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SYNTHESIS AND MOLECULAR DOCKING STUDIES OF 1, 3-THIAZOLIDINE 4 ONES AS ANTICANCER AGENTS

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ABSTRACT
Cancer is a major cause of death all over the globe. Controlling cell division by inhibition of mitosis is the most successful clinical strategy for cancer treatment. The development of novel anticancer agents is the most important area in medicinal chemistry and drug discovery research. Thiazolidine is the multifunctional nucleus which shows a number of pharmacological activities like anticancer, anti-inflammatory, antioxidant, antibacterial, antifungal, antidiabetic, antihyperlipidemic and antiarthritic.
Methods: In a present study series of 3-[5-(2-Chloro-phenyl)-isoxazol-3-ylmethyl]-2-Substituted-thiazolidin-4-one (CMT-1 to CMT-12) were designed, synthesized by the microwave-assisted system, and characterized by melting point, IR, IR, ^{1}H NMR, and mass spectroscopy. All the newly synthesized compounds were examined for their *in vitro* anticancer activity against breast cancer cell line MCF-7 by Sulforhodamine B (SRB) assay, antioxidant activity by DPPHassay and angiogenesis activity by chorioallantoic membrane (CAM) of chick embryos assay.
Results: The compounds CMT-12 (GI_{50}: 28.5 µg/ml) and CMT-6 (GI_{50}: 50.7 µg/ml)& CMT-4 (GI_{50}: 53.1 µg/ml) exhibited significant cell growth inhibitory activity.
Conclusion: These results indicate that compound CMT-12 and CMT-6 as related polo-like kinase 1inhibitors compounds could be lead compounds for further development of anticancer agents. The antioxidant activity by DPPH radical scavenging assay the compounds CMT-7 (IC_{50}:11.96 µg/ml), CMT-9 (IC_{50}:10.67 µg/ml) and CMT-10 (IC_{50}:9.08 µg/ml)exhibited excellent radical scavenging activities comparedto ascorbic acid (IC_{50}:13.04 µg/ml). The compounds CMT-4, CMT-8 and CMT-10 at 10 nM test drug concentration and the CMT-6 compounds at 100 nM test drug concentration shows the maximum capillary growth inhibitory activity as compared with thalidomide as standard drug
Conclusion: These results indicate that compound CMT-6, CMT-8, CMT-10 and CMT-12 as related polo-like kinase 1inhibitors compounds could be lead compounds for further development of anticancer agents.

Key Words: Anticancer activity, Antioxidant activity, Angiogenesis activity**,** MCF-7 cell line, Molecular Modelling, Polo-like kinase 1 inhibitors, Synthesis, Thiazolidine-4-one

INTRODUCTION

Cancer is causes an uncontrolled growth of cell divisions in which normal body cells transmute into cancerous cells out of control. As per World Health Organization (WHO) report on cancer 2021 the global cancer burden is significant and increasing and also causes second most fatality worldwide. About 2.26 million cases and 6, 85,000 deaths occurred due to breast cancer in 2020 [1]. Polo-like kinase 1 (PLK1) is a preserved mitotic serine-threonine protein kinases [2]. PLK1 is the most scientifically studied member of the PLK family that interprets a significant role in cell cycle progression. This is required to regulate the various steps involved in the cell cycle progression [3]. PLK1 overexpression may lead to carcinogenesis and represents a specific target for the prevention of various steps involved in tumor cell growth such as mitosis, centrosome maturation, spindle formation, mitotic entry, mitotic exit, chromatin segregation, and cytokinesis [4]. Currently, more than 51 kinase inhibitors are approved for the effective management of various types of cancer [5]. In the current situation, some PLK1 inhibitors are undergoing preclinical and clinical trials in phase 1 and phase 2.

The literature survey reveals that native ligand 1, 3-thiazolidine-4-ones shows a diversity of biological response like anticancer [6], anti-inflammatory [7], antioxidant [8], antibacterial [9], antifungal [10], antidiabetic [11] and antihyperlipidemic [12] activity. The native ligand 1, 3-thiazolidine-4-ones used as an inhibition of polo like kinase 1 enzyme for the uncontrolled growth of cell division into the cancer [13].

In the present study, we propose a series of 1, 3 thiazolidine-4-ones synthesis, structural elucidation and molecular docking studies, followed by screening for their anticancer activity by Sulforhodamine B (SRB) assay on breast cancer cell line MCF-7, Angiogenesis activity on chorioallantoic membrane (CAM) of chick embryos assay**,** Antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH).

