl)-3-(2-chlorophenyl)prop-2-en-1-one
^{1}H NMR (400 MHz, d_6-DMSO): δ 7.36 (s, 1H, Ar-H), 7.42-7.54(m, 5H, Ar-H), 7.65-7.68 (d, J=12Hz, 1H, Ar-H), 7.72 (s, 1H, Ar-H), 7.76-7.78 (t, 1H, J=4 Hz, Ar-H), 7.81-7.83 (d, 1H, J=8Hz, =C-H), 7.85(s, 1H, Ar-H), 7.88 (s, 1H, Ar-H), 7.93-7.95 (d, J=8 Hz, 1H, =CH) 7.99-8.08 (m, 1H, Ar-H) 8.23-8.26(d, J=12 Hz,1H,Ar-H). LCMS (m/z, %), 319 (M+1,35), 293 (M+2,15) 197 (C_{14} H_{13} O, 100).

C10:(2E)-1-(Biphenyl-4-yl)-3-(3-bromo-4-methoxyphenyl)prop-2-en-1-one
^{1}H NMR (400 MHz, d_6-DMSO): δ 7.39 (s, 1H, Ar-H), 7.41-7.54(m, 5H, Ar-H), 7.67-7.67 (d, J=8Hz, 1H, Ar-H), 7.73 (s, 1H, Ar-H), 7.76-7.78 (t, 1H, J=4 Hz, Ar-H), 7.80-7.82 (d, 1H, J=8Hz, =C-H), 7.86(s, 1H, Ar-H), 7.89 (s, 1H, Ar-H), 7.94-7.96 (d, J=8 Hz, 1H, =CH) 8.23-8.26(d, J=12 Hz, 1H, Ar-H) 3.9(s, 3H, OCH_3). LCMS (m/z, %), 393 (M, 80), 197 (C_{14} H_{13} O, 100).

Determination of antioxidant activity by DPPH scavenging assay
The DPPH assay was carried out according using standard method with some modifications. The stock solution was prepared by dissolving 24 mg DPPH with 100mL methanol and then stored at -20 °C until needed. The working solution was obtained by mixing 10mL stock solution with 45mL methanol to obtain an absorbance of 1.17 ±0.02

units at 517 nm using the spectrophotometer. The solution of compounds at 15µg/ml concentration was prepared in DMSO. It was diluted to 4 ml using distilled water. To this 1ml of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) solution in methanol was added. The mixed solution was incubated at room temperature for 30 min. The absorbance of stable DPPH• was read at 517 nm using UV-vis. spectrophotometer and the remaining DPPH was calculated [14].

Modulator effect of compounds against oxidative stress induced by gamma radiation

Bacterial Strain, Culture Conditions and preparation of bacteria for irradiation

Stocks of *E. coli* K12 were routinely maintained on slopes of nutrient agar and sub cultured into LB broth, which was incubated overnight at 37˚C under the moderate aeration. An overnight broth culture [0.2 ml.] was inoculated into 5 ml. broth, which was incubated for 1.5 hr. From this culture 0.2 mL was inoculated into a fresh bottle containing 5 mL LB broth, which was rotated in the incubator for 1.7 hr. The suspensions were centrifuged and washed three times with phosphate buffer (pH 6.9) and finally resuspended in buffer. This was used for further studies with suitable dilutions. *E. coli* K12 taken from the stock was grown aerobically in liquid LB broth at 37ºC at 150 r/min. The compounds **C5, C7** and **C8** at the concentration of 20µg/ml was added to cultures at the same time of inoculation which were then incubated for 24 h before harvesting for enzyme activity studies. DMSO solvent was used to dissolve **C5, C7** and **C8** hence 200µl DMSO (control) was also added to cultures that were incubated for 24 h in liquid LB medium [15].

Irradiation of bacteria sample

Irradiations of bacteria samples in polypropylene vials were done under telecobalt radiotherapy unit [Phoenix (#P44)] at Kasturba Medical College Hospital, Attavar, Mangalore. This unit is of medical use for the treatment of malignant diseases which is having a Cobalt- 60 radioactive source [produces gamma radiation energy of 1.17 MeV & 1.33 MeV; with an activity of 170 RMM (Roentgen per minute at one meter)]. Different treated Bacteria sample was irradiated for 0.2 and 0.4 Gy.

Bacteria Harvest and Lysis Procedures

Bacteria cells were harvested from 6 mL liquid culture by centrifugation and washed twice using ice-cold 0.9% sodium chloride solution. Pellets were re-suspended in 3 mL 0.9% sodium chloride solution and then subjected to 99 cycling of sonication in an ice water bath for 3s followed by cooling for another 4s. Cellular debris was removed by centrifugation at 10000 g at 4ºC. The supernatants were collected for SOD, CAT, and TBARS levels were determined.

Enzymatic activity measurements

Bacteria homogenate were used for Catalase activity measurements. Dismutation of hydrogen peroxide by catalase was registered using spectrophotometer at 240nm [16].The activity of SOD was assayed at 406 nm as the inhibition of quercetin oxidation by superoxide anion [17]. Protein estimation was carried out using bacteria homogenate by Lowery's method [18].

