1, 3-Thiazolidine-4-Ones: Copper Nickel Oxide Bimetallic Nanoparticle Catalyzed Synthesis, Anticancer Potential and Molecular Modeling Studies

Ramesh L. Sawant

Padmashri Doctor Vithalrao Vikhe Patil Foundation's College of Pharmacy <https://orcid.org/0000-0003-0064-7122>

Manisha R. Sawant

New Arts Commerce and Science College

Jyoti B. Wadekar

Padmashri Doctor Vithalrao Vikhe Patil Foundation's College of Pharmacy

Rushikesh D. Ukirde

Padmashri Doctor Vithalrao Vikhe Patil Foundation's College of Pharmacy

Ganesh D. Barkade (✉ ganeshbarkade7@gmail.com)

Padmashri Doctor Vithalrao Vikhe Patil Foundation's College of Pharmacy <https://orcid.org/0000-0003-3836-3125>

Anjali S. Rajbhoj

Dr Babasaheb Ambedkar Marathwada University

Research Article

Keywords: Nanocatalytic synthesis, Copper nickel oxide nanoparticles, Thiazolidine-4-ones, Anticancer activity, Angiogenesis activity, Antioxidant activity

Posted Date: September 9th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-872668/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Cancer is a leading cause of death worldwide. Inhibiting mitosis is the most effective clinical technique for cancer treatment. The most critical field of medicinal chemistry and drug development research is the discovery of innovative anticancer drugs. Thiazolidine is a multifunctional nucleus with anticancer, anti-inflammatory, antioxidant, antibacterial, antifungal, antidiabetic, antihyperlipidemic, and antiarthritic properties.

Methods: In this investigation, copper-nickel oxide nanoparticles synthesized by electrochemical synthesis yielded a significant yield. The capping was done with cetyltrimethyl ammonium bromide (CTAB), and the characterization was done with UV, FTIR, XRD, SEM-EDS, and TEM SAED. The biologically significant 2-(2-substituted-4-oxo-thiazolidine-3-yl)-1, 9-dihydro-purin-6-ones (GB-1 to GB-12) have been synthesized using copper-nickel oxide nanoparticles as a catalyst and by applying a microwave-assisted tool. It was characterized by melting point, IR, ^1H NMR, ^{13}C NMR and LC-HRMS/MS spectroscopy. Using Sulforhodamine B (SRB) test, all newly synthesized compounds were evaluated *in vitro* for anti-cancer, anti-inflammatory, antioxidant, and angiogenesis activities.

Results: The compounds GB-6 (GI_{50} : 30 μM), GB-8 (GI_{50} : 10 μM) and GB-10 (GI_{50} : 30 μM) exhibited significant cell growth inhibitory activity. The compounds GB-6, GB-8, GB-10 exhibited significant *in vitro* anti-inflammatory activity in the range of IC_{50} :179.65-194.59 $\mu\text{g/ml}$ as compared with aceclofenac (IC_{50} :191.19 $\mu\text{g/ml}$) and the antioxidant activity by DPPH radical scavenging assay the compounds GB-6 (IC_{50} :11.96 $\mu\text{g/ml}$), GB-8 (IC_{50} :10.67 $\mu\text{g/ml}$) and GB-10 (IC_{50} : 9.08 $\mu\text{g/ml}$) exhibited excellent radical scavenging activities compared to ascorbic acid (IC_{50} :13.04 $\mu\text{g/ml}$) and by the KMnO_4 radical scavenging assay the compounds GB-2 (IC_{50} :15.33 $\mu\text{g/ml}$), GB-4 (IC_{50} :23.60 $\mu\text{g/ml}$), GB-8 (IC_{50} :24.93 $\mu\text{g/ml}$), GB-10 (IC_{50} :24.96 $\mu\text{g/ml}$) exhibited good radical scavenging activities compared to ascorbic acid (IC_{50} : 26.55 $\mu\text{g/ml}$). The compounds GB-4, GB-8 and GB-10 at 10 nM test drug concentration and the GB-6 compounds at 100 nM test drug concentration show the maximum capillary growth inhibitory activity compared with thalidomide as a standard drug.

Conclusion: Further development of anticancer drugs may be enabled by the discovery of related compounds to the anticancer agent, such as compound (GB-6), compound (GB-8), and compound (GB-10) as polo-like kinase 1 inhibitors.

Introduction

Cancer causes uncontrolled growth of cell divisions in which normal body cells transmute into cancerous cells out of control. As per the World Health Organization (WHO) report on cancer 2021, the global cancer burden is significant and increasing and causes the second most fatality worldwide. In 2020, breast cancer will account for around 2.26 million new cases and 6,855,000 deaths [1]. Polo-like kinase 1 (PLK1) is a preserved mitotic serine-threonine protein kinase [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 result in carcinogenesis and is a specific target for inhibiting numerous stages involved in tumor cell proliferation, including mitosis, centrosome maturation, spindle formation, mitotic entry, mitotic exit, chromatin segregation, and cytokinesis [4]. At the moment, over 51 kinase inhibitors have been licensed to treat various forms 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 responses 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 is used to inhibit polo-like kinase 1 enzyme for the uncontrolled growth of cell division into cancer [13]. Metallic nanocatalysts have played a vital role in the chemical industry; optimizing their catalytic activity enables significant cost reduction [14]. Due to the extraordinary features of nanoparticles, such as optical, catalytic, and electric capabilities that may be adjusted depending on the particle's size, size distribution, and morphology [15], nanoparticles have been extensively explored. Nanoparticles (NPs) are typically spherical particles with a 1–100 nm and NPs have a larger surface-to-volume ratio than non-nano scale particles of the same material, making them more reactive [16]. Cu-NiO, mixed metal oxide nanoparticles, can operate as a catalyst in a variety of organic reactions, including methanol production and non-enzymatic glucose sensing [17]. Numerous methods for preparing copper-nickel oxide nanoparticles have been reported in the literature, including spray pyrolysis, electro-deposition via the sol-gel process, pulsed laser deposition, pulsed plasma deposition, co-evaporation, DC magnetron sputtering, and RF magnetron sputtering [18]. The present study proposes the electrochemical synthesis of copper-nickel oxide nanoparticles and their analysis and the synthesis, structural elucidation, and molecular docking of 1, 3 thiazolidine-4-ones, followed by screening for anticancer activities against the breast cancer cell line MCF-7 using the Sulforhodamine B (SRB) assay.

Materials And Methods

Chemistry

Copper-nickel oxide nanoparticles were analyzed using a UV-visible spectrophotometer (UV 1800 Shimadzu), a Fourier transform infrared spectrophotometer (FTIR Affinity 1 Shimadzu), a thermogravimetric analyzer (TGA 50 Thermoanalyzer Shimadzu), a high-end X-ray diffractometer, scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS), and transmission electron microscopy elected area electron diffraction (TEM-SAED) techniques. Flash chromatography was used to purify the titled compounds. Melting points (m.p.) were obtained in uncorrected open capillary tubes using a Gallenkamp electric melting point device. Thin-layer chromatography (TLC, Silica gel 60 F254, Merck) was used to monitor the reaction's course and determine the purity of the chemicals identified via UV light and iodine vapor absorption. IR, ^1H NMR, and ^{13}C NMR spectroscopy were used to characterize all compounds, where as LC-HRMS/MS (small molecules) spectroscopy was used to characterize two compounds. On a Jasco IR Affinity-1 spectrophotometer, IR spectra (KBr discs) were recorded. Bruker 500

MHz ^1H NMR spectra were acquired in chloroform (CDCl_3) and dimethyl sulfoxide (DMSO-d_6) solvents, with tetramethylsilane (TMS) serving as an internal standard. Chemical shifts are expressed in terms of values (ppm). Signals are denoted by the letters s (singlet), d (doublet), t (triplet), q (quintet), and m (multiplet) (multiplet). Bruker 500 MHz ^{13}C NMR spectra were acquired in chloroform (CDCl_3) and dimethyl sulfoxide (DMSO-d_6) solvents, with tetramethylsilane (TMS) used as an internal standard. Chemical shifts are expressed in terms of values (ppm). The LC-HRMS/MS data were acquired using an Impact II UHR-TOF Mass Spectrometer System and a Dionex UHPLC Ultimate 3000 System spectrometer. The molecular ion for the majority of the substances was identified as $(\text{M}+1)^+$. Commercially available compounds were obtained from Sigma Aldrich, S. D. Fine Chemicals, Loba, and Spectrochem.