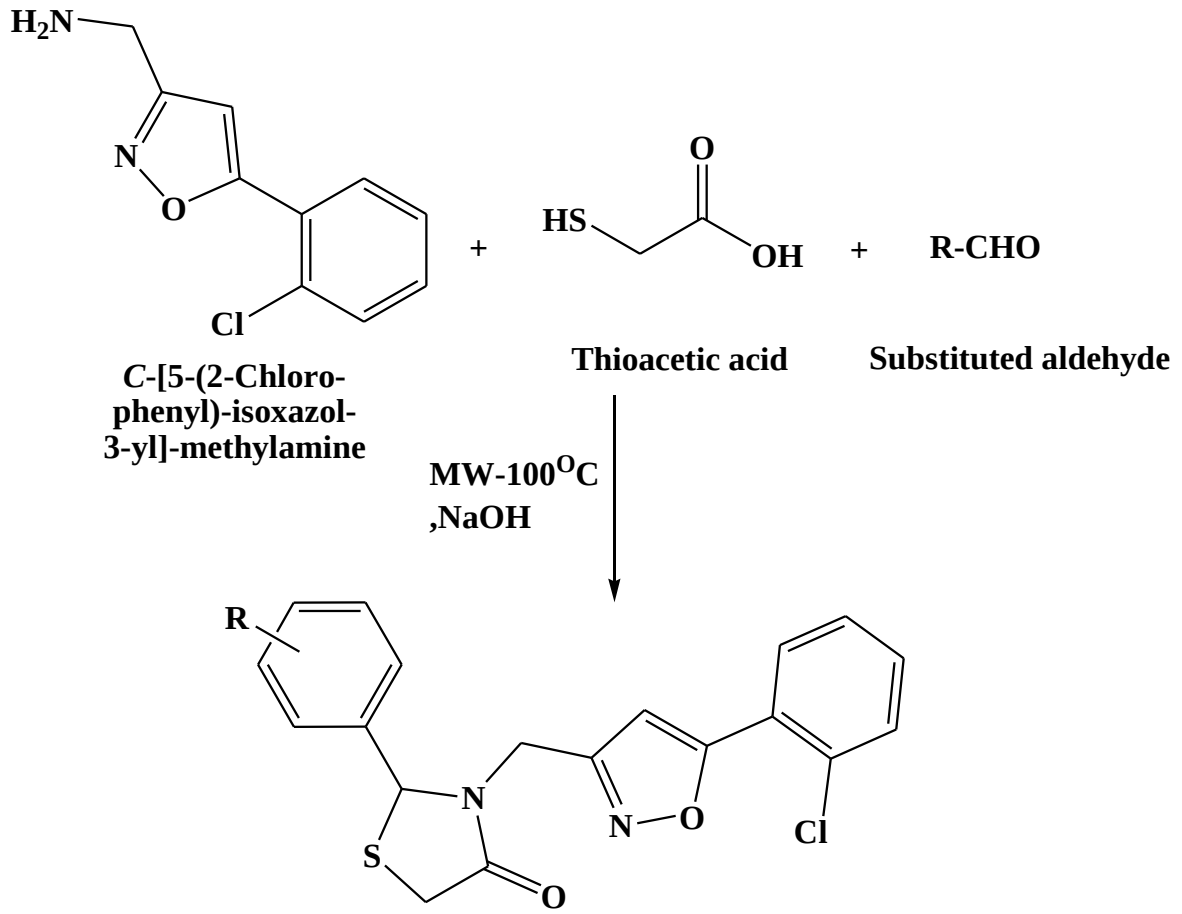
MATERIALS AND METHODS

General

The synthesized compounds were Physical constant (Melting point) was determined by Gallenkamp electric melting point apparatus. Thin layer chromatography (TLC, Silica gel 60 F254, Merck) was used to monitor the progress, amount of untreated starting material and assess the purity of the compounds that were detected under UV light and iodine vapour. All synthesized compounds were characterized by ^{1}H NMR and IR and mass spectroscopy. IR spectra (KBr discs) were recorded on Jasco Infrared Affinity-1 spectrophotometer. ^{1}H NMR, spectra recorded by using Bruker 500 MHz spectrometers (Bruker Bioscience, Billerica, MA, USA) 500 MHz in chloroform ($CDCl_3$) and dimethyl sulfoxide (DMSO-d_6) used as a solvent,tetramethylsilane (TMS) was used as an internal standard. Chemical shifts are shown as δ values (ppm). Signals are shown as s (singlet), d (doublet), t (triplet), q (quintet) or m (multiplet). The chemicals were purchased commercially from Sigma Aldrich, S. D. Fine chemicals, Loba Chemicals and Spectrochem Chemicals.

General method for the synthesis of *3-[5-(2-Chloro-phenyl)-isoxazol-3-ylmethyl]-2-Substituted-thiazolidin-4-ones (CMT-1 to CMT-12)*

A mixture of [5-(2-chlorophenyl) isoxazol-3-yl) methyl) amine (0.01 mol), substituted aldehydes (0.01mol), thioglycolic acid (0.01mol) and sodium hydroxide in ethanol (10 ml) was placed in a round bottom flask. The mixtures were stirred well and then allow for microwave irradiation in the microwave synthesis system (CEM, USA) at 30W for 30 min. After cooling, the mixture was dissolved into ethyl acetate and water. The ethyl acetate layer was dried. The crude product was purified by flash chromatography (BUCHI, Switzerland).The completion of the reaction was monitored by thin layer chromatography and the mobile phase used was ethyl acetate: petroleum ether (2:1).



3-[5-(2-Chloro-phenyl)-isoxazol-3-ylmethyl]-2-substituted-thiazolidin-4-one

Figure 1:Synthesis of3-[5-(2-Chloro-phenyl)-isoxazol-3-ylmethyl]-2-Substituted-thiazolidin-4-ones

3-((5-(2-chlorophenyl) isoxazol-3-yl) methyl)-2-phenylthiazolidin-4-one (CMT-1)

Brown solid; Yield 62%; R_f value 0.69; M.P.:129-131^oC; FT-IR: (KBr,cm^{-1}): 2988, 2584, 1731, 1688, 1594, 1290; ^1H NMR (500 MHz, $CDCl_3$): 7.16-7.42 (m, 4H, Ar-H), 4.46 (s, 2H, CH_2), 5.92 (s, 1H, CH), 3.28-3.38 (s, 2H, CH_2), 7.06-7.14 (m, 5H, Ar-H); MS (ESI): m/z 370.05 $(M+1)^+$; Anal. Calcdfor $C_{19}H_{15}ClN_2O_2S$; C, 61.53; H, 4.08; Cl, 9.56; N, 7.55; O, 8.63; S, 8.65; found C, 61.42; H, 4.17; Cl, 9.36; N, 7.54; O, 8.53; S, 8.68

2-(4-chlorophenyl)-3-((5-(2-chlorophenyl)isoxazol-3-yl) methyl) thiazolidin-4-one (CMT-2)

Black solid; Yield 68%; R_f value 0.72; M.P.:145-147^oC; FT-IR: (KBr,cm^{-1}): 3051, 2565, 1770, 1688, 1594, 1270, 739; ^1H NMR (500 MHz, $CDCl_3$): 7.16-7.42 (m, 4H, Ar-H), 4.46 (s, 2H, CH_2), 5.92 (s, 1H, CH), 3.28-3.38 (s, 2H, CH_2), 7.06-7.14 (m, 4H, Ar-H); MS (ESI): m/z 404.02 $(M+1)^+$; Anal. Calcd for $C_{19}H_{14}Cl_2N_2O_2S$; C, 56.31; H, 3.48; Cl, 17.49; N, 6.91; O, 7.90; S, 7.91; found C, 56.31; H, 3.50; Cl, 17.39; N, 6.92; O, 7.84; S, 7.96

2-(2-chlorophenyl)-3-((5-(2-chlorophenyl) isoxazol-3-yl) methyl) thiazolidin-4-one (CMT-3)
Black solid; Yield 68%; R_f value 0.72; M.P.:145-147ºC; FT-IR: (KBr,cm^{-1}): 3051, 2558, 1710, 1688, 1594, 1309, 824; ^{1}H NMR (500 MHz, $CDCl_3$): 7.16-7.42 (m, 4H, Ar-H), 4.46 (s, 2H, CH_2), 5.92 (s, 1H, CH), 3.28-3.38 (s, 2H, CH_2), 7.06-7.14 (m, 4H, Ar-H); MS (ESI): m/z 404.02 $(M+1)^+$; Anal. Calcd for $C_{19}H_{14}Cl_2N_2O_2S$; C, 56.31; H, 3.48; Cl, 17.49; N, 6.91; O, 7.90; S, 7.91; found C, 56.31; H, 3.50; Cl, 17.39; N, 6.92; O, 7.84; S, 7.96

2-(3-chlorophenyl)-3-((5-(2-chlorophenyl) isoxazol-3-yl) methyl) thiazolidin-4-one (CMT-4)
Black solid; Yield 58%; R_f value 0.62; M.P.:145-147ºC; FT-IR: (KBr,cm^{-1}): 3059, 2573, 1679, 1695, 15546, 1309, 716; ^{1}H NMR (500 MHz, DMSO): 7.16-7.42 (m, 4H, Ar-H), 4.46 (s, 2H, CH_2), 5.92 (s, 1H, CH), 3.28-3.38 (s, 2H, CH_2), 7.06-7.14 (m, 4H, Ar-H); MS (ESI): m/z 404.02 $(M+1)^+$; Anal. Calcd for $C_{19}H_{14}Cl_2N_2O_2S$; C, 56.31; H, 3.48; Cl, 17.49; N, 6.91; O, 7.90; S, 7.91; found C, 56.31; H, 3.50; Cl, 17.39; N, 6.92; O, 7.84; S, 7.96