Thiobarbituric acid-reactive substances

TBARS were measured in bacteria cell suspension by the standard procedure. For this, 1.0 ml cell suspension (about 2-3 µg of protein) was precipitated by the addition of 1.0 ml of 20% w:v TCA, centrifuged and the supernatants were mixed with 2.0 ml of saturated solution of thiobarbituric acid in 0.1 M HCl and 10 mM butylated hydroxytoluene. The samples were heated for 60 min at 100°C in a water bath. 1.5 ml aliquot was then removed, chilled and mixed with 1.5 ml of butanol. The mixture was centrifuged for 10 min at 4000 g. The organic fraction was removed and optical density at 535 nm was measured using spectrophotometer [19].

Colony forming unit study during post irradiation treatment

Microbial counting is useful in the basic sciences and is used determine the number of bacteria present for physiological or biochemical studies. After irradiation, the cultures were immediately stored at 4 ºC, and the changes of the microbial growth, were analyzed during the post-irradiation period. Media for an enumeration of the bacteria were prepared by Standard Plate Count Agar. The non irradiated and irradiated samples were serially diluted with sterile saline and each diluent (100 mL) was spread in triplicate on to each agar plate. The agar plates were incubated at 37ºC for 48 h and then the colony forming units (CFU) per milliliter of the sample were calculated [15].

Antimicrobial activity screening

Antifungal activity

Antifungal activity for newly synthesized compounds **C1-C10** was screened by serial plate dilution method. Activity of each compound was compared with Ciproflaxcin as standard.

Antibacterial activity

The newly synthesized chalcone derivatives **C1-C10** were screened for their antibacterial activity against bacterial strains by disc diffusion method. Fluconazole was used as a standard drug. Solvent and growth controls were kept [20].

Minimum Inhibitory Concentrations (MIC)

The MIC of all synthesized compounds **C1-C10** was determined by a micro dilution method. The respective clinical strain was spread separately on the medium. The wells were created using a stainless steel sterilized cork borer under aseptic conditions. The compounds **C1-C10** at different concentrations viz. 10, 20, 30, 40 and 50 µg was dissolved DMSO and later loaded into corresponding wells. The standard drug Ciprofloxacin (40 µg in100µL) and Fluconazole (40 µg in100µl) were used as standard drugs for comparison of antibacterial and antifungal activities respectively. The zone of inhibition was compared with standard drug after 24 h of incubation at 37^{o}C for antibacterial activity and 72 h at 25^{o}C for antifungal activity [20].

Docking calculations with ICM™ (Internal Coordinate Mechanics) dock.

All the docking calculations of compounds **C1-C10** in this article were performed using the ICM™ docking module with the default setup as earlier mentioned [21].

Preparations of the inhibitors and target molecules

The 2D structures of the chalcones (in mol file formats) have been converted to 3D and energy minimized at the 3D space of ICM environment. The atom types using local chemical environment, Merck Molecular Force Field (MMFF) 3-9 formal charges and 3D topology were assigned. The lowest energy conformers of chalcones were then docked into targets for antibacterials, the target was methionyl-tRNA synthetase (metRS) (PDB ID: 1A8H).

Docking process

All the docking calculations were performed using the 'interactive docking' menu at the ICM environment. After docking the stack of docking poses were checked visually. Multiple stack conformations were selected based on their docking energies, rmsd values (compared between the docked model and x-ray conformation) and similarities to closely related x-ray crystal structures from PDB. Then the best conformations for each of the compounds were finally chosen, and then their binding energies were calculated using ICM script (briefly described in the following 'Calculation' section).The correlation between the activity profiles and the binding energies (Cal.ΔG) are presented in Result and discussion section.

Calculations of free energies of binding

For each of the individual docked complexes the free energies of binding (Cal.ΔG) between the protein and ligand was calculated using ICM script utilizing the equations 2 and 3.

$$\Delta G = \Delta GH + \Delta GEL + \Delta GS + C \quad \text{.....(1)}$$

$$\Delta G = \Delta GH + \Delta GCOUL + \Delta GDESOLV + \Delta GS + C \quad \text{.....(2)}$$

Here ΔGH is the hydrophobic or cavity term, which accounts for the variation of water/non-water interface area. ΔGEL is the electrostatic term composed of coulombic (ΔGCOUL) interactions and desolvation (ΔGDESOLV) of partial charges transferred from an aqueous medium to a protein core environment. ΔGS is the entropic term which results from the decrease in the conformational freedom of functional groups buried upon complexation; and finally the C is a constant accounts for the change of entropy of the system due to the decrease of free molecules concentration (cratic factor), and loss of rotational/translational degrees of freedom [22].

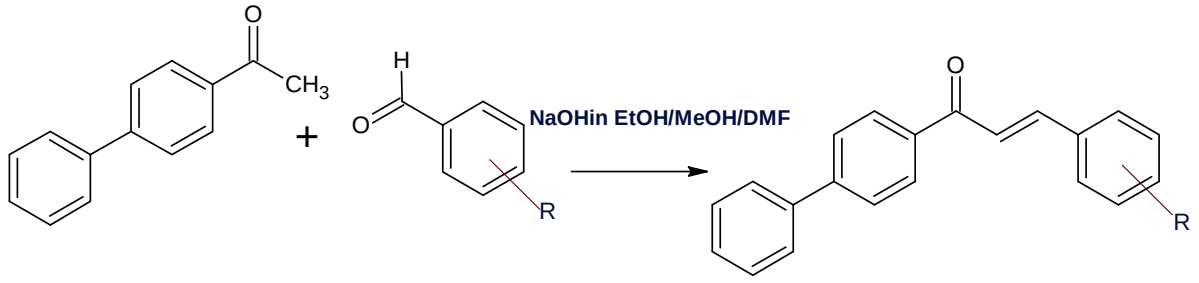
Interpretations of intermolecular interactions

To study the intermolecular interactions between the targets and the compounds LigPlot12 were used to plot the interactions from 3D to 2D. Beside LigPlot, ICM (www.molsoft.com) and Discovery Studio Visualizer (www.accelrys.com) also been used to analyze the interactions in 3D space.