Electrochemical synthesis:

To prepare copper-nickel oxide nanoparticles, cetyltrimethyl ammonium bromide (CTAB) was dissolved in water to obtain 0.01 N solutions. A 50 mL electrolysis cell vessel was filled with the solution mentioned above as one of the electrodes, a 1 cm x 1 cm sheet of copper and nickel was used. Another 1 cm x 1 cm platinum sheet serves as an inert electrode. Two electrode sheets were immersed in a 0.01 N solution of CTAB. CTAB works as a supporting electrolyte and stabilizing agent during this synthesis process, preventing the generated nanoparticles from growing further. The copper and nickel technique metals are oxidized and transformed to copper and nickel ions during this electrochemical process to create nanoparticles. Copper ions generated in this way migrate towards the cathode. They produce nanoscale oxides of copper-nickel in the electrolyte solution and at the contact between the cathode's surface and the electrolyte solution. Electrolysis was carried out at various current densities. The applied current densities in mA/cm^2 were 5, 10, 15, and 20, and the electrolysis was carried out for 2 hours. The color of the solution varies during this electrolysis process. The colorless solution first changed to a bright blue hue, a dirty green hue, and eventually a dark brown residue. After a two-hour interval, the electrolysis process was ended. The brown precipitate-containing solution was then collected in a bottle. Permitted the solid particles to settle and decantation was used to separate the solid obtained in this manner. Three to four times with water washed the separated solid to achieve complete elimination of excess capping agent.

General procedure for the synthesis of 2-(2-substituted-4-oxo-thiazolidin-3-yl)-1, 9-dihydro-purin-6-ones (GB-1 to GB-12)

In a round bottom flask, guanine (0.01 mol), substituted aldehydes (0.01 mol), thioglycolic acid (0.01 mol), and sodium hydroxide (0.01 mol) were combined in ethanol (10 ml). The mixture was vigorously agitated and then refluxed for 30 minutes at 40W using a microwave synthesizer system (CEM, USA). Once the reaction mixture was reduced to ambient temperature, it was placed in crushed ice and stirred

for 5 minutes. The resulting compounds were filtered. Purification of the crude product was accomplished using flash chromatography. The completeness of the reaction was monitored using thin-layer chromatography.

2-(4-Oxo-2-phenyl-thiazolidin-3-yl)-1, 9-dihydro-purin-6-one (GB-1)

White solid; Yield: 64.07%; mp: 160–162⁰C; FT-IR (KBr) Vmax: 3323, 2927, 2561, 1714, 1659, 1356 cm⁻¹; ¹H NMR (DMSO-d₆, 500 MHz): δ 4.28 (s, 2H), 7.06–7.14 (m, 5H), 7.97(s, 1H), 5.92(s, 1H), 3.33(s, 2H); ¹³C NMR (DMSO-d₆, 500 MHz) δ 171.216, 167.290, 159.544, 139.544, 129.135, 128.677, 127.943, 120.172, 114.629, 53.173, 40.263, 39.929, 39.763, 34.466; LC-HRMS/MS (ESI): m/z, calcd (M +) 314.06, found 314.0071.

2-[2-(4-Chloro-phenyl)-4-oxo-thiazolidin-3-yl]-1, 9-dihydro-purin-6-one (GB-2)

Yellow solid; Yield: 59.22%; mp: 144–146⁰C; FT-IR (KBr) Vmax: 3323, 2927, 2615, 1714, 1651, 1348, 757 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz): δ 4.28 (s, 2H, NH), 7.00–7.15 (m, 4H), 7.97(s, 1H), 5.92(s, 1H, CH), 3.33(s, 2H); ¹³C NMR (CDCl₃ 500 MHz) δ 130.035, 129.156, 128.081, 127.834, 127.262, 127.118, 126.505, 77.269, 77.016, 76.742, 43.599, 39.788, 33.179, 24.345; LC-HRMS/MS (ESI): m/z, calcd (M +) 348.02, found 348.019.

2-[2-(3-Methoxy-phenyl)-4-oxo-thiazolidin-3-yl]-1, 9-dihydro-purin-6-one (GB-3)

Black solid; Yield: 47.36%; mp: 139–141⁰C; FT-IR (KBr) Vmax: 3329, 2920, 2619, 1718, 1656, 1340, 1240 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz): δ 4.28 (s, 2H), 6.50–7.03 (m, 4H), 7.97(s, 1H), 5.92(s, 1H), 3.33(s, 2H), 3.73(s, 3H); ¹³C NMR (CDCl₃, 500 MHz) δ 191.199, 154.532, 149.557, 148.954, 133.724, 130.757, 129.846, 126.964, 126.447, 119.311, 111.850, 110.199, 77.483, 66.464, 64.883; LC-HRMS/MS (ESI): m/z, calcd (M +) 344.07, found 344.01.

2-[2-(4-Nitro-phenyl)-4-oxo-thiazolidin-3-yl]-1, 9-dihydro-purin-6-one (GB-4)

Black solid; Yield: 68.03%; mp: 167–169⁰C; FT-IR (KBr) Vmax: 3306, 2928, 2735, 1725, 1656, 1533, 1340 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz): δ 4.28 (s, 2H), 7.32–8.07 (m, 4H), 7.97(s, 1H), 5.92(s, 1H), 3.33(s, 2H); ¹³C NMR (CDCl₃, 500 MHz) δ 153.894, 131.695, 131.322, 131.082, 130.683, 130.497, 129.892, 124.357,

124.252, 116.492, 113.092, 112.600, 40.124, 39.791; LC-HRMS/MS (ESI): m/z, calcd (M +) 359.05, found 359.024.

2-[2-(4-Dimethylamino-phenyl)-4-oxo-thiazolidin-3-yl]-1, 9-dihydro-purin-6-one (GB-5)

Yellow solid; Yield: 52.29%; mp: 128–130⁰C; FT-IR (KBr) Vmax: 3329, 2928, 2619, 1725, 1656, 1340 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz): δ 4.28 (s, 2H), 6.47–6.88 (m, 4H), 7.97(s, 1H), 5.92(s, 1H), 3.33(s, 2H), 2.85(m, 6H); ¹³C NMR (CDCl₃, 500 MHz) δ 190.290, 176.992, 154.336, 131.960, 131.960, 125.123, 124.870, 111.198, 110.976, 110.715, 77.327, 77.073, 76.819, 45.299, 40.052, 34.582; LC-HRMS/MS (ESI): m/z, calcd (M +) 357.11, found 357.27.