3-((5-(2-chlorophenyl) isoxazol-3-yl) methyl)-2-(2-nitrophenyl) thiazolidin-4-one (CMT-5)
Black solid; Yield 52%; R_f value 0.72; M.P.:158-160ºC; FT-IR: (KBr,cm^{-1}): 3020, 2573, 1718, 1679, 1509, 1540, 1278; ^{1}H NMR (500 MHz, $CDCl_3$): 7.16-7.42 (m, 4H, Ar-H), 4.46 (s, 2H, CH_2), 5.92 (s, 1H, CH), 3.28-3.38 (s, 2H, CH_2), 7.06-7.14 (m, 4H, Ar-H); MS (ESI): m/z 415.04 $(M+1)^+$; Anal. Calcd for $C_{19}H_{14}ClN_3O_4S$; C, 54.88; H, 3.39; Cl, 8.53; N, 10.10; O, 15.39; S, 7.71; found C, 54.78; H, 3.49; Cl, 8.49; N, 10.09; O, 15.39; S, 7.21

3-((5-(2-chlorophenyl) isoxazol-3-yl) methyl)-2-(3-nitrophenyl) thiazolidin-4-one (CMT-6)
Yellowish solid; Yield 71%; R_f value 0.72; M.P.:158-160ºC; FT-IR: (KBr,cm^{-1}): 3059, 2596, 1772, 1678, 1540, 1509, 1278; ^{1}H NMR (500 MHz, $CDCl_3$): 7.16-7.42 (m, 4H, Ar-H), 4.46 (s, 2H, CH_2), 5.92 (s, 1H, CH), 3.28-3.38 (s, 2H, CH_2), 7.06-7.14 (m, 4H, Ar-H); MS (ESI): m/z 415.04 $(M+1)^+$; Anal. Calcd for $C_{19}H_{14}ClN_3O_4S$; C, 54.88; H, 3.39; Cl, 8.53; N, 10.10; O, 15.39; S, 7.71; found C, 54.78; H, 3.49; Cl, 8.49; N, 10.09; O, 15.39; S, 7.21

3-((5-(2-chlorophenyl) isoxazol-3-yl) methyl)-2-(4-nitrophenyl) thiazolidin-4-one (CMT-7)
Yellowish solid; Yield 55%; R_f value 0.72; M.P.:158-160ºC; FT-IR: (KBr,cm^{-1}): 3071, 2584, 1736, 1694, 1518, 1540, 1267; ^{1}H NMR (500 MHz, $CDCl_3$): 7.16-7.42 (m, 4H, Ar-H), 4.46 (s, 2H, CH_2), 5.92 (s, 1H, CH), 3.28-3.38 (s, 2H, CH_2), 7.06-7.14 (m, 4H, Ar-H); MS (ESI): m/z 415.04 $(M+1)^+$; Anal. Calcd for $C_{19}H_{14}ClN_3O_4S$; C, 54.88; H, 3.39; Cl, 8.53; N, 10.10; O, 15.39; S, 7.71; found C, 54.78; H, 3.49; Cl, 8.49; N, 10.09; O, 15.39; S, 7.21

2-(3-bromophenyl)-3-((5-(2-chlorophenyl) isoxazol-3-yl) methyl) thiazolidin-4-one (CMT-8)
Yellowish solid; Yield 83%; R_f value 0.82; M.P.:135-137ºC; FT-IR: (KBr,cm^{-1}): 3010, 2607, 1739, 1689, 1633, 1267, 666; ^{1}H NMR (500 MHz, $CDCl_3$): 7.16-7.42 (m, 4H, Ar-H), 4.46 (s, 2H, CH_2), 5.92 (s, 1H, CH), 3.28-3.38 (s, 2H, CH_2), 7.06-7.14 (m, 4H, Ar-H); MS (ESI): m/z 449.96 $(M+1)^+$; Anal. Calcd for $C_{19}H_{14}BrClN_2O_2S$; C, 50.74; H, 3.14; Br, 17.77; Cl, 7.88; N, 6.23; O, 7.11; S, 7.13; found C, 50.74; H, 3.14; Br, 17.77; Cl, 7.88; N, 6.23; O, 7.11; S, 7.13

2-(4-bromophenyl)-3-((5-(2-chlorophenyl) isoxazol-3-yl) methyl) thiazolidin-4-one (CMT-9)
Brown solid; Yield 74%; R_f value 0.67; M.P.:145-147ºC; FT-IR: (KBr,cm^{-1}): 3010, 2607, 1739, 1689, 1633, 1267, 666; ^{1}H NMR (500 MHz, $CDCl_3$): 7.16-7.42 (m, 4H, Ar-H), 4.46 (s, 2H, CH_2), 5.92 (s, 1H, CH), 3.28-3.38 (s, 2H, CH_2), 7.06-7.14 (m, 4H, Ar-H); MS (ESI): m/z 449.96 $(M+1)^+$; Anal. Calcd for $C_{19}H_{14}BrClN_2O_2S$; C, 50.74; H, 3.14; Br, 17.77; Cl, 7.88; N, 6.23; O, 7.11; S, 7.13; found C, 50.74; H, 3.14; Br, 17.77; Cl, 7.88; N, 6.23; O, 7.11; S, 7.13

3-((5-(2-chlorophenyl) isoxazol-3-yl) methyl)-2-(3-hydroxyphenyl) thiazolidin-4-one (CMT-10)
Brown solid; Yield 52%; R_f value 0.59; M.P.:165-167ºC; FT-IR: (KBr,cm^{-1}): 2951, 2581, 2200, 1695, 1687, 1309; ^{1}H NMR (500 MHz, $CDCl_3$): 7.16-7.42 (m, 4H, Ar-H), 4.46 (s, 2H, CH_2), 5.92 (s, 1H, CH), 3.28-3.38 (s, 2H, CH_2), 4.46 (s, 2H, CH_2), 7.06-7.14 (m, 4H, Ar-H); MS (ESI): m/z 386.05 $(M+1)^+$; Anal. Calcd for $C_{19}H_{15}ClN_2O_3S$; C, 58.99; H, 3.91; Cl, 9.16; N, 7.24; O, 12.41; S, 8.29; found C, 58.78; H, 3.91; Cl, 9.14; N, 7.24; O, 12.41; S, 8.25

