Anticancer, antioxidant activities and molecular docking study of thiazolidine-4-one and thiadiazol derivatives

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ABSTRACT
Liver cancer accounts for a major portion of the global cancer burden. In many nations, the prevalence of this condition has risen in recent decades. New series of thiazolidinones and thiazolidine have been designed, synthesized, and evaluated for potential antioxidant and antihepatocarcinogenic activity. The antioxidant activity was evaluated using a DPPH assay. Furthermore, we examined the compounds against Hepg-2 cells using MTT assay, flow cytometry analysis through the cell cycle, reactive oxygen species, and apoptosis. The result showed that compound 6b has the highest antioxidant activity with IC50 = 60.614 ± 0.739 μM. The anticancer activity showed that compounds 5 and 6b have significant toxicity against liver cancer cells Hepg2, IC50 values (9.082 and 4.712) μM, respectively. Flow cytometry experiments revealed that compound 5 arrested Hepg-2 cells in the S process, while compound 6b arrested Hepg-2 cells in the G1. Compound 6b had a greater reduction in reactive oxygen species and late apoptosis than compound 5. Substantially, compound 5 had affinity energies of −7.6 and −8.5 for Akt and CDK4 proteins, respectively, but compound 6b had affinity energies of −7.8 and −10.1 for Akt1 and CDK4 proteins, respectively. Consequently, compound 6b had lower binding energies than compound 5. In this work, we used multiple bioinformatics methods to shed light on the prospective therapeutic use of these series as novel candidates to target immune cells in the tumor microenvironment of hepatocellular carcinomas such as CD8+ T cells, endothelial cells, and hematopoietic stem cells.

ARTICLE HISTORY
Received 16 January 2022
Accepted 27 March 2022

KEYWORDS
HepG-2; thiazolidinone; AKT1; CDK4; apoptosis; cell cycle; bioinformatics immunomodulation

1. Introduction
The incidence of cancer is increasing, according to the annual report of the Health Organization, as there are millions of new cases (Cronin et al., 2018). Since 1980, the incidence of liver cancer has more than quadrupled, while the mortality rate has more than doubled. In the United States, there are expected to be 42,230 new cases and 30,230 deaths from liver cancer in 2021 (https://www.cancer.org). Liver hepatocellular carcinoma (LIHC) is fatal cancer with no viable therapy. The Cyclin-dependent kinases 4/6 (CDK4/6) and PI3K/AKT signaling pathways are important in carcinogenesis and offer prospective therapeutic options for
Akt1 ablation has been shown in genetic research in mice to significantly reduce hepatic carcinogenesis. In terms of Akt1, a 2019 study found that Akt1-mediated phosphorylation of mTORC2 is critical for starting hepatocarcinogenesis in humans and animals, as it activates c-Myc, thus promoting cellular growth (Zhao et al., 2017). Strong evidence suggests that CDK4 is a clinical prognostic marker for LIHC patients (Lu et al., 2013). As a result, the development of novel potential medicines targeting AKT1 and CDK4 may represent an unmet medical need. Tumor microenvironment (TME) is a dynamic biological environment surrounded by tumors that include macrophages, stroma, fibroblasts, dendritic cells, stem cells, lymphocytes, pericytes, adipocytes, and blood vessels (El-Araby et al., 2020). However, no studies on the connection between AKT1 and CDK4 and invading immune cells in the TME have been undertaken.

Heterocyclic compounds have medicinal uses, considered as privileged scaffolds in the discovery of drugs (Xiao et al., 2020). The researchers try to find new ways to design and synthesize new derivatives of heterocyclic compounds (Zhang et al., 2020). Sulfur-containing substances such as 1,3,4-thiadiazolines and thiazolidinone have received great attention from medicinal chemists due to their biological activities and remarkable pharmacological properties and there are commercial drugs such as methazolamide acetazolamide and etozoline (Belluco et al., 1990; Eissa et al., 2021; Lu et al., 2020). 1,3,4-thiadiazole and thiazolidinone are the best heterocyclic compounds that have a wide spectrum of biological activity. Such as activity as an antibacterial, antifungal (Abu-Bakr & Hashem, 2019; Alho et al., 2000; Wu et al., 2020), anti-HIV-1, inhibitors of cancer and central nervous system (CNS) (Abdelhameid et al., 2020; Appalanaidu et al., 2016; Carvalho et al., 2004; Aly et al., 2020; Prabhakar et al., 2006; Prashantha Kumar et al., 2012; Tahlan et al., 2019; Weis et al., 2019). And have a strong biological activity, therefore widely used in the pharmaceutical, medicinally, and as pesticides (Almandil et al., 2019; Pushkarevsky et al., 2012; Semenov et al., 2013). Therefore, for the synthesis of new 1,3,4-thiadiazolines and thiazolidinone derivatives, there are several methods to obtain 1,3,4-thiadiazoles, mostly achieved by condensation substituted thiodyrazide with thiocyanate, also by the reaction between thiosemicarbazone with acetic anhydride (Ebrahimi et al., 2015; Janowska et al., 2018; Kakekochi et al., 2020; Saiz et al., 2009; Trotsko et al., 2019). The heterocyclic thiazolidinone is mostly achieved by the reaction from the reaction azomethine group with thioglycolic acid (Bade et al., 2017), also a reaction chloroacetic acid with thiosemicarbazones (Feitoza et al., 2012; Gomha & Khalil, 2012).

Given these findings, the current study’s goal is to synthesize thiazolidinone and thia diazoline derivatives. The synthesized heterocyclic compounds were characterized using spectroscopic methods such as IR, $^{13}$C, $^1$H NMR, and mass spectroscopy. They were then evaluated as antioxidants, anticancer (HepG-2), molecular docking studies of a target protein Akt1, and CDK4, and bioinformatic analysis of immune cell infiltration in the TME of LIHC patients.