2-[2-(3, 4-Dimethoxy-phenyl)-4-oxo-thiazolidin-3-yl]-1, 9-dihydro-purin-6-one (GB-6)

Black solid; Yield: 42.02%; mp: 155–157⁰C; FT-IR (KBr) Vmax: 3346, 2935, 2615, 1729, 1656, 1348, 1240 cm⁻¹. ¹H NMR (500 MHz, CDCl₃): δ 4.28 (s, 2H), 6.46–6.54 (m, 3H), 7.97(s, 1H), 5.92(s, 1H), 3.33(s, 2H), 3.73(s, 6H); ¹³C NMR (CDCl₃, 500 MHz) δ 190.355, 177.324, 169.748, 154.357, 144.642, 141.120, 131.999, 125.172, 110.998, 77.283, 77.029, 76.776, 45.712, 43.243, 40.087, 8.560; LC-HRMS/MS (ESI): m/z, calcd (M +) 374.08, found 374.019.

2-(2-Methyl-4-oxo-thiazolidin-3-yl)-1, 9-dihydro-purin-6-one (GB-7)

Brown solid; Yield: 58.44%; mp: 121–123⁰C; FT-IR (KBr) Vmax: 3346, 2927, 2615, 1714, 1651, 1348 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz): δ 4.28 (s, 2H), 7.97(s, 1H), 4.81(s, 1H), 3.33(s, 2H), 1.54(s, 3H); ¹³C NMR (CDCl₃, 500 MHz) δ 144.854, 144.015, 129.835, 123.618, 114.477, 112.788, 112.560, 63.676, 40.292; LC-HRMS/MS (ESI): m/z, calcd (M +) 252.05, found 252.045.

2-[2-(2-Nitro-phenyl)-4-oxo-thiazolidin-3-yl]-1, 9-dihydro-purin-6-one (GB-8)

Yellow solid; Yield: 38.51%; mp: 173–175⁰C; FT-IR (KBr) Vmax: 3346, 2927, 2615, 1714, 1651, 1533, 1341 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz): δ 4.28 (s, 2H), 7.32–8.07 (m, 4H), 7.97(s, 1H), 5.92(s, 1H), 3.33(s, 2H); ¹³C NMR (CDCl₃, 500 MHz) δ 163.244, 155.310, 149.314, 139.148, 121.698, 121.181, 111.198, 110.976, 110.715, 77.282, 77.028, 76.774, 28.317; LC-HRMS/MS (ESI): m/z, calcd (M +) 359.05, found 359.014.

2-[2-(2-Chloro-phenyl)-4-oxo-thiazolidin-3-yl]-1, 9-dihydro-purin-6-one (GB-9)

Yellow solid; Yield: 56.11%; mp: 166–168⁰C; FT-IR (KBr) Vmax: 3351, 3095, 2735, 1718, 1625, 1348, 824 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz): δ 4.28 (s, 2H), 7.00–7.15 (m, 4H), 7.97(s, 1H), 5.92(s, 1H), 3.33(s, 2H); ¹³C NMR (CDCl₃, 500 MHz) δ 198.834, 161.638, 141.351, 140.202, 133.832, 131.060, 129.785, 128.098, 127.275, 126.617, 125.512, 77.296, 45.303, 37.250; LC-HRMS/MS (ESI): m/z, calcd (M +) 348.02, found 348.28.

2-[2-(3-Chloro-phenyl)-4-oxo-thiazolidin-3-yl]-1, 9-dihydro-purin-6-one (GB-10)

Yellow solid; Yield: 61.35%; mp: 141–143⁰C; FT-IR (KBr) Vmax: 3346, 2919, 2615, 1722, 1628, 1341, 718 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz): δ 4.28 (s, 2H), 6.94–7.08 (m, 4H), 7.97(s, 1H), 5.92(s, 1H), 3.33(s, 2H); ¹³C NMR (CDCl₃, 500 MHz) δ 198.545, 161.367, 145.244, 134.582, 130.179, 129.602, 128.099, 127.397, 126.994, 125.412, 77.296, 77.043, 76.789, 40.434; LC-HRMS/MS (ESI): m/z, calcd (M +) 348.02, found 348.087.

2-[2-(3-Bromo-phenyl)-4-oxo-thiazolidin-3-yl]-1, 9-dihydro-purin-6-one (GB-11)

Yellow solid; Yield: 71.09%; mp: 183–185⁰C; FT-IR (KBr) Vmax: 3385, 3082, 2732, 1722, 1620, 1333, 672 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz): δ 4.28 (s, 2H), 7.00–7.24 (m, 4H), 7.97(s, 1H), 5.92(s, 1H), 3.33(s, 2H); ¹³C NMR (CDCl₃, 500 MHz) δ 198.471, 161.293, 145.541, 131.197, 130.446, 130.135, 129.935, 126.614, 125.880, 123.021, 122.841, 77.297, 77.043, 40.320; LC-HRMS/MS (ESI): m/z, calcd (M +) 391.45, found 391.97.

2-[2-(3-Nitro-phenyl)-4-oxo-thiazolidin-3-yl]-1, 9-dihydro-purin-6-one (GB-12)

Black solid; Yield: 67.19%; mp: 153–155⁰C; FT-IR (KBr) Vmax: 3354, 2926, 2618, 1725, 1623, 1536, 1339; ¹H NMR (CDCl₃, 500 MHz): δ 4.28 (s, 2H), 7.40–8.00 (m, 4H), 7.97(s, 1H), 5.92(s, 1H), 3.33(s, 2H); ¹³C NMR (CDCl₃, 500 MHz) δ 190.297, 151.153, 140.060, 130.901, 130.499, 129.495, 124.328, 124.009, 121.861, 77.289, 77.035, 76.781, 45.736, 8.547; LC-HRMS/MS (ESI): m/z, calcd (M +) 359.26, found 359.48.

Anticancer activity (Sulforhodamine B assay)

RPMI 1640 medium supplemented with 10% fetal bovine serum and 2 mM L-glutamine was used to culture the cells. For the current screening experiment, cells were injected into 96 well microtiter plates in

90 L at a density of 5000 cells per well. Before adding experimental drugs, the microtiter plates were incubated for 24 hours at 37°C, 5% CO₂, 95% air, and 100% relative humidity. The experimental drugs were dissolved in a suitable solvent to form a stock solution with a concentration of 10⁻². Four 10-fold serial dilutions of full media were conducted during the experiment. Aliquots of 10 µl of each of these various drug dilutions were added to the appropriate microtiter wells that had been previously filled with 90 µl of a medium, yielding in the required final drug concentrations. Plates were incubated under usual conditions for 48 hours after drug addition, and the test was stopped by adding cold TCA. Cells were gently fixed in situ for 60 minutes at 4°C using 50 µl of cool 30% (w/v) TCA (final concentration, 10% TCA). After discarding the supernatant, the plates were rinsed and dried five times with tap water. Sulforhodamine B (SRB) solution (50 µl) was added to each well at a concentration of 0.4 percent (w/v) in 1% acetic acid, and plates were incubated at room temperature for 20 minutes. Following staining, the unbound dye was recovered, and the remaining dye was removed using a five-step wash with 1% acetic acid. The plates were dried in the air. After eluting the bound dye with 10 mM trizma base, the absorbance was measured using an Elisa plate reader at a wavelength of 540 nm with a reference wavelength of 690 nm.