3-((5-(2-chlorophenyl) isoxazol-3-yl) methyl)-2-(4-hydroxyphenyl) thiazolidin-4-one (CMT-11)
Brown solid; Yield 52%; R_f value 0.69; M.P.:165-167ºC; FT-IR: (KBr,cm^{-1}): 2951, 2581, 2200, 1695, 1687, 1309; ^{1}H NMR (500 MHz, $CDCl_3$): 7.16-7.42 (m, 4H, Ar-H), 4.46 (s, 2H, CH_2), 5.92 (s, 1H, CH), 3.28-3.38 (s, 2H, CH_2), 4.46 (s, 2H, CH_2), 7.06-7.14 (m, 4H, Ar-H); MS (ESI): m/z 386.05 $(M+1)^+$; Anal. Calcd for $C_{19}H_{15}ClN_2O_3S$; C, 58.99; H, 3.91; Cl, 9.16; N, 7.24; O, 12.41; S, 8.29; found C, 58.78; H, 3.91; Cl, 9.14; N, 7.24; O, 12.41; S, 8.25

3-((5-(2-chlorophenyl) isoxazol-3-yl) methyl)-2-(3-methoxyphenyl) thiazolidin-4-one(CMT-12)
Black solid; Yield 52%; R_f value 0.55; M.P.:152-154ºC; FT-IR: (KBr,cm^{-1}): 3067, 2581, 1795, 1687, 1556, 1301; ^{1}H NMR (500 MHz, $CDCl_3$): 7.16-7.42 (m, 4H, Ar-H), 4.46 (s, 2H, CH_2), 5.92 (s, 1H, CH), 3.28-3.38 (s, 2H, CH_2), 4.46 (s, 2H, CH_2), 3.73 (s, 3H, CH_3), (7.06-7.14 (m, 4H, Ar-H); MS (ESI): m/z 400.06 $(M+1)^+$; Anal. Calcd for $C_{20}H_{17}ClN_2O_3S$; C, 59.92; H, 4.27; Cl, 8.84; N, 6.99; O, 11.97; S, 8.00; found C, 59.96; H, 4.27; Cl, 8.84; N, 6.72; O, 11.84; S, 8.02

Molecular docking studies
Docking procedure aims to identify the correct binding poses and binding interaction within the binding site of the protein. Molecular docking is performed with VLife MDS software. The crystal structure of Polo-like kinase 1 (PDB entry code-2rku) was extracted from protein data bank database (http://www.rcsb.org/pdb). The 2D structures were drawn and converted into 3D structures. The 3D structure was optimized by using optimization tool up to the rms gradient of 0.01 using MMFF. Protein structure was optimized. Cavity no.1 is selected for docking procedure. The active site of protein was defined as all atoms within 5A˚ radius. Docking studies were performed by batch docking to get docking score and interactions between ligand and target protein.

Anticancer activity (Sulforhodamine B assay)
The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96 well microtiter plates in 90 µL at 5000 cells per well. After cell inoculation, the microtiter plates were incubated at 37°C, 5%CO2, 95% air and 100 % relative humidity for 24 h prior to addition of experimental drugs. Experimental drugs were solubilized in appropriate solvent to prepare stock of 10^{-2} concentration. At the time of experiment four 10-fold serial dilutions were made using complete medium. Aliquots of

10 µl of these different drug dilutions were added to the appropriate micro-titer wells already containing 90 µl of medium, resulting in the required final drug concentrations. After compound addition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 µl of cold 30 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4 % (w/v) in 1 % acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1 % acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on an Elisa plate reader at a wavelength of 540 nm with 690 nm reference wavelength.

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells * 100. Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. The dose response parameters were calculated for each test article. Growth inhibition of 50 % (GI50) was calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from Ti = Tz. The LC50 (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50. Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the values for that parameter were expressed as greater or less than the maximum or minimum concentration tested [14, 15].

Angiogenesis activity by *chorioallantoic membrane (CAM) of chick embryos assay*

The chicken chorioallantoic membrane (CAM) assay was performed using eight-day-old fertilized chicken eggs. A 1-cm diameter window was created in the shell of each egg and the surface of the dermic sheet was removed to expose the CAM. A 0.5-cm diameter filter paper was placed on top of the CAM, and a volume of 1 nM, 10 nM, 100 nM drug (control, Thalidomide) was placed on the center of the filter paper. Then the windows in the shell were closed using sterilized bandages. The eggs were incubated at 37˚C at 90% relative humidity for 48 h. Following fixation with stationary solution (a mixture of methanol and acetone with a volume ratio of 1:1) for 15 min, the CAM was excised and imaged using a digital camera. The morphology of chicken blood vessels with different treatments was detected [16].

Antioxidant activity

DPPH radical scavenging assay

The standard solution was prepared by dissolving 100 mg of ascorbic acid in methanol to give the concentration of 10, 20, 30, 40 and 50µg/mL. The tests solutions were prepared by dissolving 10 mg of compounds (**CMT-1** to **CMT-12**) in 10 mL of methanol to give 100 µg/mL of stock solution of each compound. The concentrations 10, 20, 30, 40 and 50µg/mL were prepared using this stock solution. To each dilution 150 µL of DPPH was added and kept in dark for 30 min. The 150 µL DPPH solution was added to 10 mL methanol and absorbance was taken immediately at 517 nm as control reading. 10 mL of different concentrations of test sample (10, 20, 30, 40, 50 µL) prepared with methanol were taken and 150 µL DPPH solution was added to each test tube. Absorbance was taken at 517 nm in UV visible spectrophotometer (Jasco, UV-630) after 15 min using methanol as a blank.

The free radical scavenging activity (FRSA) (% inhibition) was calculated using the following equation. The % inhibition for different log concentrations were plotted to obtain a concentration *vs.* % inhibition graph from which IC_{50} value was calculated [17].

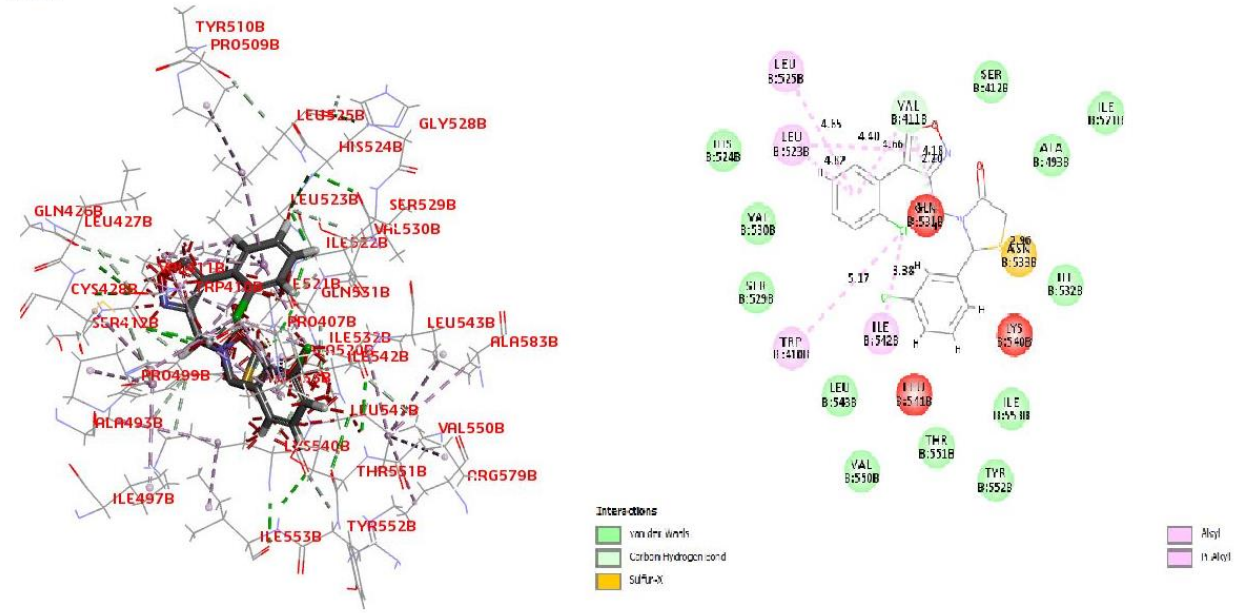
$$\% \textbf{ Inhibition} = \frac{(\textbf{Absorbance of control} - \textbf{Absorbance of test})}{\textbf{Absorbance of control}} \boldsymbol{X}\ \mathbf{100}$$