RESULTS AND DISCUSSION

Chemistry

The Claisen-Schmidt condensation is an important C-C bond formation for the synthesis of 1,3-diaryl-2-propen-1-ones (chalcones). It is generally carried out using strong bases such as NaOH or KOH in polar solvents (MeOH/EtOH or DMF). The yield of the synthesized compounds was found to be significant. The structure of the synthesized compounds was confirmed by ^{1}H NMR, LCMS and elemental analysis. Elemental analysis showed that the percentage of the hydrogen and carbon was found to be experimentally equivalent to the calculated values for all compounds. LCMS of the synthesized chalcones gave peak corresponding to the molecular mass. In ^{1}H NMR(400MHz) spectrum of **C5** a singlet appeared at δ 3.83 was due to OCH_3 substituent of the phenyl ring. The signal due to two olefinic protons appeared as doublets at δ 7.86-7.88(J=8Hz) and 8.24-8.26 (J=8Hz) respectively. The signals appeared at δ 7.02-7.05 (d, 1H, J=12 Hz Ar-H), 7.42-7.49(m, 6H, Ar-H), 7.51-7.54 (t, 1H, J=4 Hz Ar-H), 7.51-7.54 (m, 5H, Ar-H), 7.83(s, 1H, Ar-H), 7.96(s, 1H, Ar-H), 7.98-8.04 (m, 1H, Ar-H) account for the thirteen aromatic protons present in the molecule. Hence the spectral data confirmed the formation of the compound 1- biphenyl -3- (4-methoxyphenyl)-prop-2-en-1-one (**C5**). The spectral characterizations of all the compounds are given in the Experimental section. The synthetic pathway is presented in Scheme 1 and physicochemical data for the synthesized compounds are given Table 1.



R = H; 4-Cl; 4-Br; 4-F; 4-OCH_3; 3,4,5-$(OCH_3)_3$; 2,5-$(OCH_3)_2$; 3,4-$(OCH_3)_2$; 2-Cl;4-OCH_3,3-Br

Scheme 1. Reaction scheme for synthesis of chalcone derivatives C1-C10

Table 1 Analytical data of chalcone derivatives C1-C10.

Compound		MP (ºC)	% Yield	Elemental analysis Found [Cald]	
	R			C	H
C1	H	220-02	80	88.70[86.45]	5.67[5.24]
C2	4-Cl	170-83	85	79.12[82.44]	4.74[4.81]
C3	4-Br	176-76	78	69.44[71.12]	4.16[4.23]
C4	4-F	223-28	75	83.42[52.90]	5.00[5.09]
C5	4-OCH_3	180-88	85	84.05[85.12]	5.77[5.85]
C6	3,4,5- $(OCH_3)_3$	211-18	89	76.99[77.08]	5.92 [6.03]
C7	2,5- $(OCH_3)_2$	221-27	87	80.21 [81.07]	5.85[6.01]
C8	3,4-$(OCH_3)_2$	197-03	79	80.21 [81.12]	5.85[6.03]
C9	2-Cl	206-16	84	76.58[76.72]	4.74[4.89]
C10	3-Br; 4-OCH_3	168-71	80	67.19[68.11]	4.36[4.41.]

Biological evaluation

Antioxidant activity by DPPH scavenging assay

DPPH is a stable radical that has a high absorption at 517 nm. When a odd electron of DPPH is paired up with electron taken from phenolic compounds, the absorption at 517nm decreases. The degree of decolorization is a measure of the reducing capacity of compounds and whereby it enables to evaluate their antioxidant activity. The compounds with two or more electron donating groups have lower anodic peak potentials and higher antioxidant abilities than mono-substituted phenols [23]. The ascorbic acid was taken as standard and it exhibited 79.43±0.23 % DPPH scavenging. Among the tested compounds **C5, C7** and **C8** showed 79.95±0.44, 73.46±0.65 and 71.66±0.59 % DPPH scavenging at 15µg/mL. The presence of OCH_3 substituent in the phenyl ring might be contributing for its antioxidant activity. The compounds with two or more electron donating groups have higher antioxidant abilities than mono-substituted phenols [23]. The enhanced radical scavenging capacity of these compounds might be due to the presence of methoxy substitution in the phenyl ring. The DPPH scavenging ability of chalcone derivatives is given in the **Fig. 1.**