On a plate-by-plate basis, percent rise was assessed for test wells relative to control wells. Percent Growth was computed as the average absorbance of the test well to the average absorbance of the control well multiplied by 100. The percentage growth rate at each of the four drug concentration levels was calculated using the six absorbance measurements [time zero (T_z), control growth (C), and test growth in the presence of the four-drug concentration levels (T_i)]. For each test article, dosage response parameters were computed. The 50% growth inhibition (GI₅₀) was calculated as $[(T_i - T_z) / (C - T_z)] \times 100 = 50$, which is the drug concentration that results in a 50% reduction in the net protein increase (as shown by SRB staining) in control cells during drug incubation. The medication concentration required to suppress total growth (TGI) was determined using T_i = T_z. The LC₅₀ value (drug concentration that results in a 50% drop in the measured protein at the end of treatment compared to the beginning) indicates a net loss of cells following treatment when $[(T_i - T_z) / T_z] \times 100 = -50$. If the desired level of activity was not achieved or was surpassed, values for each of these three parameters were stated as higher or less than the maximum or minimum concentration tested [19,20].

***In vitro* Anti-inflammatory activity**

The anti-inflammatory efficacy of the synthesized compounds was determined employing the inhibition of the albumin denaturation procedure. Aceclofenac was used as the standard drug. The standard and test substances were dissolved in a small amount of dimethylformamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4). In all solutions, the final concentration of DMF was less than 2.5 percent. A test solution (1 ml) containing various drug doses was mixed with 1 ml of a 1% mM albumin solution in phosphate buffer and incubated for 15 minutes at 27 ± 10 °C in a BOD incubator.

Denaturation was induced by maintaining the reaction mixture on a water bath at 60 ± 10 °C for 10 minutes. After cooling, the turbidity was determined using a double beam UV-visible spectrophotometer at 660 nm. The percentage inhibition of denaturation was determined by comparing it to a control condition

in which no medication was added. Each experiment was repeated twice, and an average was calculated. The percent inhibition can be computed as follows: percent inhibition = $100 ((V_c / V_t) - 1)$, where V_t represents the absorbance value of the test group and V_c represents the absorbance value of the control group. The 50% Inhibitory Concentration (IC_{50}) was derived using the percentage inhibition [21].

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

The chicken chorioallantoic membrane (CAM) experiment was performed using fertilized chicken eggs that were eight days old. Each egg's shell was drilled with a 1-cm diameter hole, and the dermic sheet's surface was removed to expose the CAM. A filter paper with a diameter of 0.5 cm was placed on top of the CAM, and a volume of 1 nM, 10 nM, or 100 nM drug (control, Thalidomide) was deposited in the center. The shell's windows were then sealed with sterile bandages. For 48 hours, the eggs were incubated at 37°C and 90% relative humidity. Following 15 minutes of fixation with stationary solution (a 1:1 combination of methanol and acetone), the CAM was removed and photographed using a digital camera. The morphology of chicken blood arteries was determined following various treatments [22].

Antioxidant activity

DPPH radical scavenging assay

The standard solution was made by dissolving 100 mg ascorbic acid in methanol to get 10, 20, 30, 40, and 50 µg/mL concentrations. The tests solutions were prepared by dissolving 10 mg of compounds (**GB-1 to GB-12**) in 10 mL of methanol to give 100 µg/mL of stock solution of each compound. The 10, 20, 30, 40 and 50 µg/mL concentrations were prepared using this stock solution. To each dilution, 150 µL of DPPH was added and kept in the dark for 30 min. The 150 µL DPPH solution was added to 10 mL methanol, and the absorbance at 517 nm was measured immediately as a control. 10 mL of different test sample concentrations (10, 20, 30, 40, 50 µL) prepared with methanol were taken, and 150 µL DPPH solution was added to each test tube. After 15 minutes, absorbance at 517 nm was determined using methanol as a blank in a UV visible spectrophotometer (Jasco, UV-630). The following equation was used to compute the free radical scavenging activity (FRSA) (% inhibition). The % inhibition for different log concentrations was plotted to obtain a concentration vs. % inhibition graph from which IC_{50} value was calculated [23].

KMnO₄ radical scavenging assay

The experiment was conducted using a Jasco 630 UV-Spectrophotometer. A fresh stock solution of potassium permanganate (0.05N) was produced and stored in a dark place until use. The sulphuric acid

stock solution (2N) was prepared for Mn²⁺ ion separation. L-ascorbic acid (70 mg/100 ml) was produced in distilled water as a positive control, and successive dilutions [10, 20, 30, 40, 50 µg/ml] were made. The compounds were dissolved in methanol (10 mg/10 ml) and serially diluted. At room temperature and in a dark environment, test solutions were allowed to react with KMnO₄ solution, and absorbance values at 528 nm were determined compared to a blank. The radical scavenging activity (percent inhibition) was computed and expressed as a percentage of KMnO₄ radical elimination [24].

Molecular docking studies

The docking approach is used to determine the proper binding poses and interactions inside the protein's binding site. The docking of molecules is carried out using the VLife MDS 4.4 program. Polo-like kinase 1 crystal structure (PDB code 1q4k) was obtained from the protein data bank database (<http://www.rcsb.org/pdb>). The initial crystal structure contained the bound ligand; this was removed, and missing loops were inserted using the same software's homology modeling package. The two-dimensional structures were drawn and translated to three-dimensional structures. The three-dimensional structure was optimized using MMFF up to an rms gradient of 0.01. The structure of the protein was optimized. Cavity number one is chosen for the docking procedure. All atoms within a 5Å⁰ radius were defined as the active site of a protein. Batch docking was used to determine the docking score and interactions between the ligand and target protein.

Results And Discussion

Electrochemical synthesis:

Copper-nickel oxide nanoparticles were synthesized significantly by an electrochemical reduction process with the capping agent cetyltrimethyl ammonium bromide. The capping compounds are organic surface-active agents employed to bind selectively to a particular plane of a nonmaterial crystal. Cetyltrimethyl ammonium bromide is a surfactant that plays a critical function in preventing particle aggregation and flocculation. Due to the capping of the surface of nanoparticles, surface-active substances limit growth pace, affecting the morphological features of nanoparticles and orienting them during crystal formation. The rate of surface-active agent adhesion and diffusion on the surface determines the growth rate of nanoparticles. UV-visible spectral investigations were used to determine the size of nanoparticles after 15 minutes of electrolysis.

Analysis of copper nickel oxide nanoparticles

UV-visible studies:

Copper-nickel oxide nanoparticles were scanned in a twin beam spectrophotometer for UV-visible spectroscopic investigation in the range of 200-1100 nm. The scanning revealed that all samples had wavelength maxima in the UV area. The UV-visible spectra of copper-nickel oxide nanoparticles produced in water using cetyltrimethyl ammonium bromide as a capping agent are shown in Figure 1 at current densities of 5 and 10 mA/ cm², respectively. According to the analysis, the wavelength maxima are smaller at a current density of 10 mA/ cm² than 5 mA/ cm². It confirmed that as current density increases, particle size falls, and a blue shift occurs. After 15 minutes of electrolysis, the absorbance of the sample was found to be greater than after 2 hours of electrolysis. These observations demonstrate that the particle size at a lower electrolysis time is less than at a larger electrolysis time. The particle size increased as the electrolysis time increased.

FT-IR Studies:

FTIR spectroscopy was used to determine the capping effectiveness of copper-nickel oxide nanoparticles electrochemically produced using cetyltrimethyl ammonium bromide. The FTIR spectrum of prepared copper-nickel oxide nanoparticles with cetyltrimethyl ammonium bromide (Figure 2) reveals peaks at 2970, 2864, 2832, 3462, and 3111 cm⁻¹, corresponding to C-H stretching vibrations, 642 cm⁻¹ Br-C stretching vibrations, and 1342-1266 cm⁻¹ N-C stretching vibrations. It demonstrated the presence of cetyltrimethyl ammonium bromide on the surface of nanoparticles. The peaks at 3640 and 3483 cm⁻¹ showed the existence of water due to the ligand's hygroscopic nature. The FTIR spectrum of calcinated copper-nickel oxide a nanoparticle (Figure 3) at 500 °C in a muffle furnace demonstrates that peaks related to the ligand have vanished or have become weak, indicating that the ligand has been removed during the calcination process. At 550 cm⁻¹, the broadband suggests the presence of a CuNi-O bond.