RESULTS AND DISCUSSION

Molecular docking studies:

The molecular docking study was carried out to explore binding modes of our derivatives with the PLK1 enzyme. The preceding results encouraged us to study the molecular docking of the most active compoundsCMT-6, CMT-8, CMT-10 and CMT-12 using PLK1, which are overexpressed in numerous tumors such as prostate (PC-3), breast (MCF-7), hepatocellular carcinoma (HepG2), and human cervical (HeLa) cancer cell lines. All docking calculations were performed using VLife MDS 4.4 software. The docked compounds CMT-1 to CMT-12 with (Protein Data Bank [PDB] code 1q4k) into the putative active site of PLK1 is shown in Figure 2.The molecular modelling results of the compound, CMT-6, CMT-8, CMT-10 and CMT-12demonstrated an approximate orientation of the molecule inside the putative binding site of receptor pocket with some additional hydrophobic and hydrogen bonding interactions with surrounding amino acids.These docking results showed the compounds with phenyl ring containing *meta*-methoxy groups correctly shown to exhibit good binding interaction and elucidate the good docking score (-5.30), the compound with phenyl ring containing *meta*-chloro(-4.64) groups shows better docking score than the phenyl ring containing *ortho*-bromogroups. The phenyl ring containing *meta*-bromo group elucidates good protein-ligand binding interactions and good docking score (-4.77) than the phenyl ring containing *meta* (-3.33) and *para* (-3.59) hydroxyl phenyl containing groups. The dock scores (kcal/moles) of all compounds were shown in Table1. The nitrogen atom of isoxazol ring forms hydrophobic bonding with amino acid residues LEU525B, LEU523B. The chlorine group substituted on isoxazol containing phenyl ring shows a hydrophobic bonding with amino acid residues ILE542B, LEU525B, TRP520B, TRP410B, LEU523B, VAL550B, LEU541B as well as VAL411B, LEU543B, ALA493B. The binding site of interactions between the most active title compound CMT-12 with the polo like kinase 1 (PLK 1) was recorded. The binding interaction of the title compound CMT-12 at the binding sites showed 5 hydrophobic bonding with the amino acid residues. According to molecular modelling studies thiazolidine-4-ones are a good inhibitor of polo-like kinase-1. On the basis of molecular docking study also reveals that the compound CMT-6, CMT-8, CMT-10 and CMT-12 shows a good binding interaction and significant anticancer activity. The molecular docking study shows similar results in some extent.

CMT-4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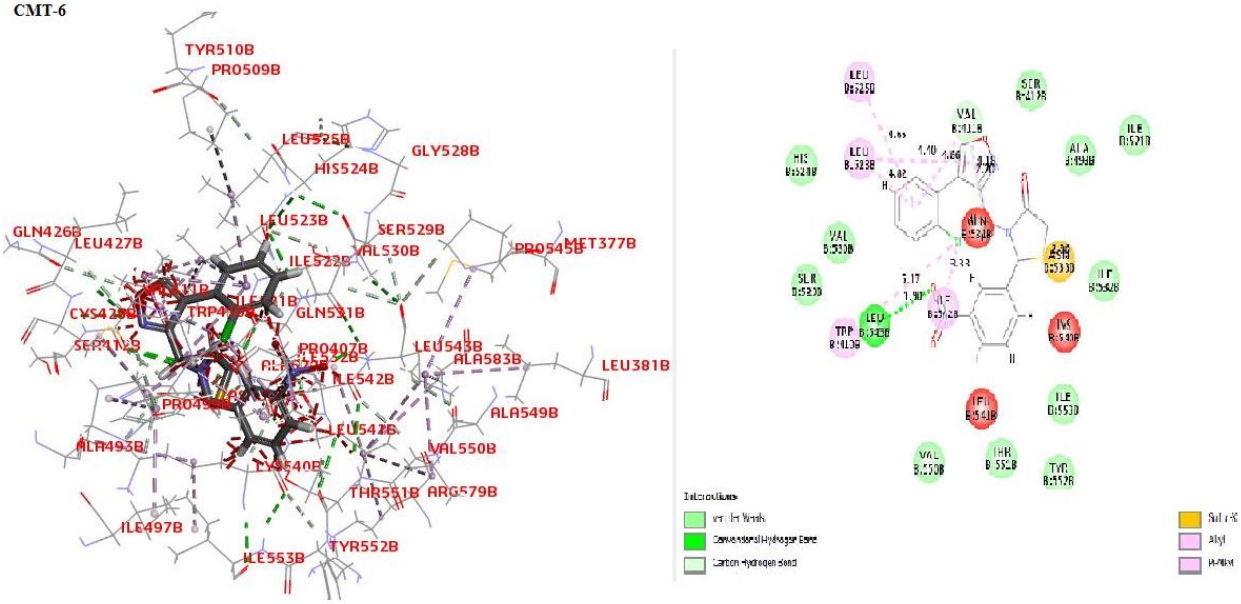
Sulfur-X
Alkyl