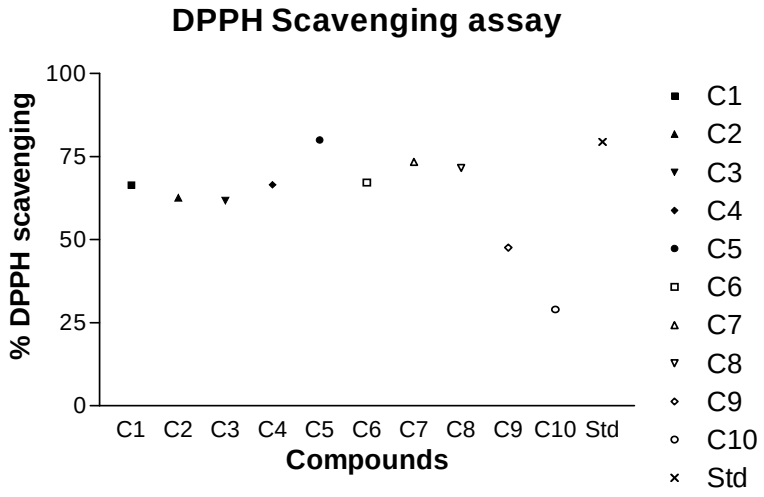
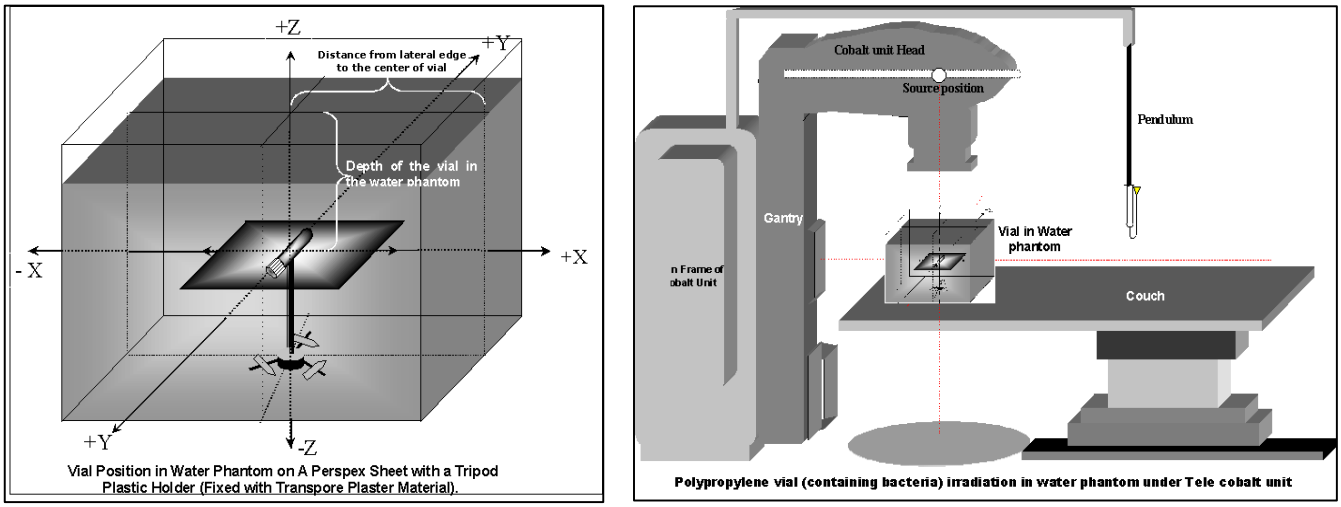


Fig.(1). DPPH scavenging ability of the compounds C1-C10

Modulatory effect of compounds against oxidative stress induced by gamma radiation in E.coli K12

Radiation dosimetry (absorbed dose to a specific point in Gray or centi-Gray: Gy) was carried out using International Atomic Energy Agency (IAEA) Technical Report Series–398 protocol under the cobalt. Measurements were taken with a calibrated ionization chamber kept inside the water phantom (under full scatter conditions) using the above protocol. The dosimetry of tele cobalt unit is audited periodically under supervision of IAEA / WHO. Polypropylene vials (containing bacteria culture in LB media) were kept under water phantom at a specified depth as shown in **Fig.2a**. The vials were irradiated to doses of 0.2Gray and 0.4 Gray under cobalt unit which is illustrated Fig.2b. The audit results are within ±0.2%. The compounds (2E)-1-(biphenyl-4-yl)-3-(4-methoxyphenyl)prop-2-en-1-one **C5,** (2E)-1-(biphenyl-4-yl)-3-(2,5-dimethoxyphenyl)prop-2-en-1-one **C7** and *(*2E)-1-(biphenyl-4-yl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one **C8** were selected to evaluate modulatory effect on oxidative stress markers on *E.coli K12* bacteria, which was based on DPPH scavenging efficacy. The lethal concentration of the compounds for the study was determined by evaluation of minimum inhibitory concentration (MIC). Determination of radioprotective property against radiation induced oxidative stress in *E. coli* was carried out below MIC of the compounds.



a **b**

Fig. (2). Irradiation of Bacteria samples

a. Irradiation of bacteria sample in water plank set up; b. Cobalt Radiation Unit

Antioxidant Enzyme activity

Most environmental bacteria experience oxidative stress from a variety of sources. The reactive oxygen species (ROS) can be produced in cells not only during microbial aerobic growth as byproducts of normal cellular metabolism but also under stress situations [24]. The variation in the level of antioxidant cellular enzymes like SOD and CAT occurs under stress conditions. The supplemented antioxidants modulate their levels. In the present work, the modulatory effect of newly synthesized biphenyl chalcone derivatives **C5, C7** and **C8** on antioxidant enzymes of irradiated E. coli K12 bacteria were determined.