Thermal studies:

As shown in Figure 4, the weight of copper-nickel oxide nanoparticles above 200 °C was constant; indicating that the capping agent cetyltrimethyl ammonium bromide was utilized to create the nanoparticles was not present.

XRD studies:

After two hours of calcination at 500 °C, the XRD pattern of produced copper-nickel oxide nanoparticles was obtained (Figure 5). The measured diffraction peaks indicate that copper-nickel oxide nanoparticles have a biclinic structure. The ten distinctive peaks with lattice parameters $a = 4.679$, $b = 3.431$, and $c = 5.136$ at $\beta = 99.262$ with $2\theta = 32.889, 35.990, 39.177, 49.182, 54.113, 58.795, 62.094, 68.627, 72.968$, and

75.731 were detected, which correspond to miller indices of (101), (031), (130), (141), (042), (132), were observed which accurately coincide with the JCPDS no. 80-0076 of copper-nickel oxide. The average particle size was determined using Debye-equation, Scherrer's, which specifies that $D = k\lambda/\beta\cos\theta$. In this equation, k denotes the shape constant, λ denotes wavelength, and θ denotes the diffraction angle, where as β denotes the whole width half maximum. Copper-nickel oxide nanoparticles with an average size of 7.79 nm were estimated using XRD data and are given in Table 1.

SEM-EDS studies:

Copper-nickel oxide nanoparticles were studied using scanning electron microscopy at various resolutions in this work. The scanning electron microscope image (Figure 6) illustrates the morphological characteristics of copper-nickel oxide nanoparticles. The SEM image demonstrates the presence of uniform, regular, and irregularly shaped particles. Additionally, it established the creation of nanoscale crystalline particles as well as randomly oriented aggregates. Calcination leads to an increase in the crystal size of particles. As shown in Figure 7, the copper-nickel oxide nanoparticles were examined qualitatively and quantitatively using EDS. The EDS spectra confirmed copper, nickel, and oxygen in the composition, as shown in Table 2.

TEM-SAED analysis:

The various colors are the result of the varying densities revealed by TEM images (Figure 8). The TEM image shows particles with diameters of 27.84, 25.70, 24.47, 31.00, 45.62, 38.23, and 27.18 nm, with an average of 31.43 nm and maximum particles with diameters of 20-30 nm, which is consistent with the XRD study. The image demonstrates the randomly distributed metal nanoparticle aggregates. The electron diffraction pattern of a selected section of the nanoparticle, as shown in Figures 9 and 10 and 11 confirms its crystalline character due to the absence of dispersed holes often associated with amorphous phases.

Synthesis of 2-(2-substituted-4-oxo-thiazolidin-3-yl)-1, 9-dihydro-purin-6-ones (GB-1 to GB-12)

The title compounds have been synthesized as per the reaction shown in Figure 12 (Scheme 1). Thin layer chromatography is used to monitor reaction progress. The physical and chemical properties of the title compounds have been recorded as shown in Table 3. The calcinated copper nickel oxide nanoparticles were used successfully as a catalyst to synthesis of 2-(2-substituted-4-oxo-thiazolidin-3-yl)-1, 9-dihydro-purin-6-ones (GB-1 to GB-12) using starting material guanine, substituted aldehydes, thioglycolic acid, and sodium hydroxide were combined in ethanol in microwave synthesis system (CEM, USA).

Anticancer activity

In this study, a novel series of 2-(2-substituted-4-oxo-thiazolidine-3-yl)-1, 9-dihydro-purin-6-ones were designed and synthesized. All synthesized compounds were screened by sulforhodamine (SRB) assay against breast cancer MCF-7 cell line, compared to adriamycin as a reference drug. The results are reported in Table 7, expressed as GI_{50} μ M (growth inhibitory concentration at 50%). Anticancer screening found that compounds GB-6 (GI_{50} : 30 M) and GB-10 (GI_{50} : 30 M) displayed considerable cytotoxic activity against the MCF-7 breast cancer shown in Figure 13 and 14. The most promising compound, GB-8 (GI_{50} : 10 μ M), showed excellent activity against MCF-7 cell lines.

In-vitro anti-inflammatory activity

We have devised a novel methodology for synthesizing (2-substituted-4-oxo-thiazolidine-3-yl)-1, 9-dihydro-purin-6-ones (**GB-1 to GB-12**) in this work. All synthesized compounds were compared to standard aceclofenac for in vitro anti-inflammatory efficacy utilizing the inhibition of the albumin denaturation technique. Derivatives GB-4, GB-6, GB-8, and GB-9, GB-10 showed significant in vitro anti-inflammatory activity in the range of IC_{50} :179.65-194.59 μ g/ml as compared with aceclofenac (IC_{50} :191.19 μ g/ml). The satisfactory results for anti-inflammatory activity have been correctly reported in Table 4. The anticancer activity study shows similar results to some extent.

Chorioallantoic membrane (CAM) of chick embryos assay

The CAM assay results of 2-(2-substituted-4-oxo-thiazolidine-3-yl)-1, 9-dihydro-purin-6-ones (**GB-1 to GB-12**) gives a significant blood vessel growth inhibitory activity of in chick embryo eggs. The 10 nM drug concentration of GB-4, GB-8 and GB-10 compounds shows an excellent capillary growth inhibitory activity. The 100 nM drug concentration of GB-6 compound shows a good capillary growth inhibitory activity shown in Figure 15.

Antioxidant activity

Antioxidant activity was determined appropriately in vitro utilizing the DPPH and $KMnO_4$ radical scavenging assays. The effective concentration (IC_{50}) of each chemical was determined using linear regression. The satisfactory results were expressed as an IC_{50} value, accurately depicting the concentration at which the investigated chemicals diminish half of the substrate.

DPPH radical scavenging assay

The antioxidant activity of the title compounds 2-(2-substituted-4-oxo-thiazolidine-3-yl)-1, 9-dihydro-purin-6-ones (**GB-1 to GB-12**) and the parent compound ascorbic acid were evaluated using DPPH free radical scavenging assay. Antioxidant chemicals react with the DPPH radical via the hydrogen atom donation mechanism, resulting in a change in the color of DPPH from violet to yellow as determined by a UV-visible spectrophotometer. The satisfactory result of the antioxidant activity at different test concentrations and IC_{50} value in $\mu\text{g/ml}$ of the synthesized compounds (**GB-1 to GB-12**) are demonstrated in **Table 5**. The data shows that among all the synthesized compounds in IC_{50} value in ($\mu\text{g/ml}$) GB-6: 11.96, GB-8: 10.67, GB-10: 9.08, GB-11: 10.30, GB-12: 10.63 exhibited excellent radical scavenging activities compared to ascorbic acid: 13.04 $\mu\text{g/ml}$. The reason for the higher antioxidant activity of compound **GB-10, GB-11, and GB-12** may be due to the chloro, bromo and pyridine substituents on the phenyl ring attached with thiazolidine moiety; whereas compounds **GB-2, GB-6** and **GB-8** exhibited moderate antioxidant potential, and anticancer activity study shows similar results in some extent. Therefore, these molecules could be developed for antioxidant agents and can be potential polo-like kinase 1 inhibitors and promising anticancer agents.