## 2. Material and methods

### 2.1. Instrumentation and spectral measurements

The IR spectra were collected by using FTIR-Affinity-1 spectrophotometer in the region 4000–400 cm$^{-1}$ in KBr pellet. The mass spectra were scanned by the EI technique at 70 eV with an Agilent Technologies 5975 C spectrometer. The experimental values of $^1$H and $^{13}$C NMR spectra for the studied compounds were scanned on a Bruker Avance 500 MHz spectrometer. TMS as the internal standard. DMSO-d6 was used as a solvent. Melting points were measured on an electrothermal apparatus. Elemental analysis (CHNS) was measured by using elementaryVario MICRO. UV-Visible were measured by spectrophotometer type PG-instrument T80+. Microplate Reader Instrument using STAT FAX 2100, BioTek, Winooski, USA.

### 2.2. Chemistry section

Two types of heterocyclic derivatives were prepared from thiosemicarbazone. The first type is thiazolidine-4-one and its derivatives, the second type is thia diazoline derivatives.

#### 2.2.1. Procedure for synthesis 2-(diphenylmethylene)hydrazine-1-carbothioamide (1)

Thiosemicarbazide (0.914 g, 10 m.mol) was added to hot ethanol and (1.822 g, 10 m.mol) of benzophenone with 1 mL of glacial acetic acid, the reaction mixture was refluxed for 3 h. Then cool down to room temperature, the solid product was filtered, dried and finally recrystallization from ethanol.

White crystals, yield: 90%, m.p.: 173–172 °C.$^1$H NMR (DMSO, δ ppm): 7.34 (t, 3H, J = 5 Hz, Ar-H); 7.38 (t, 3H, J = 5 Hz, Ar-H); 7.64 (d, 2H, J = 5 Hz, Ar-H); 7.67 (d, 2H, J = 5 Hz, Ar-H). 8.37 (s, 2H, NH$_2$) 8.64 (s, H,NH).$^{13}$C NMR (DMSO, δ ppm) 128, 130, 131, 136.7, 149.5, 178.3. IR (DMSO, δ cm$^{-1}$): 3412, 3346, 2553, 255.34 [M], 238, 222.2, 195.2, 180.1, 165.1, 77.1. Anal. Calcd. for C$_4$H$_4$N$_3$S:C, 68.65; H, 5.83; N, 17.46; S, 13.26, found: C, 66.96; H, 5.33; N, 16.92; S, 12.87.

#### 2.2.2. Procedure for synthesis 2-(diphenylmethylene) hydrazineylidene) thiazolidin-4-one (2)

Compound 2 was prepared from thiosemicarbazone (1) with cyclization agent chloroacetic acid and sodium acetate. The thiosemicarbazone(1) (1.2 g, 4 m.mol) was dissolved in 50 mL of benzophenone with 1 mL of glacial acetic acid, the reaction mixture was refluxed for 3 h. Then cool down to room temperature, the solid product was filtered, dried and finally recrystallization from ethanol.

Pale yellow crystals, yield: 73%, m.p.: 185–186°C.$^1$H NMR (DMSO, δ ppm): 3.86 (S,2H), 7.23 (t, 3H, J = 5 Hz,Ar-H), 7.42 (t,
An equimolar amount of thiazolidine-4-one (2) and the corresponding aldehyde (0.2 m mol) Dissolved in glacial acetic acid (20 mL), then added anhydrous sodium acetate (1.2 g) the was heated at 120 °C in an oil bath, the reaction mixture was monitored by TLC using (chloroform: ethanol) (8:2 v/v), after cool down to room temperature, the mixture reaction was poured to (100 mL) ice water and kept it overnight, the solid product was filtered, recrystallization from an appropriate solvent.

2.2.3.5. (5z)-5-(benzo[d][1,3]dioxol-5-yl)methylene)acrylamide (4) 

Thiosemicarbazone (1) (1.82 g, 7.156 m mol) was dissolved in an oil bath at 118 °C, the reaction mixture was heated in acetic anhydride, the reaction mixture was monitored by TLC using (benzene: ethyl acetate), (6:4 v/v), the reaction mixture was cool down to room temperature and poured to 200 mL ice water, then was kept it at room temperature, then the filtered it, was recrystallized from ethylacetate, yield: 51%, m.p.: 169–170 °C. 1H NMR (DMSO, δ ppm): 2.54(s, 3H, CH3) 7.22(s, 1H, olefinic protons), 7.26 (d, 2H, J = 10Hz, Ar-H), 7.32 (d, 2H, J = 10Hz, Ar-H), 7.41(t, 3H, J = 5Hz, Ar-H), 7.44(t, 3H, J = 10Hz, Ar-H), 7.49(d, 2H, J = 5Hz,Ar-H), 7.61(2H, J = 10Hz,Ar-H). 13C NMR (DMSO, δ ppm): 16.7, 128.3–133.8, 136.7, 136.7, 139.3, 149.9, 161.4, 178, IR (ν, cm⁻¹): 3431, 3078, 2920, 1705, 1612, 1583, 1492, 1444, 1317, 1165, 696. MS (70 eV, m/z): 429.6 [M], 294.2, 195.2, 180.1, 165, 149.2, 77, 43. Anal. Calcd. for: C24H17N3OS2: 67.11; H, 4.46; N, 9.78, S, 11.49, found: C, 66.15; H, 4.19; N, 9.06; S, 14.42.

2.2.3.4. 5-(5Z)-(3,4-dihydroxybenzylidene)-2-((diphenylmethylene)hydrazinylidene) thiazolidin-4-one (3d).

Pale yellow crystals, recrystallized from ethylacetate, yield: 51%, m.p.: 169–170 °C. 1H NMR (DMSO, δ ppm): 2.54(s, 3H, CH3) 7.22(s, 1H, olefinic protons), 7.26 (d, 2H, J = 10Hz, Ar-H), 7.32 (d, 2H, J = 10Hz, Ar-H), 7.41(t, 3H, J = 5Hz, Ar-H), 7.44(t, 3H, J = 10Hz, Ar-H), 7.49(d, 2H, J = 5Hz,Ar-H), 7.61(2H, J = 10Hz,Ar-H). 13C NMR (DMSO, δ ppm): 16.7, 128.3–133.8, 136.7, 136.7, 139.3, 149.9, 161.4, 178, IR (ν, cm⁻¹): 3431, 3078, 2920, 1705, 1612, 1583, 1492, 1444, 1317, 1165, 696. MS (70 eV, m/z): 429.6 [M], 294.2, 195.2, 180.1, 165, 149.2, 77, 43. Anal. Calcd. for: C24H17N3OS2: 67.11; H, 4.46; N, 9.78, S, 11.49, found: C, 66.15; H, 4.19; N, 9.06; S, 14.42.