There was a significant elevation in the level of SOD in dose dependant manner in case of irradiated sample in comparison with control could be attributed to the induced oxidative stress by gamma radiation. Where as in case of **C5** and **C8** pretreated and irradiated (0.2 Gy) bacteria homogenate, the level of SOD restored significantly to near basal level. But in the bacteria samples irradiated with the higher dosage of 0.4 Gy, There was decrease in the level of SOD but not significant with respect to the normal control. In case of **C7** pretreated and 0.4 Gy irradiated sample showed demolition in the level of SOD but not restored to near basal level. The data is given in the Fig. 3.

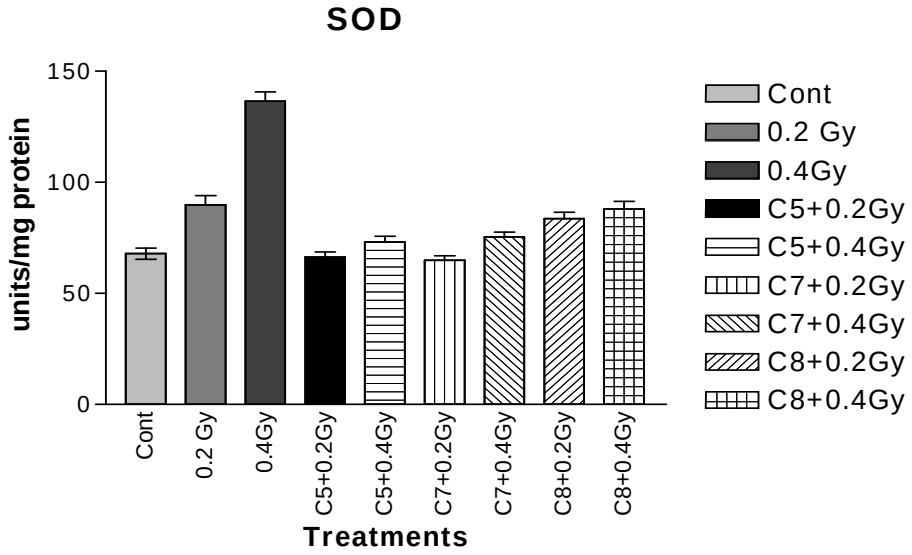


Fig.(3). Modulatory effect of C5, C7 and C8 on SOD by radiation induced oxidative stress in *E.Coli* K12 bacteria

The level of CAT was brought to near basal in case of **C5, C7** and **C8** pretreated bacteria and irradiated at 0.2 Gy. But in case of compound pretreated irradiated at 0.4 Gy the level of CAT decreased but not significantly. The data is given in the Fig. 4.

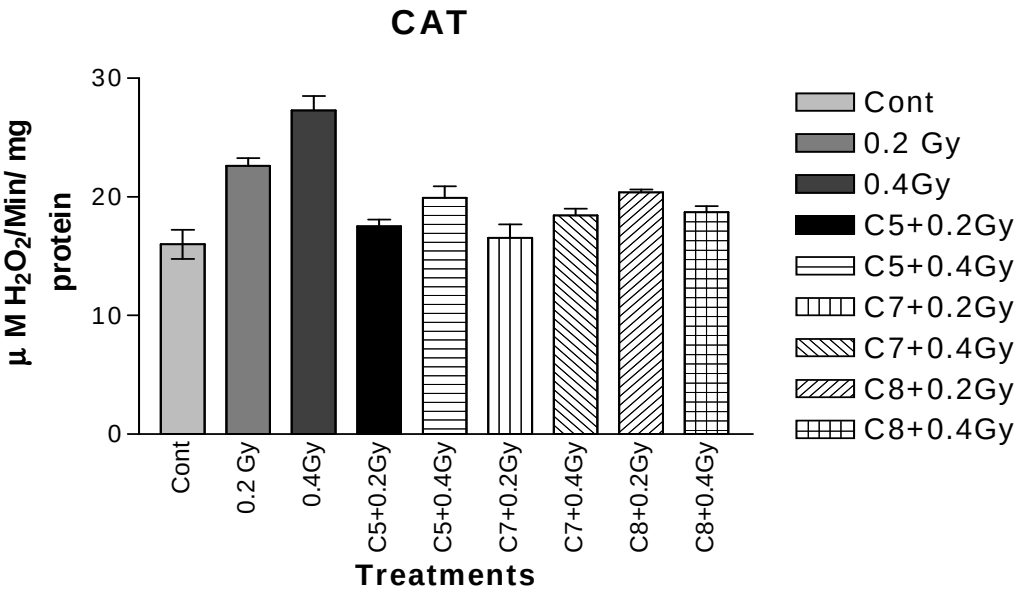


Fig.(4). Modulatory effect of C5, C7 and C8 on CAT by radiation induced oxidative stress in *E.Coli* K12 bacteria

These results indicate that bacteria pretreated with **C5, C7** and **C8** are successful in bringing the level of SOD and CAT to near basal level at 0.2 Gy dose significantly. In order to antagonize ROS, defensive enzyme systems, such as CAT and SOD are involved. In case of compound treated and irradiated samples the antioxidant levels are restored to near basal level, hence it can be presumption that the molecules may be acting as antioxidant. The antioxidant property of the molecules might be due to the presence of OCH_3 substituent on the phenyl ring. The mono substituted compounds exhibit better activity compared to disubstituted compounds could be due to easy permeability of the small molecules than bulkier molecules. Hence the effective concentration of the mono substituted molecule might be more inside the bacterial cell. However, further studies are essential to determine the exact mechanism of action of the molecules.