CMT-6


TYR510B
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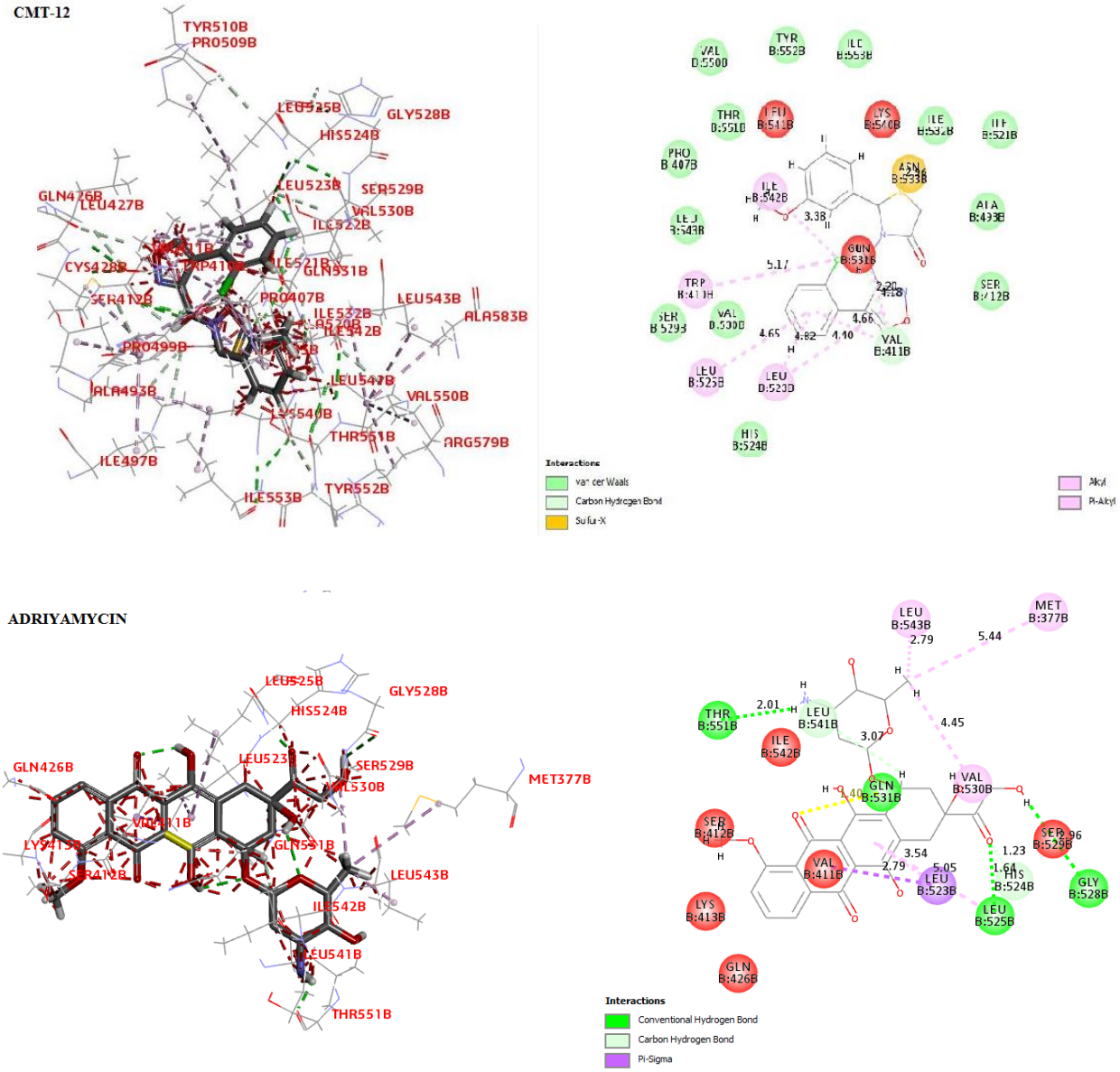

Figure 2: Molecular docking interactions images of 1, 3-thiazolidine analogues with polo like kinase-1

Table 1:Binding free energy and key interactions at the binding site of PLK1 and GI_{50} value (µg/ml) on MCF-7 human breast cancer cell line

		BINDING FREE ENERGY AND KEY INTERACTIONS AT THE BINDING SITE			**GI_{50} (µg/ml) MCF-7 cell lines**
Compounds	**R**	**Docking score**	**Hydrophobic bonding**	**Hydrogen bonding**	
CMT-1	$-C_6H_5$	-4.44	LEU525B, LEU523B,	VAL411B, ALA493B,	>80

			TRP410B, ILE542B	LEU543B, THR551B	
CMT-2	4-Cl-C_6H_5	-4.07	LEU525B, LEU523B, TRP410B, ILE542B, VAL550B, ILE542, LEU541B	VAL411B, LEU543B, ALA493B, VAL530B	>80
CMT-3	2-Cl-C_6H_5	-2.56	LEU525B, LEU523B, TRP410B, ILE542B	LEU543B, VAL411B, ALA493B, THR551B	53.7
CMT-4	3-Cl-C_6H_5	-3.46	LEU525B, LEU523B, TRP410B, ILE542B	ASN533B, VAL411B, LEU541B	53.1
CMT-5	4-NO_2-C_6H_5	-1.98	TRP410B, LEU525B, LEU523B, ILE542B	ASN533B, VAL411B, VAL530B, ILE553B	>80
CMT-6	3-NO_2-C_6H_5	-4.64	LEU525B, TRP410B, ILE542B, LEU523B	VAL411B, ALA493B, LEU543B	50.7
CMT-7	4-NO_2-C_6H_5	-2.74	LEU525B, TRP410B, ILE542B, LEU523B	VAL411B, ALA493B, LEU543B	>80
CMT-8	3-Br-C_6H_5	-4.77	LEU525B, TRP410B, ILE542B, LEU523B	VAL411B, ALA493B, LEU543B	79.5
CMT-9	4-Br-C_6H_5	-3.86	LEU525B, LEU523B, TRP410B, VAL550B, ILE542B, LEU541B	VAL411B, ALA493B, LEU543B	64.2
CMT-10	3-OH-C_6H_5	-3.33	LEU525B, TRP410B, ILE542B, LEU523B	VAL411B, ALA493B, LEU543B	>80
CMT-11	4-OH-C_6H_5	-3.59	LEU525B, TRP410B, ILE542B, LEU523B	VAL411B, ALA493B, LEU543B	>80
CMT-12	3-OCH_3-C_6H_5	-5.30	LEU525B, TRP410B, ILE542B, LEU523B	VAL411B, ALA493B, LEU543B	28.5
ADR	-	-	-	-	<10

ADR: Adriamycin, Adriamycin was used as standard anticancer drug.

Anticancer potential

In this study a novel series of 3-[5-(2-Chloro-phenyl)-isoxazol-3-ylmethyl]-2-Substituted-thiazolidin-4-oneswere designed and synthesized. All synthesized compounds were screened by sulforhodamine (SRB) assay against breast cancer MCF-7 cell line, in comparison with adriyamycin as reference drug. Results are summarized in Table 1 and are expressed as GI_{50}**(µg/ml)** (50% growth inhibitory concentration) values. The anticancer screening of the tested compounds revealed that compound CMT-6 (GI_{50}:50.7 **(µg/ml)**) and CMT-4 (GI_{50}:53.1**(µg/ml)**) exhibited a significant cytotoxic activity against MCF-7 breast cancer. The most promising compound CMT-12 (GI_{50}: 28.5**(µg/ml)**) displayed excellent activity against MCF-7 cell lines,