KMnO₄ radical scavenging assay

The scavenging activity of synthesized compounds 2-(2-substituted-4-oxo-thiazolidine-3-yl)-1, 9-dihydro-purin-6-ones (**GB-1 to GB-12**) at different concentrations 10, 20, 30, 40 and 50 $\mu\text{g/ml}$. were evaluated. The high scavenging activity levels imply a strong antiradical action. The results are summarized in Table 6 as IC_{50} values ($\mu\text{g/ml}$). Low IC_{50} values indicate a greater ability to scavenge. The nitro group substituted at phenyl ring *para* GB-4: 23.60 $\mu\text{g/ml}$ and *ortho* GB-8: 24.93 $\mu\text{g/ml}$ positions exhibited good antioxidant activity. The phenyl ring containing *para* GB-2: 15.33 $\mu\text{g/ml}$ and *meta* GB-10: 24.96 $\mu\text{g/ml}$ chlorophenyl-containing groups exhibited the highest antioxidant potential compared to ascorbic acid: 26.55 $\mu\text{g/ml}$ as a standard. The phenyl ring containing *meta* methoxy phenyl GB-3: 20.21 $\mu\text{g/ml}$ containing groups exhibited good radical scavenging activity. These desired outcome results reveal that the compounds GB-8 and GB-10 display good free radical scavenging activity, same as anticancer activity.

Molecular docking studies:

The docking analysis was performed to determine the mechanisms of binding of our compounds to the PLK1 enzyme. The previous results prompted us to investigate the molecular docking among the most bioactive compound, GB-6, GB-8, and GB-10, using PLK1, which is overexpressed in a variety of cancer cell lines, including prostate (PC-3), breast (MCF-7), hepatocellular carcinoma (HepG2), and human

cervical (HeLa) cancer cell lines. Figure 13 illustrates the docked compounds GB-1 to GB-12 with (1q4k) into the putative active site of PLK1. The molecular modeling results for the compounds GB-6, GB-8, and GB-10 indicated that the molecule was approximately oriented into the predicted binding location of the receptor pocket, with some extra hydrophobic and hydrogen bonding interactions with adjacent amino acids. These docking results demonstrate that compounds with a phenyl ring containing meta-nitro groups exhibit a favorable binding interaction and elucidate the good docking score (-5.22).

In contrast, compounds with a phenyl ring containing meta and para-dimethoxy (-4.70) groups exhibit a more favorable docking score than compounds with a phenyl ring containing meta-methoxy groups. The phenyl ring containing a meta-chlorophenyl group elucidates more favorable protein-ligand interactions and a higher docking score (-4.78) than the phenyl ring containing ortho (-4.20) or para chlorophenyl groups (-4.20). Table 7 summarises the docking scores (kcal/moles) of all compounds. The nitrogen atom of the guanine ring creates hydrophobic bonds with the LEU511B and LEU523B amino acid residues. The nitro group substituted at the *meta* position of the phenyl ring produces hydrophobic bonds with amino acid residues LEU511B, LEU523B, LEU543B, ILE542B, and LEU541B, as well as hydrogen bonds with amino acid residues LEU525B, VAL411B, HIS524B, and VAL530B.

The dimethoxy group (electron-donating) substituted on the phenyl ring at meta and para positions developed hydrophobic bonds with amino acid residues LEU541B, ILE542B, LEU543B, LEU523B and LEU511B, as well as hydrogen bonds with amino acid residues ALA549B, VAL411B and LEU525B. The electron-withdrawing group (chlorine) substituted on phenyl ring at *meta* position forms as hydrophobic bonding with amino acid residues MET377B, LEU543B, ILE542B, LEU41B, LEU523B and LEU511B as well as hydrogen bonding with amino acid residues VAL411B, LEU525B, HIS524B and VAL530B. The molecular docking study also reveals that the compound GB-6, GB-8 and GB-10 shows a good binding interaction and significant anticancer activity shown in Figure 16.

Structure activity relationship (SAR)

Including a nitro group to the *para*, *ortho*, or *meta* position of the phenyl ring (GB-4, GB-8, GB-12) enhances anticancer activity against the breast cancer MCF-7 cell line. Meta-chlorophenyl compounds (GB-10) are more active against cancer cell lines than ortho- and para-chlorophenyl compounds (GB-2 & GB-9). The 3, 4-dimethoxy groups on the *meta* and *para* positions of the phenyl ring (GB-6) enhance anticancer and anti-inflammatory action. The antitumor activity of meta-chlorophenyl (GB-10) and 3, 4-dimethoxy phenyl compounds (GB-6) was comparable. The bromo group is substituted in the *meta* position of the phenyl ring (GB-11), resulting in a more potent anticancer agent than the para and *ortho* chlorophenyl analogs (GB-2 & GB-9). Unsubstituted phenyl compounds (GB-1) were less effective against a cancer cell line.

Conclusion

In the present work, we synthesized some novel 2-(2-substituted-4-oxo-thiazolidine-3-yl)-1, 9-dihydro-purin-6-ones supported by a newly synthesized copper nickel-oxide nanocatalyst. The newly synthesized compounds were adequately evaluated for their anti-cancer, anti-inflammatory, antioxidant activity and anti-angiogenetic activity. The experimental and docking data were found to be well correlated, indicating that compounds GB-6, GB-8, and GB-10 exhibited significant anti-cancer action against the breast cancer cell line MCF-7, as well as anti-inflammatory, antioxidant, and anti-angiogenic activities. Additionally, docking studies indicate that the drugs' effect may be attributable to their inhibition of polo-like kinase 1. These findings suggest that compounds GB-6, GB-8, and GB-10, similar polo-like kinase 1 inhibitors could serve as lead compounds for future anti-cancer drug development.

Declarations

Acknowledgment

We are thankful to the Indian Council of Medical Research (ICMR), a constituent of the Ministry of Health and Family Welfare, New Delhi, autonomous for Medical Research in India for financial support of this work.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This work was supported by the Indian Council of Medical Research, New Delhi (Grant number: BIC/12(12), 2015).

References

1. World Health Organization, Global Cancer Deaths Report for the <https://www.who.int/news-room/fact-sheets/detail/cancer/> 2020. Accessed 3 March 2021.
2. Shakeel I, Basheer N, Hasan GM, Afzal M, Hassan MI. Polo-like Kinase 1 as an Emerging Drug Target: Structure, Function and Therapeutic Implications. *J Drug Target* 2021; 29 (2):168-184. doi:org/10.1080/1061186X.2020.1818760.