TBARS assay

In biological membranes, lipid peroxidation is frequently a consequence of free radical attack. The peroxidation of unsaturated fatty acids of cells produces many reactive species, such as free radicals, hydroperoxides, and carbonyl compounds, which may cause damage to proteins and DNA. Lipid peroxidation is a continual process in living aerobic cells; it is maintained at a low level; and it can be prevented from entering into the autocatalytic phase by protective enzymes and antioxidants [25]. Thiobarbituric acid-reactive substances (TBARS) were determined as an independent measurement of lipid peroxidation. The samples were evaluated for malondialdehyde (MDA) production using a lipid peroxidation assay. The level of TBARS was elevated in case of irradiated bacteria sample indicated the presence of oxidative stress. The level of TBARS was found to be decreased significantly in case of **C5** treated and irradiated bacteria samples. In the case of **C7** and **C8** treated and irradiated samples also showed significant decrease in the levels of TBARS compared to irradiated bacteria samples. This result supports the antioxidant properties of the compounds. The data is given in the Fig. 5.

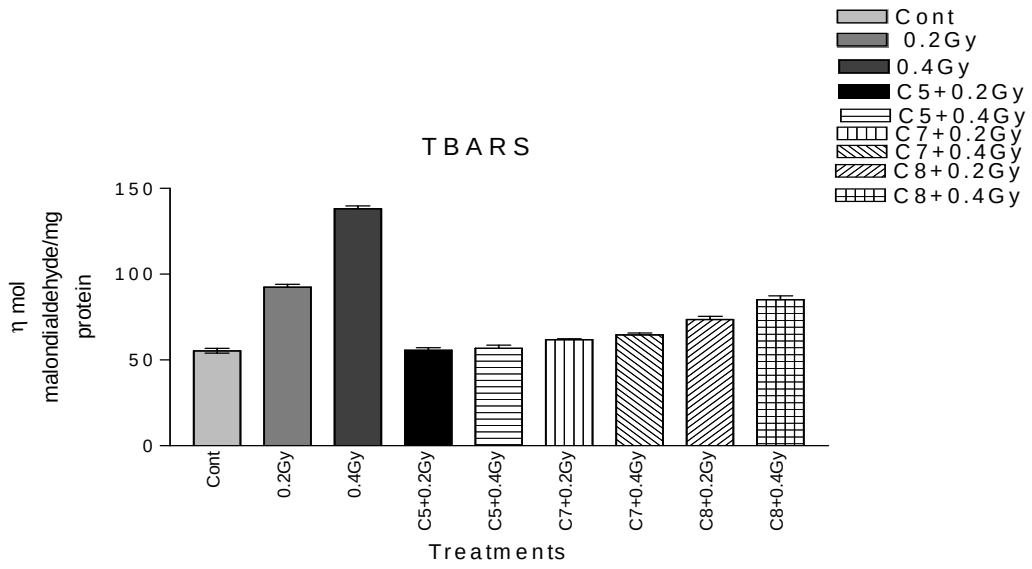


Fig.(5). Modulatory effect of C5, C7 and C8 on TBARS by radiation induced oxidative stress in *E. Coli* K12 bacteria

Colony forming units (CFU) studies

There are numerous papers in the literature dealing with modification of radiation damage to micro-organisms by treatment both before and after irradiation. A number of investigations were also made with *Escherichia coli*, and its response to radiation found to be typical in some respects and presumably also in its biochemical behavior. 'Survival' of microorganisms after irradiation is commonly defined as the ability to form visible colonies on the surface of solid media [26]. Each colony forming unit represents a bacterium that was present in the sample. This study was basically carried out to see the survival of the bacteria under radiation. The count of CFU in compound treated and irradiated bacteria samples reveal the protective effect of compounds. In irradiated bacteria sample the CFU were reduced significantly. Where as in compound treated and irradiated samples significant increase in CFU was observed. This study further supports the protective nature of the compounds against radiation. The data is depicted in the Table 2**.**

Table 2 Colony forming unit studies of E. coli K12 during post radiation treatments.

Control	Irradiated		**C 5** + radiation		**C7** + radiation		**C8** + radiation	
(DMSO)	0.2Gy	0.4 Gy	0.2 Gy	0.4 Gy	0.2 Gy	0.4 Gy	0.2 Gy	0.4 Gy
$1.92x10^7$	$0.87x10^7$	0.67×10^7	1.71×10^7	1.64×10^7	1.24×10^7	1.05×10^7	1.16×10^7	1.09×10^7

Antifungal studies

Compounds **C1-C10** were screened for their antifungal activity against *Aspergillus niger* and *Candida albicans*. The compounds were dissolved in DMSO and antimicrobial activity was determined by serial plate dilution method Among the tested compounds, the compound (2*E*)-1-(biphenyl-4-yl)-3-(4-bromophenyl) prop-2-en-1-one **C3,** (2*E*)-1-(biphenyl-4-yl)-3-(4-fluorophenyl) prop-2-en-1-one **C4** and (2*E*)-1-(biphenyl-4-yl)-3-(2-chlorophenyl) prop-2-en-1-one **C9** have showed significant activity against all tested microorganisms. The other molecules also exhibited moderate to good activity. The data is given in the Table.3**.**

Antibacterial studies

The newly synthesized compounds were also screened for their antibacterial activity against *Escherichia coli K-12*, *Staphyllococcus aureus*, *Salmonella typhi*, and *Bacillus subtilis* bacterial strains by disc diffusion method. Among the tested compounds, the compounds **C3**, **C4**, **C9** and **C10** have emerged as active against all tested microorganisms.