2.2.3.2. 5-(5Z)-(3,4-dihydroxybenzylidene)-2-((diphenylmethylene)hydrazinylidene) thiazolidin-4-one (3b).

Pale yellow crystals, recrystallized from ethanol, yield: =60%, m.p.: 165–166 °C. 1H NMR (DMSO, δ ppm): 7.11 (s, 1H, Ar-H), 7.12 (s, H, Ar-H), 7.24(d, H, J = 5Hz, Ar-H),7.42 (d, H, J = 10Hz,Ar-H)7.45 (t,6H, J = 5Hz, Ar-H), 7.53 (d, 2H, J= 10Hz, Ar-H), 7.57 (d, 2H, J= 10Hz, Ar-H), 10.13(s,OH),11.08 (s, OH), 11.90(s, H, NH). 13C NMR (DMSO, δ ppm): 122.3–136.2, 137.2, 154.7, 161.6, 162.7, 164, 166.5, 187.7. IR (ν, cm⁻¹):3431, 3143, 3062, 2937, 1705, 1612, 1585, 1494, 1444, 1317, 696. MS (70 eV, m/z): 415.47 [M], 398.1, 294.1, 222.2, 180.1, 165.2 77.2. Anal. Calcd. for:C24H17N3O3S2: 66.49; H, 4.12; N, 10.11; S, 7.72; found: C, 65.61; H, 3.93; N, 9.66; S, 7.14.

2.2.3.3. 5-(5Z)-(3,4-dihydroxybenzylidene)-2-((diphenylmethylene)hydrazinylidene) thiazolidin-4-one (3c).

Pale yellow crystals, recrystallized from methanol, yield: 69%, m.p.: 157–159 °C. 1H NMR (DMSO, δ ppm): 7.09 (s, 1H, Ar-H), 7.12 (s, Ar-H), 7.24(d, H, J = 5Hz, Ar-H), 7.36 (d, H, J = 10Hz, Ar-H), 7.42 (t, 3H, J = 5Hz, Ar-H), 7.47 (t, 3H, J = 10Hz, Ar-H), 7.53 (d, 2H, J = 10Hz, Ar-H), 7.79 (d, 2H, J = 10Hz, Ar-H, 10.21(s, 1H, OH), 11.08(s, 1H, OH), 11.90(s, 1H, NH). 13C NMR (DMSO, δ ppm): 128.3–138.1, 147.7, 156.6, 162.1, 165.9, 172, 174.3, 186. IR (ν, cm⁻¹): 3441, 3123, 3064, 2937, 1705, 1622, 1585, 1492, 1429, 696. MS (70 eV, m/z):415.47 [M], 398.1, 338, 294.1, 222.1, 195.2, 65, 77.2. Anal. Calcd. for: C16H13N3OS: 63.70; H, 5.05; N, 12.38; S, 9.45, found: C, 62.95; H, 4.87; N, 11.66; S, 882.
2.2.5. Procedure for synthesis 5,5-diphenyl-1,3,4-thiadiazoline-2-amine (5)

Compound (4) (0.678 g, 8 mmol) was dissolved in 10 mL of ethanol, hydrazine mono hydrate (6.8 mL) was added to the reaction mixture drop wise with stirring, slowly raising the temperature to the reflux for 3 hrs. the progress of the reaction was monitored by TLC using (ethanol; chloroform) (2:8 v/v), after completion the reaction, the reaction mixture was cool down to room temperature, then it was filtered and recrystallized from ethanol. yellow crystals, yield: 62%, m.p.: 90–91 °C. 1H NMR (DMSO, δ ppm): 6.23(s, 2H, NH2), 7.23(t, 2H, J = 10Hz, Ar-H), 7.28(t, 2H, J = 10Hz, Ar-H), 7.33(d, 2H, J = 10Hz, Ar-H), 7.49(d, 2H, J = 5Hz, Ar-H), 7.56(t, 2H, J = 5Hz, Ar-H). 13C NMR (DMSO, δ ppm): 25.9, 127.6–129.8, 129.8, IR (υ, cm⁻¹): 3421, 3271, 3080. 1610, 1492, 1442, 1336, 1180, 696. MS (70 eV, m/z): 255.34 [M] 195.3, 180.1, 165.2, 77.2. Anal. Calcd. for: C14H17N3O2S, C, 70.17; H, 4.77; N, 11.69; S, 8.92. found: C, 69.21; H, 4.13; N, 11.06; S, 8.02.

2.2.6. General procedure for synthesis 1,3,4-thiadiazoline derivatives (6a, 6b)

5,5-diphenyl-1,3,4-thiadiazoline-2-amine (5) (0.635 g, 2.5 mmol) was dissolved in 20 mL ethanol, corresponding aldehyde (2.5 mmol) and 0.5 mL glacial acetic acid was added to the reaction mixture, the mixture was refluxed 5 hrs, forming of the products was confirmed TLC using (ethanol; ethyl acetate) (1:9 v/v), after completion the reaction was cool down to room temperature, then it was filtered and recrystallized from an appropriate solvent.

2.2.6.1. (Z)-4-(((5,5-diphenyl-1,3,4-thiadiazoline-2-yl)iminomethyl)phenol (6a). Yellow crystals, recrystallized from ethanol, yield: 60%, m.p.: 175–177 °C. 1H NMR (DMSO, δ ppm): 6.79(s, H, NH), 6.80–7.61(m, 14 H, J = 10Hz, Ar-H), 8.49 (s, H, CH = N), 10.03(s, 1H, OH). 13C NMR (DMSO, δ ppm): 116, 128.1–138.2, 159.5, 160.7, 164.8. IR (υ, cm⁻¹): 3342, 3124, 1608, 3055, 1585, 1516, 1492, 1305, 694. MS (70 eV, m/z): 359.45 [M]+, 283.2, 239.1, 195.1, 165.1, 77.2. Anal. Calcd. for: C16H17N3S, C, 70.17; H, 4.77; N, 11.69; S, 8.92. found: C, 69.21; H, 4.13; N, 11.06; S, 8.02.