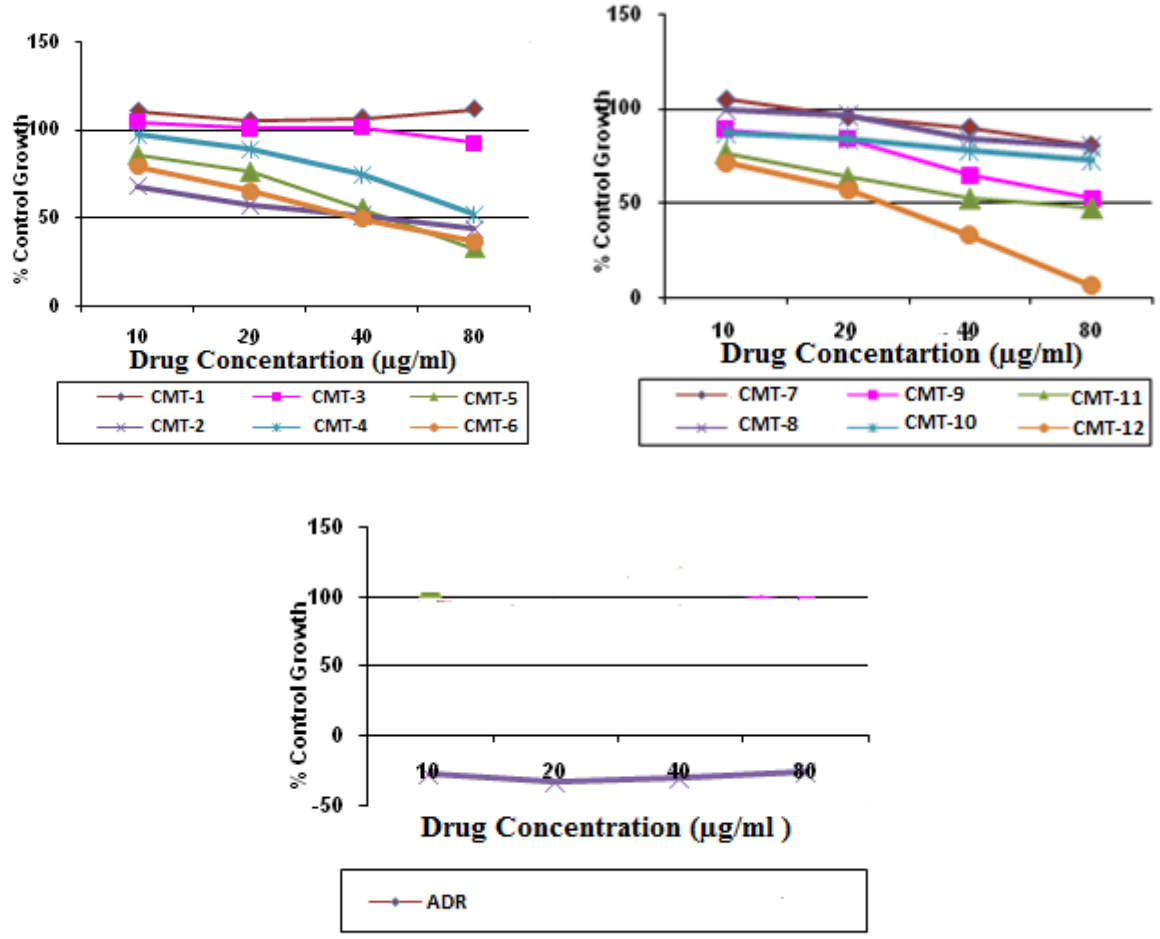

Figure 3: Cell cytotoxicity determined by Sulforhodamine B assay. The title compound (CMT-1 to CMT-12) tested with various concentrations on MCF-7 (Human breast cancer cell line) and compared with standard drug of Adriamycin. Graph showed growth curve of human breast cancer cell line MCF-7.

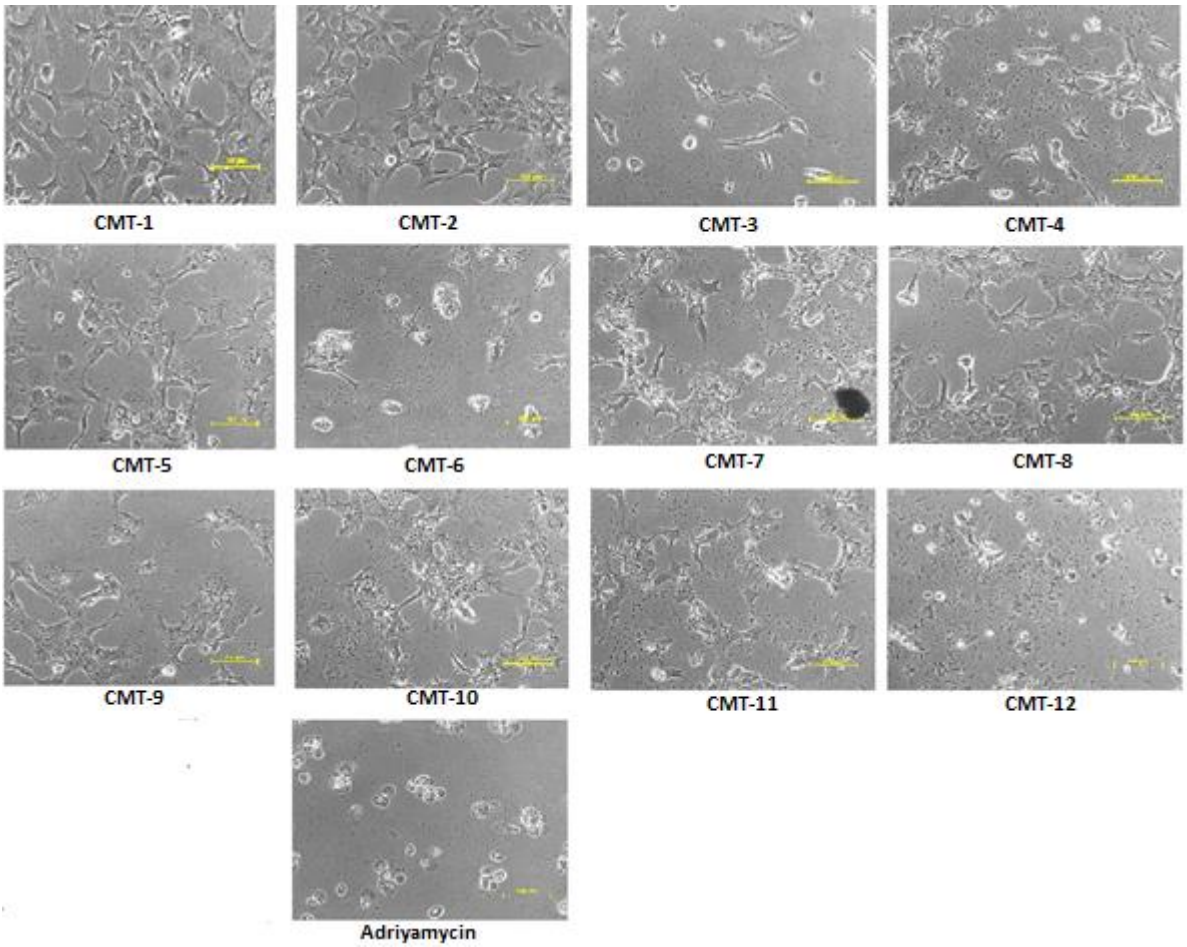

Figure 4: Morphological changes of the human breast cancer cell line MCF-7 after treatment. MCF-7 cells were treated with title compounds (CMT-1 to CMT-12) for 48 h and the morphological changes of cells were observed under a transmission electron micrograph.