3. Jeyapal P, Krishnasamy G, Suzuki R, Venkatesh C, Chandrasekar M. In-Silico Design and Synthesis of N9-Substituted β -Carbolines as PLK-1 Inhibitors and Their In-Vitro/In-Vivo Tumor Suppressing Evaluation. *Bioorg Chem* 2019; 88: 1-8. doi.org/10.1016/j.bioorg.2019.04.007.
4. Weiß L, Efferth T. Polo-Like Kinase 1 as Target for Cancer Therapy. *Exp Hematol* 2012; 1(1):38:1-6. doi.org/10.1186/2162-3619-1-38.
5. Frost A, Mross K, Steinbild S, Hedbom S, Unger C, Kaiser R, Munzert G. Phase I Study of the PLK1 Inhibitor BI2536 Administered Intravenously on Three Consecutive Days in Advanced Solid Tumours. *Curr Oncol*. 2012;19(1):e28-35. doi.org/10.3747/co.19.866.
6. Horishny V, Chaban T, Matychuk V. Synthesis and Primary Antitumor Screening of 5-ylidene Derivatives of 3-(Morpholin-4-yl)-2-Sulfanylidene-1,3-Thiazolidin-4-One. *Russ J Org Chem*. 2020;56: 454–457. doi.org/10.1134/S1070428020030148.
7. Horishny V, Mandzyuk L, Lytvyn R, Bodnarchuk O, Matychuk V, Obushak M. Synthesis and Biological Activity of Pyrazolo [1, 5-c][1,3]Benzoxazines Containing a Thiazolidin-4-One Fragment. *Russ J Org Chem*. 2020; 56(4):588–595. doi.org/10.1134/S1070428020040053.
8. Isloor A, Sunil D, Shetty P, Malladi S, Pai K, Maliyakkl N. Synthesis, Characterization, Anticancer, and Antioxidant Activity of Some New Thiazolidin-4-Ones in MCF-7 Cells. *Med Chem Res*. 2012;22(2):758–767. doi.org/10.1007/s00044-012-0071-5.
9. Ahani Z, Nikbin M, Maghsoodlou MT, Farhadi-Ghalati F, Valizadeh J, Beyzaei H, Moghaddam-Manesh M. Semi-Synthesis, Antibacterial and Antifungal Activities of Three Novel Thiazolidin-4-Ones by Essential Oil of *Anethum Graveolens* Seed as Starting Material. *J Iran Chem Soc*. 2018;15: 2423-2430. doi.org/10.1007/s13738-018-1431-y.
10. Panzariu A, Apotrosoaei M, Vasincu IM, Dragan M, Constantin S, Buron F, Tuchilus C. Synthesis and Biological Evaluation of New 1,3-Thiazolidine-4-One Derivatives of Nitro-L-Arginine Methyl Ester. *Chem Cent J*. 2016; 10(1):1-14. doi.org/10.1186/s13065-016-0151-6.

11. Ottana R, Maccari R, Giglio M, Corso AD, Cappiello M, Mura U, Settimo FD. Identification of 5-Arylidene-4-Thiazolidinone Derivatives Endowed with Dual Activity as Aldose Reductase Inhibitors and Antioxidant Agents for the Treatment of Diabetic Complications. *Eur J Med Chem.* 2011;46(7): 2797-2806. doi:org/10.1016/j.ejmech.2011.03.068.
12. Raza S, Srivastava S, Srivastava D, Srivastava A, Haq W, Katti S. Thiazolidin-4-One and Thiazinan-4-One Derivatives Analogous to Rosiglitazone as Potential Antihyperglycemic and Antidyslipidemic Agents. *Eur J Med Chem.* 2013;63: 611–620. doi:org/10.1016/j.ejmech.2013.01.054.
13. Sawant R, Wadekar J, Ukirde R, Barkade G. Synthesis, Molecular Docking and Anticancer Activity of Novel 1,3-Thiazolidin-4-Ones. *Pharm Sci.* 2020;27(3): 339-346. doi:org/ 10.34172/PS.2020.95.
14. Shao J, Liu M, Wang Z, Li K, Bao B, Zhao S, Zhou S. Controllable Synthesis of Surface Pt-Rich Bimetallic AuPt Nanocatalysts for Selective Hydrogenation Reactions. *ACS. Omega.* 2019;4: 15621-15627. doi:org/10.1021/acsomega.9b02117.
15. Roldan MV, Pellegrini N, Sanctis OD. Electrochemical Method for Ag-PEG Nanoparticles Synthesis. *J Nanoparticl.* 2013;1-7. doi:org/10.1155/2013/524150.
16. Ozin GA, Arsenault AC, Cademartiri L. *Nanochemistry: A Chemical Approach to Nanomaterials.* Royal Society Chemistry, United Kingdom: 2009.
17. Meloni D, Monaci R, Solinas V, Auroux A, and Dumitriu E. Characterization of the Active Sites in Mixed Oxides Derived from LDH Precursors by Physicochemical and Catalytic Techniques. *Appl Catal.* 2008; 350(1):86-95. doi:org/10.1016/j.apcata.2008.08.005.
18. Ravindra K, Reddy HM, Uthanna S. Studies on the Structural, Electrical and Optical Properties of Thermally Oxidized Copper Nickel Oxide Thin Films. *Front Nanosci.* 2017; 3(2):1-7. doi:org/10.15761/FNN.1000154.

19. Vichai V, Kirtikara K. Sulforhodamine B Colorimetric Assay for Cytotoxicity Screening. *Nat Protoc.* 2006;1: 1112–1116. doi:org/10.1038/nprot.2006.179.
20. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. New Colorimetric Cytotoxicity assay for Anticancer Drug Screening. *J Natl Cancer Inst.* 1990; 82(13):1107-1112. doi:org/10.1093/jnci/82.13.1107.
21. Banerjee AG, Das N. Design, Synthesis, Evaluation and Molecular Modelling Studies of Some Novel 5,6-Diphenyl-1,2,4-Triazin-3(2H)-Ones Bearing Five Member Heterocyclic Moieties as Potential COX-2 Inhibitors: A Hybrid Pharmacophore Approach. *Bioorg Chem.* 2016;69: 102-120. doi:org/10.1016/j.bioorg.2016.10.003.
22. Kacan T, Nayir E, Altun A, Kilickap S, Babacan NA. Antitumor Activity of Sorafenib on Colorectal Cancer. *J Oncol Sci.* 2016:1-5. doi:org/10.1016/j.jons.2016.07.008.
23. Ukirde RD, Patil RB, Sawant SD. Design, Synthesis and Evaluation of Antioxidant Activity of Some Coumarin Derivatives. *Asian j org med chem.* 2019; 4(3):138-143. doi:org/10.14233/ajomc.2019.
24. Rani PS, Vunguturi S. In-Vitro Study of Antioxidant Activities of Medicinal Plant Extracts using KMnO_4 and DPPH-a Comparison. *Int j innov sci eng technol.* 2015;2(6):116-119.

Tables

Table 1: Average size of copper nickel oxide nanoparticles

h	k	l	2Theta	d (Å)	FWHM	Size (nm)
1	0	1	36.537	2.45732	0.8740	9.65
0	3	1	39.733	2.26673	0.9042	9.23
1	3	0	49.853	1.82771	0.9333	8.62
1	4	1	54.559	1.68066	0.9660	8.16
0	4	2	59.200	1.55949	0.9571	8.06
1	3	2	62.646	1.48175	0.9629	7.87
2	0	0	67.171	1.39248	1.1902	6.19
0	5	2	69.053	1.35905	1.0547	6.92
1	0	3	73.348	1.28973	1.0351	6.86
		2 3 1	76.214	1.24820	1.1078	6.29
Average particle size						7.79 nm

Table 2: EDS data of copper nickel oxide nanoparticles

Element	Weight percentage	Atomic percentage
O k	34.06	67.87
Ni k	11.50	6.24
Cu k	51.60	25.89
Total	97.15	

Table 3: Chemical structures and physicochemical characteristics of title compounds (GB-1 to GB-12)

Compound	R	Molecular formula	Molecular weight	Melting point (°C)	Yield (%)	Rf value
GB-1		C ₁₄ H ₁₁ N ₅ O ₂ S	313.33	160-162	64.07	0.59
GB-2		C ₁₄ H ₁₀ ClN ₅ O ₂ S	347.77	144-146	59.22	0.62
GB-3		C ₁₅ H ₁₃ N ₅ O ₃ S	343.36	139-141	47.36	0.67
GB-4		C ₁₄ H ₁₀ N ₆ O ₄ S	358.33	167-169	68.03	0.60
GB-5		C ₁₆ H ₁₆ N ₆ O ₂ S	356.10	128-130	52.29	0.58
GB-6		C ₁₆ H ₁₅ N ₅ O ₄ S	373.08	155-157	42.02	0.55
GB-7	-CH ₃	C ₉ H ₉ N ₅ O ₂ S	251.04	121-123	58.44	0.65
GB-8		C ₁₄ H ₁₀ N ₆ O ₄ S	358.33	173-175	38.51	0.54
GB-9		C ₁₄ H ₁₀ ClN ₅ O ₂ S	347.77	166-168	56.11	0.61
GB-10		C ₁₄ H ₁₀ ClN ₅ O ₂ S	347.77	141-143	61.35	0.67
GB-11		C ₁₄ H ₁₀ BrN ₅ O ₂ S	392.23	183-185	71.09	0.71
GB-12		C ₁₄ H ₁₀ N ₆ O ₄ S	358.33	153-155	67.19	0.52