2.2.6.2. (Z)-4-(((5,5-diphenyl-1,3,4-thiadiazoline-2-yl)iminomethyl)benzene-1,3-diol (6b). Yellow crystals, recrystallized from methanol, yield: 75%, m.p.: 168–169 °C. 1H NMR (DMSO, δ ppm): 6.35(s, 1H, NH), 6.37(s, H, Ar-H), 7.28(d, 1H, J = 10Hz, Ar-H), 7.36(d, 9H, J = 10Hz, Ar-H), 7.52(t, 3H, J = 10Hz, Ar-H), 7.65 (d, 4H, J = 10Hz, Ar-H), 8.81(s, H, CH = N), 10.20(s, H, NH), 11.22(s, H, COH), 13C NMR (DMSO, δ ppm): 102.7, 108.5, 110.7, 128.6–137.2, 161.6, 164.1, 166.5. IR (υ, cm⁻¹): 3317, 3142, 1631, 3055, 1606, 1506, 1489, 1323, 692. MS (70 eV, m/z): 375.45 [M]+, 316.2, 299.1, 239.1, 180.1, 165.1, 77.2. Anal. Calcd. for: C21H17N3O2S, C, 66.55; H, 5.26; N, 10.56; S, 7.82.

2.3. DPPH radical scavenging assay

The antioxidant activity of all the synthesized compounds was an evaluation by determining the ability of radical scavenging according to the Blois method (Blois, 1958). The inhibitory activity of DPPH was measured by using the spectrophotometer method by mixing 1 mL of different concentrations (50, 75, 100, and 200) μM of solution of synthesized compounds with 1 mL of 200 μM of DPPH solution, the absorbance was measured at 517 nm after 70 min. The inhibition percentage calculates by using the following equation:

\[ \text{inhibition percentage} \% = \frac{\text{Ac} - \text{As}}{\text{As}} \times 100 \]

Ac = control absorbance, the absorbance of DPPH without sample
As = sample absorbance, the absorbance of DPPH with sample

2.4. MTT assay

The cell line was mainly grown in 96-well plates at 1 x 10⁵ cells per well for 24 hours under optimum conditions (37°C, 5% CO₂ in a humidified incubator). Next, the cells were washed twice with PBS after the removal of the growth medium (10% FBS). New culture media containing the studied compounds at a concentration (5, 10, 25, 25) μM were added to the cells followed by incubation for 24, 48, and 72 h. Quinnet wells were analyzed for each concentration. A 10 μL solution of freshly prepared (5 mg/mL) MTT in PBS was added to each well and then incubated for an additional 4 hours. After successful incubation, the resulting medium was aspirated and the MTT formazan which has been generated in this step was dissolved in the 100 μL of DMSO. The solubilization of formazan crystals was obtained by gently shaking the plates. A microplate reader was then employed to measure the absorbance at 545 nm. The cellular toxicity percentage, as well as the half-maximal inhibitory concentration (IC₅₀) were calculated by the following equation:

\[ \text{Viability} \% = \frac{\text{mean of OD sample}}{\text{mean of OD control}} \times 100 \]

2.5. Flow cytometry analysis

2.5.1. Cell cycle

According to Al-Shawi et al. (2020), flow cytometry analysis identified the G1, S, and G2 phases for compounds 5 and 6b. System with some modifications. The IC₅₀ values of compounds 5 and 6b were added to Hepg-2 cells for 48 hours. The cells were washed in PBS followed by fixation in 70% ice-cold ethanol overnight at 4°C. After being washed twice with PBS, cells were then stained with a solution containing 50 g/mL PI and 100 g/mL RNase A for 30 minutes in the dark at room temperature. Flow cytometry was employed to investigate the labeled cells (Beckman Coulter, Epics XL).
2.5.2. Reactive oxygen species
The cells were stained with 2,7-dichlorofluorescein-diacetate to determine the changes within the intracellular reactive oxygen species generation, as described by Khan et al. with some modifications (Khan et al., 2012). (DCFH-DA) Formalized adverbial in a nutshell, Hepg-2 cells were cultured in 6 well culture plates overnight. The cells were examined 48 hours after treatment with or without the IC_{50} value of compounds 5 and 6b. The cells were then incubated for 30 minutes at 37°C with 10 mol/L DCFH-DA, per the manufacturer's instructions. The cells were then incubated for 30 minutes at 37°C with 10 mol/L DCFH-DA, as directed by the manufacturer. In the positive control group, DCFH-DA labeled cells were treated with 1 L rose for 30 minutes. The cells were then harvested, rinsed, resuspended in PBS, filtered through 300 apertures, and flow cytometry was used to search for DCF fluorescence (FCM).

2.5.3. Apoptosis
Flow cytometry analysis, which was adapted from the Wang et al. method (Wang et al., 2017), was used to identify early and late apoptosis. Compounds 5 and 6b IC_{50} values were applied to Hepg-2 cells for 48 hours. The cells were collected, resuspended twice with PBS, and labeled with 5 μL FITC-conjugated annexin V, as directed by the manufacturer. After being incubated in the dark for 10 minutes and then labeled with PI. Samples were then analyzed on a flow cyrometer (Beckman Coulter, Epics XL).