Angiogenesis activity

Chorioallantoic membrane (CAM) of chick embryos assay

The CAM assay results of 3-[5-(2-Chloro-phenyl)-isoxazol-3-ylmethyl]-2-Substituted-thiazolidin-4-ones **(CMT-1 to CMT-12)** gives 100% inhibitions of blood vessel growth in chick embryo eggs. The 1 nM drug concentration of CMT-2, CMT-3, CMT-6, CMT-9, and CMT-10 compounds shows a excellent 100 % capillary growth inhibition as well as 10 nM drug concentration of CMT-2, CMT-3, CMT-4, CMT-6, CMT-8, CMT-9, and CMT-10 compounds shows an excellent capillary growth inhibitory activity. The 100 nM drug concentration of CMT-2, CMT-3, CMT-4, CMT-6, CMT-8, CMT-9, CMT-10 compounds shows a good capillary growth inhibitory activity.

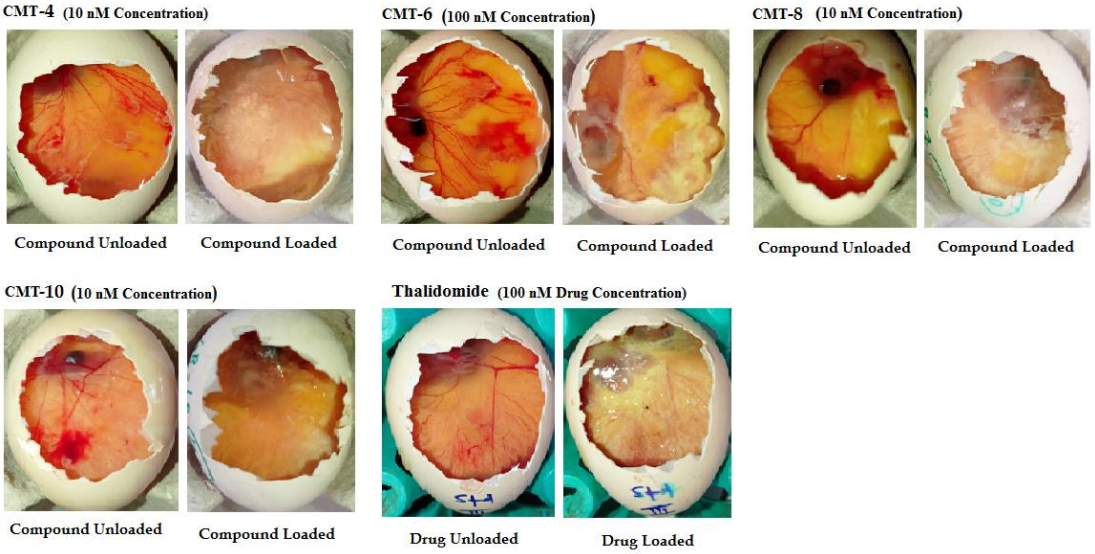

Figure 5: Angiogenesis activity chorioallantoic membrane (CAM) of chick embryos

Antioxidant activity

The antioxidant activity was properly evaluated using in vitro tests: DPPH radical scavenging assays. For each compound it was calculated effective concentration (IC_{50}) by linear regression. The satisfactory results were expressed as IC_{50} value which correctly represents the concentration where half of the substrate is being reduced by the tested compounds.

DPPH radical scavenging assay

The antioxidant activity of the title compounds 3-[5-(2-Chloro-phenyl)-isoxazol-3-ylmethyl]-2-Substituted-thiazolidin-4-ones **(CMT-1 to CMT-12)** and the parent compound ascorbic acid were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay method. The antioxidant compounds reacts with DPPH radical through the hydrogen atom donation mechanism and producing the alteration of DPPH's color from violet to yellow measured by UV-visible spectrophotometer. The satisfactory result of the antioxidant activity at different test concentrations and IC_{50} value in µg/ml of the synthesized compounds (**CMT-1 to CMT-12**) are demonstrates in**Table 2**. The data shows that among all the synthesized compounds in IC_{50} value in (µg/ml) CMT-6: 11.96, CMT-8: 10.67, CMT-10: 9.08, CMT-11: 10.30, CMT-12: 10.63exhibited excellent radical scavenging activities comparedto ascorbic acid: 13.04µg/ml. The reason for higher antioxidantactivity of compound **CMT-10, CMT-11, and CMT-12** may be due to the methoxy &hydroxyl substituents on the phenyl ring attached with thiazolidine moiety; where as in compounds **CMT-2, CMT-6** and **CMT-8**exhibited moderate antioxidant potential and anticancer activity study shows similar results in some extent. There for, thesemolecules could be developed for antioxidant agent and canbe potential polo like kinase 1 inhibitors and can be promisinganticancer agents.

Table 2: Antioxidant activity by DPPH assay

Comp. ID	% inhibition					IC_{50} (µg/ml)
	Concentration in µg/ml					
	10	20	30	40	50	
CMT-1	38.28	49.42	66.00	75.89	80.71	19.17
CMT-2	45.52	55.24	65.73	66.60	80.21	14.33

CMT-3	41.54	48.63	60.40	65.36	79.04	20.20
CMT-4	43.24	42.45	67.51	72.93	83.42	19.26
CMT-5	39.96	55.21	61.24	66.96	78.76	18.34
CMT-6	47.47	53.89	60.55	84.00	91.30	15.19
CMT-7	51.57	57.18	63.61	71.19	91.91	11.96
CMT-8	36.83	61.11	73.07	78.39	85.54	15.19
CMT-9	44.09	65.24	72.33	80.24	88.52	10.67
CMT-10	50.86	55.33	71.96	72.86	81.56	9.08
CMT-11	50.86	57.42	70.03	77.55	88.86	10.30
CMT-12	56.36	55.33	56.81	67.45	89.00	10.63
Ascorbic acid	41.20	64.54	67.97	78.88	86.52	13.04

CONCLUSION:

In this report, we synthesized some novel 3-[5-(2-Chloro-phenyl)-isoxazol-3-ylmethyl]-2-Substituted-thiazolidin-4-ones **(CMT-1 to CMT-12).** The newly synthesized compounds were properly evaluated for their anticancer, antioxidant activity and anti-angiogenetic activity. The results of the experimental and molecular docking data found in good correlation and demonstrated that compound **GB-6, GB-8** and **GB-10** exhibited good anticancer potential against breast cancer cell line MCF-7 and also showed good antioxidant and anti-angiogenic activity. Molecular docking studies also suggest that the activity of the compounds may be due to the inhibition of polo-like kinase 1. These results indicate that compound **GB-4, GB-6** and **GB-12** as related polo-like kinase 1 inhibitors compounds could be lead compounds for further development of anticancer agents.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest

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