The antifungal and antibacterial activities of the **C3, C4** and **C9** could be attributed to the presence of halogen substituent. However, based on this promising observation, it is immature to arrive at the conclusion on structure activity aspect of these molecules and further evaluation is needed to recommend them for clinical use. The data is given in the Table 3.

Table 3 Antimicrobial activity of new chalcones C1-C10

Compounds	*Staphylococcus aureus*	*Bacillus subtilis*	*Salmonella typhi*	*Escherichia coli*	*Aspergillus niger*	*Candida albicans*
C1	21(30)	21(30)	20(40)	24(30)	23(30)	20(30)
C2	22(30)	22(40)	22(30)	22(30)	23(20)	21(30)
C3	26(30)	27(30)	26(30)	26(20)	24(30)	21(40)
C4	24(30)	25(30)	23(40)	24(30)	21(30)	23(20)
C5	21(30)	19(40)	23(30)	18(30)	21(40)	17(40)
C6	19(40)	21(30)	20(40)	21(40)	18(20)	17(30)
C7	17(30)	19(30)	17(40)	18(40)	22(20)	11(20)
C8	21(40)	21(30)	23(20)	21(40)	22(40)	22(30)
C9	24(30)	26(20)	24(30)	22(20)	24(30)	24(30)
C10	25(40)	27(30)	26(30)	25(10)	24(10)	19(40)
Standard	24	23	23	25	25	24
DMSO(Control)	0	0	0	0	0	0

Note: - Standard drug used: Bacteria (Ciprofloxcin), Fungal (Fluconazole) (40 µg in100µl).
Compounds used : (40 µg in 10mL- based on MIC concentration).
Control : DMSO (dimethyl sulphoxide)
Zone of Inhibition in mm; MIC in µg/mL (data given in the parenthesis is for MIC).

Putative molecular interactions with metRS by molecular docking simulations

The chalcones **C1-C10** have been docked into the active site of the methionyl-tRNA synthetase (metRS) (PDB ID: 1A8H). The calculated docking and binding (ΔG) energies (in Kcal/mol) of the compounds are shown in Table 4. Based on the calculated binding energies compounds **C4, C9** and **C10** should be potent antibacterial.

Carbon atoms from the ring systems of the compounds **C1, C2** and C**3** showed hydrophobic interactions with Glu138, Pro145, Ile146 and Thr55. Other than the hydrophobic interactions the ketonic oxygen of compounds **C4** and **C5** formed hydrogen bonds with the NH_2 (2.36 Å) and NE (2.43 Å) atoms of Arg132. Compound **C6** also have very similar hydrophobic interactions in addition to Val140 with the side of trimethoxyphenyl part of the compound and hydrogen bonds are also same, but little longer in distance, NH_2 (2.42 Å) and NE (2.63 Å) atoms of Arg132. In the cases of compounds **C7** and **C8** hydrogen bonds are NH_2 (~2.51 Å) and NE (~2.35 Å) atoms of Arg132.Compound **C9** exhibited similar hydrophobic interactions with Glu138, Pro145, Ile146 and Thr55. In the cases of compound **C10** in addition to the hydrophobic interactions like other compounds (with Glu138, Pro145, Ile146 and Thr55) hydrogen bonds are NH_2 (~2.75 Å) and NE (~2.68 Å) atoms of Arg132. The molecular interactions between the chalcones and the active site residues of metRS at 3D space are shown in Fig. 6 different compounds in different panels, accordingly.

Table 4 Calculated binding (ΔG) energies of the compounds against metRS

Compounds	Calculated binding Energies (ΔG in Kcal/mol)
C1	-3.5
C2	-1.7
C3	-4.7
C4	-0.8
C5	-1.1
C6	-1.3
C7	-1.0
C8	-0.7
C9	-4.1
C10	-5.2