2.6. Molecular docking
As usual, the receptor input files were created. To begin, we removed any water molecules, ligand atoms, and ions that did not belong to the receptor’s active site from the PDB file. After that, hydrogen atoms, protons, and partial charges from amino acid side chains were added (following the protonation state at the physiological). This was followed by a local minimization to relieve potential bad contacts. Minimization was performed in the presence of restraints to maintain the protein conformation. The structure of ligands (compound 5 and 6b) was drawn using Chem Draw Ultra from the Chem Office software package. Then, it was copied into Chem3D Ultra (same program package), before using Auto Dock tools (ADT), it is a good idea to check that the file contains all hydrogen atoms. ADT now immediately computes Gasteiger charges (empirical atomic partial charges) and distinguishes between hybridization states and atom groups after opening the ligand. The software specifies the ligand’s rotatable bonds as part of the preparation process so that different conformers for docking can be produced. Where it was subjected to a simplified energy minimization search to a minimum root mean standard deviation gradient of 0.100. The obtained structure with local minimum was saved in convenient mol2 format.

Docking studies (Jin et al., 2020) was confuted using Auto Dock vena to obtain binding interaction between the ligand (compound 5 and 6b) and binding pockets of two distinct proteins of liver cancer cell (Hepg-2). From the protein data bank (https://www.rcsb.org/). The crystal structure of Hepg-2 proteins was obtained: Akt1 (PDB: ID 5KCV) (Lapierre et al., 2016), and CDK4 (PDB: ID 2W96) (Day et al., 2009). Energy minimization and hydrogen bonding optimization were carried out after docking. For each ligand the docking simulation was run multiple times for further study, the highest binding scores were used in addition. Discovery studio visualizes 2019 was used to do a thorough examination of the docking effects.

2.7. Statistical analysis
IC_{50} values of the compounds were performed by plotting dose-response curves versus the concentrations using GraphPad Prism version 8.1 for windows. The experiment was repeated thrice.

2.8. Bioinformatic analysis
To highlight many discoveries linked to our targets AKT1 and CDK4, we used a variety of bioinformatics approaches. First, UALCAN is a web-based resource for cancer research that is comprehensive, user-friendly, and interactive. UALCAN is meant to give users quick access to publicly available TCGA data, allowing them to find biomarkers and perform in silico validation of possible genes of interest, as well as display expression profiles and patient survival statistics in graphs and plots for various cancer types (Chandrashekar et al., 2017).

3. Result and discussion
3.1. Antioxidant potential
For many living things, oxidation is necessary for the production of energy (Pong, 2003). However, the continually generated free radical reactive oxygen (ROS) species may destructively cause RNA, DNA, causing mutations, chromosomal damage, and the oxidation of unsaturated fatty acids. ROS promotes heart disease, neurological disease, cancer, and aging, and contributes to enhancing oxidative damage (Duru et al., 2014). However, antioxidants help protect the body from free radical damage and are necessary for the human body to scavenge the radicals are produced from mitochondria leak, pollution, sunlight, ultraviolet ray, and smoking (Jothy et al., 2011).

Several antioxidant scans are collected under the subject of HAT or single electron transfer (SET). The DPPH concentration reduce with time and change in color from purple to yellow and/or colorless owing to the transfer of the atomic hydrogen, was used in the measurements of scavenging activity of studied compounds (2, 3a, 3b, 3c, 3d, 3e, 4, 6a, and 6b) at concentrations of 50, 75, 100, and 200 μM. At a concentration of 200 μM, the inhibition activity was (9.02%–77.778%). The inhibition activity percentage of all studied compounds is shown in Figure 1.

Several antioxidant scans are gathered under the topic of hydrogen atom transfer (HAT) or single electron transfer (SET). Their action can be clarified through the oxidative mechanism of phenolic antioxidants. The values of the scavenging activity of the synthesized compounds (2, 3a, 3b, 3c,
3d, 3e, 4, 6a, and 6b) at concentrations 50, 75, 100, and 200 μM, were measured by the decrease of DPPH absorbance at 517 nm with time and the change of the DPPH color from purple to yellow or colorless due to the transfer of hydrogen atom. The inhibition activity at a concentration of 200 μM within the range (9.02%–77.778%), the inhibition percentage activity of the studied compounds shows in Figure 1.

The inhibitions activity of compounds 6a, 6b, and 2 were quite high (200 μM) in DPPH. While the other compounds exhibited less inhibitory activity of DPPH, due to absence of the phenolic OH group, which provided the 6a, 6b, and 2 compounds with a strong radical activity of scavenging by giving radicals of DPPH hydrogen atom and inhibiting radical activity with HAT (Slavova-Kazakova et al., 2015). Consequently, the radical scavenging activity of compounds 6a, 6b, and 2 were in the order: 3a > 3b > 2.

The half-maximal inhibitory concentration (IC₅₀) for the studied compounds was calculated by GraphPad Prism 8.02. The IC₅₀ inhibition of 6a, 6b, and 2 compounds was 55.729 ± 0.285, 60.614 ± 0.719, and 105.56 ± 4.456 μM, respectively. The results showed that the IC₅₀ values are consistent with the inhibition activity and that compounds with a phenolic group can scavenge free radicals and inhibit the oxidizing agents via the transfer of hydrogen atomic (HATs). So the most efficient which have phenolic groups were the most efficient as an antioxidant to the radical source.

3.2. Anticancer activity

Liver cancer is one of the most common malignant tumors in the world (Ferlay et al., 2015). The fast growth in liver cancer incidence, lack of adequate treatment has led us to look for new and more efficient molecules (Zhang et al., 2019).