Table 4: *In-vitro* anti-inflammatory activity and IC₅₀ value (µg/ml)

Sr.No.	Compounds	Anti-inflammatory Activity IC ₅₀ (µg/ml)	Sr.No.	Compounds	Anti-inflammatory Activity IC ₅₀ (µg/ml)
1.	GB-1	263.96	7.	GB-7	278.39
2.	GB-2	221.87	8.	GB-8	179.65
3.	GB-3	346.30	9.	GB-9	187.67
4.	GB-4	180.65	10.	GB-10	245.55
5.	GB-5	352.80	11.	GB-11	336.53
6.	GB-6	194.59	12.	GB-12	181.98
13.	Aceclofenac	191.19			

Table 5: DPPH radical scavenging assay

Comp. ID	% inhibition					IC ₅₀ (µg/ml)
	Concentration in µg/ml					
	10	20	30	40	50	
GB-1	38.28	49.42	66.00	75.89	80.71	19.17
GB-2	45.52	55.24	65.73	66.60	80.21	14.33
GB-3	41.54	48.63	60.40	65.36	79.04	20.20
GB-4	43.24	42.45	67.51	72.93	83.42	19.26
GB-5	39.96	55.21	61.24	66.96	78.76	18.34
GB-6	47.47	53.89	60.55	84.00	91.30	11.96
GB-7	51.57	57.18	63.61	71.19	91.91	15.19
GB-8	36.83	61.11	73.07	78.39	85.54	10.67
GB-9	44.09	65.24	72.33	80.24	88.52	15.19
GB-10	50.86	55.33	71.96	72.86	81.56	9.08
GB-11	50.86	57.42	70.03	77.55	88.86	10.30
GB-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

Table 6: KMnO₄ radical scavenging assay

Comp. ID	% inhibition					IC ₅₀ (µg/ml)
	Concentration in µg/ml					
	10	20	30	40	50	
GB-1	38.27	41.79	47.90	50.24	51.87	41.26
GB-2	45.57	55.14	56.96	62.44	67.54	15.33
GB-3	40.16	44.21	64.09	76.18	82.94	20.21
GB-4	37.12	41.23	59.02	67.99	81.79	23.60
GB-5	34.87	42.66	47.12	54.21	62.02	32.79
GB-6	38.93	39.22	40.52	41.64	42.85	122.05
GB-7	41.07	42.82	47.12	54.21	56.28	34.09
GB-8	49.57	43.14	52.49	53.29	58.81	24.93
GB-9	38.86	39.74	47.69	54.09	59.48	33.71
GB-10	45.00	50.24	51.00	52.68	58.60	24.96
GB-11	39.23	40.66	46.15	53.43	56.56	35.92
GB-12	41.06	43.09	45.50	47.72	49.36	52.02
Ascorbic acid	33.42	43.18	45.15	66.14	83.37	26.55

Table 7: Binding free energy and key interactions at the binding site of PLK1 and GI50 value (µM) on MCF-7 human breast cancer cell line

BINDING FREE ENERGY AND KEY INTERACTIONS AT THE BINDING SITE					GI ₅₀ (μ M)
Compounds	R	Docking score	Hydrophobic bonding	Hydrogen bonding	MCF-7 cell lines
GB-1		-4.48	ILE542B, LEU543B, LEU523B, LEU511B	TYR425B, VAL411B, ILE532B, VAL530B, HIS524B	80
GB -2		-4.20	VAL550B, LEU541B, ILE542B, LEU543B, LEU523B, LEU511B	ILE532B, VAL530B, HIS524B, LEU525B, VAL411B, TYR425B, THR551B	80
GB -3		-4.70	LEU511B, LEU523B, LEU543B, ILE542B, LEU541B	VAL530B, LEU525B, VAL411B, HIS524B	80
GB -4		-4.44	LEU543B, LEU541B, ILE542B, LEU523B, LEU511B	VAL411B, LEU525B, HIS524B, VAL530B	40
GB -5		-4.02	LEU511B, LEU523B, LEU543B, ILE542B, LEU541B	VAL411B, LEU525B, HIS524B, VAL530B	80
GB -6		-4.53	LEU541B, ILE542B, LEU543B, LEU523B, LEU511B	ALA549B, VAL411B, LEU525B,	30
GB -7	-CH ₃	-4.00	LEU523B, LEU511B, VAL411B,	LEU541B, HIS524B, VAL530B, ILE525B, VAL411B	80
GB -8		-5.22	LEU511B, LEU523B, LEU543B, ILE542B, LEU541B	LEU525B, VAL411B, HIS524B, VAL530B	10
GB -9		-4.20	LEU541B, ILE542B, LEU543B, LEU523B, LEU511B	LEU525B, VAL411B, VAL530B, SER529B, HIS524B	80
GB -10		-4.78	MET377B, LEU543B, ILE542B, LEU41B, LEU523B, LEU511B	VAL411B, LEU525B, HIS524B, VAL530B	30
			MET377B, LEU543B, ILE542B, LEU541B,	VAL411B, LEU525B, HIS524B, VAL530B	

GB -11	-4.00	LEU523B, LEU511B		50
GB -12	-4.63	LEU543B, ILE542B, LEU541B, LEU511B, LEU523B	HIS524B, SER529B, VAL530B, LEU525B, VAL411B	40
ADR	-	-	-	0.4

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

Figures

Figure 1
 UV-visible spectrum of copper nickel oxide nanoparticles at 5 mA/cm2 capped with CTAB in water

Figure 2

FTIR spectrum of as prepared copper nickel oxide nanoparticles

Figure 3

FTIR spectrum of calcinated copper nickel oxide nanoparticles

Figure 4

TGA curve of as prepared copper nickel oxide nanoparticles

Figure 5

XRD pattern of copper nickel oxide nanoparticles after calcination

Figure 6

SEM image of calcinated copper nickel oxide nanoparticles

Figure 7

EDS spectrum of copper nickel oxide nanoparticles

Figure 8

TEM image of calcinated copper nickel oxide nanoparticles

Figure 9

TEM micrograph of calcinated copper nickel oxide nanoparticles

Figure 10

SAED pattern of calcinated copper nickel oxide nanoparticles

Figure 11

Lattice fringe image of calcinated copper nickel oxide nanoparticles

Figure 12

Scheme 1 synthesis of 2-(2-substituted-4-oxo-thiazolidin-3-yl)-1,9-dihydro-purin-6-ones (GB-1 to GB-12)

Figure 13

Cell cytotoxicity determined by sulforhodamine B assay. The title compound (GB-1 to GB-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 14

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

Figure 15

The compounds GB-4, GB-8 and GB-10 at 10 nM test drug concentration and the GB-6 compounds at 100 nM test drug concentration show the maximum capillary growth inhibitory activity compared with thalidomide as a standard drug.

Figure 16

Molecular docking interactions images

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryFiles.docx](#)