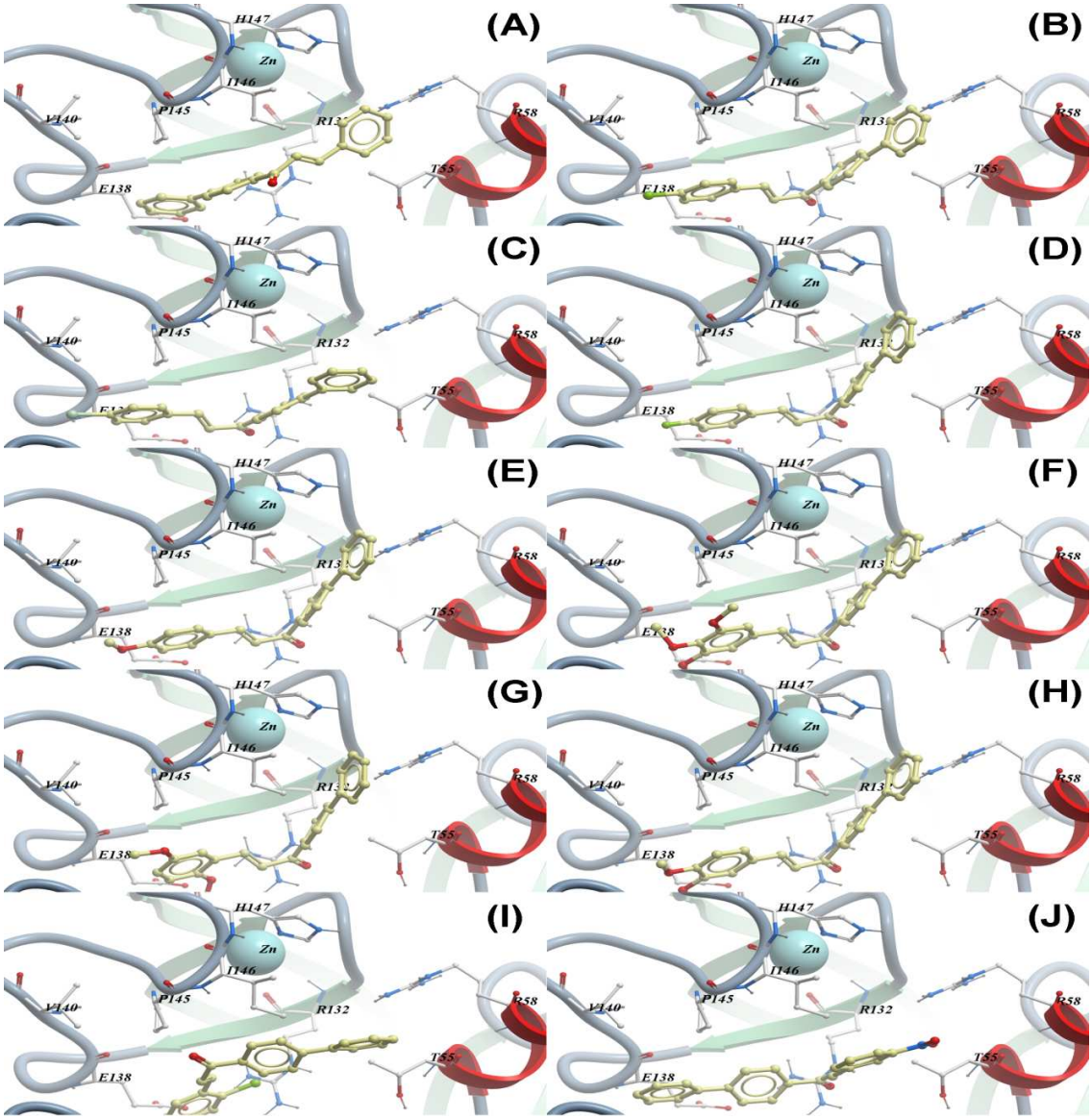


Fig.(6). Molecular interactions with of the chalcones C1 (A), C2 (B), C3 (C), C4 (D), C5 (E), C6 (F), C7 (G), C8 (H), C9 (I) and C10 (J) against methionyl-tRNA synthetase (metRS) (PDB ID: 1A8H). Yellow stick models are the chalcones docked at the active site of metRS and only polar hydrogens are shown.

CONCLUSION

A new series of chalcone derivatives **C1-C10** were synthesized by the reaction of 4- phenyl acetophenone and substituted benzaldehydes in a Claisen Schmidt condensation reaction. They were characterized by analytical and spectral studies. The compounds were screened for DPPH scavenging activity. Among the tested compounds **C5, C7** and **C8** exhibited very good activity. Based on DPPH scavenging activity these molecules were selected to study modulatory effect on gamma radiation induced oxidative stress markers in E. *coli* K12. The compound (2E)-1-(biphenyl-4-yl)-3-(4-methoxyphenyl)prop-2-en-1-one **C5** exhibited significant demolition effect in the levels of lipid peroxidation product [TBARS]. The compounds (2E)-1-(biphenyl-4-yl)-3-(2,5-dimethoxyphenyl)prop-2-en-1-one **C7** and (2E)-1-(biphenyl-4-yl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one **C8** showed good activity towards demolition effect of TBARS level compared to irradiated sample but no statistically significant result was found compared to control. All the tested compounds brought the antioxidant enzyme levels [SOD and CAT] to near basal level in comparison with non irradiated (control) and irradiated controls. The count of Colony Forming Units

(CFU) of *E coli* in the compound treated irradiated samples also supported the antioxidant effect of the chalcone derivatives. In antifungal and antibacterial property evaluation studies the compounds exhibited promising activities. Among them compounds, (2E)-1-(biphenyl-4-yl)-3-(4-fluorophenyl) prop-2-en-1-one **C4** and (2E)-1-(biphenyl-4-yl)-3-(2-chlorophenyl) prop-2-en-1-one **C9** exhibited good activity. The *in silico* inhibitor efficiency of newly synthesized compounds was evaluated with methionyl-tRNA synthetase for antibacterial activity. Among the tested compounds the compounds **C3, C9** and **C10** showed -4.7, -4.1 and -5.2 binding energies respectively. This results support the *in vitro* antimicrobial activity studies. Hence this further opens scope for screening of these newly synthesized molecules for probable antioxidant, radioprotective and antimicrobial activities in higher model system.

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