Because of the action of the thiazolidinone and thiadiazoline rings, as well as their ability to communicate with biological targets, they have a variety of biological applications, including antimicrobial and antitumor (Lu et al., 2020; Szeli, 2020). As a result, it may be a starting point for developing new anticancer agents. We tested five new thiazolidinone and thiadiazoline derivatives against liver cancer cells in this study (Hepg-2). The compounds had varying anticancer activity against the Hepg-2 cell line, Figure 2 shows the IC₅₀ values within the range (4.712–46.60) μM. The studied compounds were taken from the lowest IC₅₀. Compound 6b has the lowest IC₅₀ value of 4.712 μM, compound 5 with IC₅₀ value of 9.082μM, compound 2 with IC₅₀ 17.49μM and compound 4 and 6a has IC₅₀ value 38.616 μM and 46.60 μM respectively and the results showed substantial anti-cancer efficacy against the HepG2 cell line of the investigated substances. Therefore compounds 5 and 6b which has the lowest IC₅₀ being used in subsequent flow cytometry experiments (cell cycle, reactive oxygen species, and apoptosis). The IC₅₀ values of compounds 5 and 6b were used in cell cycle phase detection, with compound 5 arresting cells in the S phase (Figure 3(B2)) and compound 6b arresting cells in the G1 phase (Figure 3(B3)), as compared to the monitor (Figure 3(B1)). Figure 4 depicted the impact of compounds 5 and 6b on reactive oxygen species, DCFH⁺ for the control was 90.8%, which was increased to 92.8% by using the IC₅₀ value of compound 5, and raised to 96.0% by using the IC₅₀ value of compound 6b. This increase in DCFH ratio compared to the control revealed that compound 6b has a greater impact on HepG2 cells than compound 5, which was improved by the apoptosis experiment. Figure 3(A) shows that the IC₅₀ value of compound 5 has a late apoptosis ratio (Q2) of about 15.1%, compared to the control late apoptosis ratio of 42.1%, and compound 6b increased the late apoptosis ratio to 19.6% when compared to live cells (Q4). Therefore, these results suggest that compounds 5 and 6b are potential cytotoxic agents. One reason for apoptosis might be the observed capacity of compounds to cause cell cycle arrest. Cancer is a complicated illness characterized by aberrant cell growth control, chromosomal misalignment, as well as genetic instability (El-Arabey, et al., 2018). The principal regulatory mechanisms for cell growth and proliferation are known as cell cycle and apoptosis. When specified control points of the cell cycle are detained, apoptotic cell death is triggered (Pathak & Khandelwal, 2007). Accordingly,
numerous anti-cancer drugs lead to cell cycle stoppage and clinically effective cancer treatment has been demonstrated (Xiao-Ming, 2000). The majority of anticancer medicines now utilized in clinical oncology exploit intact apoptotic signaling pathways to cause cancer cell death. As a result, abnormalities in the death pathways might lead to drug resistance, decreasing the effectiveness of medicines. In this regard, a better knowledge of the apoptotic cell death signaling pathways may increase cancer therapeutic efficacy and circumvent resistance (Pistritto et al., 2016). Several studies deduced that chemotherapeutic agents may act as prooxidants to produce reactive oxygen species in overwhelming amounts with subsequent autophagy reaction in LiHC cells. In fact, these interaction from chemotherapy aims to scavenging reactive oxygen species and its inhibition accelerates cell survival after exposure to antioxidants, but promotes cell death with prooxidants (Yuan et al., 2018). Similarly, our results indicated that compound 5 and 6b may act as prooxidant (Figure 4) to decrease cell survival as shown in our apoptotic analysis (Figure 3).

**3.3. Structure activity Relationship Studies**

Our in vitro results suggest that compound 6b has a stronger impact on Hepg-2 cells than compounds 6a and 5. Compound 5 has an amino group bonded to a thiadiazolin ring and hydroxyl free. Whereas compound 6b has two aromatic rings bonded to the thiadiazolin ring and two hydroxyl groups. From a chemical point of view, the binding potential of potent with Hepg-2 to the presence of hydroxyl and heteroatoms. These groups specifically −OH group serves as an excellent candidate for hydrogen bonding and exhibits a tendency to form strong interactions with amino acid residues of the target protein (Adem, et al., 2021). This range of
functional groups boosts the compound’s efficacy against liver cancer cells, paring the way for further research.

3.4. Pharmacokinetic and drug-likeness properties

The ability to anticipate the absorption, distribution, metabolism, and excretion of possible novel therapeutic leads requires an understanding of the pharmacokinetic and drug-like features of newly developed compounds for specific targets (Savale et al., 2021). In this study physicochemical features of powerful hits, such as water solubility, lipophilicity, and pharmacokinetics, were investigated in silico by ADMA analysis (The absorption, distribution, metabolism, and excretion), using the website http://www.swissadme. The percentage of potent hits absorbed (percent ABS) from the gut was calculated as follows: percent ABS = 109 (0.345Xtpsa. The SwissADME software was used to predict the pharmacokinetic parameters of the examined compounds (Figure 5). We observed that all compounds obeyed the Lipinski’s rule of five, with molecular weights of <400 g/mol, number of hydrogen bond donors 2-4, and acceptors 17-45, log P value of < 5.5, and molar refractivity of <120, all compounds obeyed Lipinski’s rule of five (Lipinski, 2004).All compounds have a Topological Polar Surface Area (TPSA) of < 93Å2, suggesting their potential to permeate biological membranes (Shivanika et al., 2020). Furthermore, all substances have high gastrointestinal absorption and solubility. The presence of a large number of rotatable bonds (5-2) implied a high level of flexibility. The pkCSM software tool (http://structure.bioc.cam.ac.uk/pkcsim) was used to assess the toxicity of the chemicals studied (Table 1). We discovered that compound 6b was neither hepatotoxic nor carcinogenic and that its toxicity was better. The log P value within the range (5.38-3.36), interestingly, compound 6b had the highest permeation rate of all the compounds, indicating that it has the capacity to efficiently penetrate biological membranes (Abdalla et al., 2021; Mohapatra et al., 2021). This increases its ability to bind with Hepg-6 proteins and is consistent with its IC50 value.

3.5. Molecular docking results

Molecular bioinformatics docking studies in the field of drug development are currently of considerable use, reducing the money and efforts required to screen novel compounds by directing and restricting the research to possible targets/targets. A molecular docking simulation is, thus, an essential way of anticipating a substrate interacting with its receptor.

To determine whether compounds 5 and 6b inhibits Akt1 and CDK4 proteins, thus, affecting cellular migration of Hepg-2, molecular docking simulation was carried out to determine the binding mode of 5 and 6b into the Akt1 and

Figure 3. The flow cytometry analysis of A. Apoptosis analysis: A1. Untreated cells. A2. Cells treated with compound 5. A3. Cells treated with compound 6b. B. Cell cycle analysis: B1. Untreated cells. B2. Cells treated with compound 5. B3. Cells treated with compound 6b.
CDK4 active site. The 5 and 6b molecules were precisely docked into the active pocket of Akt1 and CDK4 in ADT software (El Rayes et al., 2020).

The results of the docking study showed a good fit into the binding of CDK4 protein active site with affinity energy –8.7 kJ/mol (6b) and –7.9 (5), the affinity energy of Akt1 protein with compound 6b (–10.0 kJ/mol). Moreover, the binding interactions between the amino acid residue and ligands (compounds 5 and 6b) are shown in Table 2 and Figures 6 and 7. Compound 5 formed two hydrogen bonds, as a side-chain acceptor and backbone acceptor with amino acids (GLU A75 and LYS A180) residue of CDK4 protein. In addition, several hydrophobic interactions with amino acid residue including Lys A72, Gln B183, Glu A69, Lys B180 and Thr B184. Compound 6b (Figure 6) formed four hydrogen bonds as a side-chain acceptor with amino acid (GLU A69, Thr A184, GLU A69, and GLU A75) and ionic bond with GLU A69 of CDK4 protein, in addition, hydrophobic interactions with amino acid residue including Leu B188, Gln B261, Ser B258, Leu A65, and Cys A68. The interaction of compound 6b with Akt1 target protein (Figure 7) shows two hydrogen bonds from binding with critical amino acid residues Trp A80, and Lys A268 as well as ionic interaction with Asp A292. Moreover, the hydrophobic interactions with amino acid residue including Tyr B205, Thr A82, Gin Arg B273, Val B270, Ser B205, Arg B273, and Leu B264. Compound 5 show one hydrogen bond with amino acid residue, Ile A 290, one ionic bond with Asp A 292, and one pi-pi interaction with Trp A 80 of Akt1 protein. In addition, several hydrophobic interactions with Leu B264, Leu B210, Ser B205, and Lys B268. The 3D molecular surface map of the most active compounds 5 and 6b docking into the Hepg-2 binding site is shown in Figures 6 and 7.

3.6. Molecular dynamics simulation

Molecular dynamics is an analytical approach that uses computer simulation to analyze the physical motions of a
Table 1. Physicochemical property, ADME parameter and toxicity of studied compounds.

| Comp. | Ghose | TPSA | Absorption %ABC | Water solubility Log S (ESOL) | BBB permeate | P-gp substrate | CYP isoform interact |
|-------|-------|------|-----------------|-------------------------------|--------------|----------------|---------------------|
| 5     | Yes   | 75.71| 82.88           | −5.27 soluble                 | No           | No             | No                  |
| 6a    | Yes   | 81.62| 80.84           | −5.06 poor soluble            | No           | No             | No                  |
| 6b    | Yes   | 93.06| 76.89           | −3.90 poor soluble            | No           | No             | No                  |

Predicted organ toxicity, toxicological end points and acute toxicity

| Comp. | Hepatotoxicity | Carcinogenicity | Immunotoxicity | Mutagenicity | Cytotoxicity | LD50 (mg/kg) | Acute toxicity class |
|-------|----------------|-----------------|----------------|--------------|--------------|--------------|---------------------|
| 5     | Inactive       | Active          | Inactive       | Active       | Inactive     | 73           | 3                   |
| 6a    | Inactive       | Inactive        | Inactive       | Active       | Inactive     | 650          | 4                   |
| 6b    | Inactive       | Inactive        | Inactive       | Active       | Inactive     | 1400         | 4                   |

Table 2. Affinity energy and amino acids residue of compounds 5 and 6b with a target protein.

| Target protein | PDB ID | Compound | Binding interaction (amino acid residue) | Interaction | Distance (Å) | Binding interaction energy (kcal/mol) | Affinity energy (kcal/mol) |
|----------------|--------|----------|-------------------------------------------|-------------|--------------|--------------------------------------|----------------------------|
| CDK4           | 2W96   | 6b       | Glu 69 (A)                                | H-donor     | 2.85         | −2.5                                 | −10.1                      |
|                |        |          | Thr 184 (A)                               | H-donor     | 3.44         | −0.5                                 |                            |
|                |        |          | Glu 69 (A)                                | H-donor     | 2.94         | −12.4                                |                            |
|                |        |          | Glu 75 (A)                                | H-acceptor  | 3.59         | −0.6                                 |                            |
|                |        |          | Glu 69 (A)                                | ionic       | 2.94         | −4.9                                 |                            |
|                |        |          | Glu 75 (A)                                | H-donor     | 4.03         | −1.4                                 | −8.5                       |
|                |        |          | Glu 75 (A)                                | H-acceptor  | 3.40         | −0.6                                 | −7.8                       |
|                |        |          | Lys 180 (A)                               | H-acceptor  | 3.35         | −1.3                                 |                            |
|                |        |          | Lys 268 (A)                               | H-acceptor  | 3.35         | −1.3                                 |                            |
|                |        |          | Lys 268 (A)                               | ionic       | 3.35         | −1.3                                 |                            |
|                |        |          | Asp 292 (A)                               | ionic       | 3.80         | −1.0                                 |                            |
|                |        |          | Asp 292 (A)                               | H-acceptor  | 2.95         | −1.4                                 | −7.6                       |
|                |        |          | Asp 292 (A)                               | ionic       | 3.94         | −0.6                                 |                            |
|                |        |          | Trp 80 (A)                                | pi-pi       | 3.89         | 0.0                                  |                            |
| Akt1           | 5KCV   | 6b       | Trp 80 (A)                                | H-acceptor  | 3.40         | −0.6                                 | −7.8                       |
|                |        |          | Lys 180 (A)                               | H-acceptor  | 3.35         | −1.3                                 |                            |
|                |        |          | Lys 268 (A)                               | H-acceptor  | 3.35         | −1.3                                 |                            |
|                |        |          | Asp 292 (A)                               | ionic       | 3.80         | −1.0                                 |                            |
|                |        |          | Asp 292 (A)                               | H-acceptor  | 2.95         | −1.4                                 | −7.6                       |
|                |        |          | Asp 292 (A)                               | ionic       | 3.94         | −0.6                                 |                            |
|                |        |          | Trp 80 (A)                                | pi-pi       | 3.89         | 0.0                                  |                            |

Figure 6. Two and three dimensional compounds 5 and 6b interact with the active site of target protein CDK4.
A predetermined amount of time is provided for the interaction of the atoms and molecules to reveal the system’s complicated development. The design and discovery of novel medicines, it has shown to be a helpful tool. As a result of molecular dynamics research, thermodynamics and kinetics related to the compound–protein recognition and binding may be more precisely estimated. Root mean square deviation (RMSD), Root mean square fluctuation (RMSF), and Radius of gyration data as a function of time were used to analyze the molecular dynamic simulations (Dickson et al., 2017). In addition, Figure 8 demonstrated that compound 6b-CDK4 complex has a higher ratio of water-binding with 17 amino acids for the active site of CDK4 as well as few hydrogen bonds. Compound 5-CDK4 complex demonstrated a higher ratio of hydrogen bonds and water binding. As a result, compound 6b may have an active effect on CDK4, agreeing with the IC50 value.

Figure (8C, 8D) showed the high ratio of hydrogen bond and water binding for compound 5-AKT1, while compound 6-AKT1 showed hydrogen bonds and water binding.

RMSD and RMSF were used to analyze the molecular dynamic simulations, and assess internal motions conformation change and stability of compounds 5-CDK4, 6b-CDK4, 5-AKT1, and 6b-AKT1. The RMSD and RMSF values were shown in (Figures 9 and 10). In the compound 6b-CDK4 complex the scalar distance between atoms show an upward trend fluctuation from 0-25 nanosecond and the downward trend fluctuation from 25–100 nanosecond, the value of RMSD 1.7 Å, compound 5-CDK4 complex show an upward from 20–40, and 70–90 nanosecond, and downward from 40–70 nanosecond, the value of RMSD 1.6 Å. While compound 5-AKT1, and 6b-AKT1(Figure 8(C,D)) showed the value conformation, that binding site less fluctuation. The RMSF values are used to predict the motion of compound-protein. The RMSF value of compound 5-CDK4 (Figure 10A) demonstrated broad peaks 50–150Å with a value of (1.4–2.6)Å, compound 6b-CDK4 also showed broad peaks 90–200Å with a value of (1.8–3.4) Å the RMSF of compound 5-AKT1 complex (Figure 10C) it is related that sharp peaks (10–150) Å, had higher value (1.4–4) Å, compound 6b-CDK4 complex (Figure 10D) showed sharp peaks (10–150) Å, with higher value (1.4–4.5) Å.

3.7. Bioinformatic results and discussion

In the current study, we utilized UALCAN as a bioinformatics tool to highlight the expression of AKT1 and CDK4 in LIHC.
Figure 8. Throughout the trajectory, the bar charts depict protein-compound interaction, (A) compound 5-CDK4-complex, (B) compound 6b-CDK4-complex, (C) compound 5-AKT1-complex, (D) compound 6b-AKT1-complex.

Figure 9. Root mean square deviation (RMSD), (A) compound 5-CDK4-complex, (B) compound 6b-CDK4-complex, (C) compound 5-AKT1-complex, (D) compound 6b-AKT1-complex.
Figure 10. Root mean square fluctuation (RMSF), (A) compound 5-CDK4-complex, (B) compound 6b-CDK4-complex, (C) compound 5-AKT1-complex, (D) compound 6b-AKT1-complex.

Figure 11. Bioinformatic result for AKT1.

Figure 12. Bioinformatic result for CDK4.
based on sample type (normal/primary tumor) and nodal metastasis status (N0: No regional lymph node metastasis; N1: Metastases in 1 to 3 axillary lymph nodes) using TCGA database. Substantially, our findings showed that AKT1 was expressed at much greater levels in primary tumors than in normal tissues (Figure 11A) and more abundant in N1 than in N0 and normal (Figure 11B). Similarly, CDK4 was highly expressed in primary tumors than in normal tissues (Figure 12A) and more considerable in N1 than in N0 and normal (Figure 12B). Our bioinformatic analysis (Figures 11 and 12) showed that AKT1 and CDK4 are involved in the tumorigenesis and metastasis of HLIC. In this sense, AKT1 and CDK4 are promising druggable targets to eradicate LIHC. As a consequence of the foregoing findings, it is indicated that the proposed compounds can combat LIHC and additional research on compound 6b is advised. We anticipate that the study will give ample references towards the development of a suitable scaffold for the LIHC.

3.8. Chemistry

The synthesis of two heterocyclic series of (thiazolidine-4-one and thiadiazole) derivatives are performed in multi-steps (Schemes 1 and 2). Initially, prepared thiosemicarbazone (1), then was treated with chloroacetic acid as a cyclized agent to prepare the first series from thiazolidin-4-one (Hammad et al., 2020). The derivatives of thiazolidine-4-one (3a-e) were prepared by the reaction of a corresponding aromatic aldehyde with thiazolidine-4-one and sodium acetate as a reagent. The next series of thiadiazole was prepared from the reaction thiosemicarbazone with acetic anhydride (Saiz et al., 2009), the product was treated with hydrazine monohydrate to hydrolysis the acetyl groups and prepare 2-amino thiadiazoline (5), the product was reacted with the corresponding aldehyde to form thiadiazolin derivatives (6a and 6b).

3.9. Spectral characterization

The IR spectra of the synthesized compounds exhibits show bands of expected functional groups, the IR spectrum of thiosemicarbazone (Anoop et al., 2010; Mangalam & Kurup, 2009), shows two bands at 3412 and 3346 cm\(^{-1}\) attributed to symmetric and asymmetric stretching of an amine group, strong band at 1087 cm\(^{-1}\) attributed to stretching vibration of C=S, the thiosemicarbazone behavior has been reported in the literature (Ebrahimi, 2010; Raj et al., 2013; Roeges & Baas, 1994).

Thiazolidine-4-one (2) shows a strong band within the range \(1705–1770\) cm\(^{-1}\) attributed to C=O, the absence of absorption bands of NH\(_2\) and C=S group due to participate to form the thiazole ring with the synchronous appearance of new bands assigned to the carbonyl group within range \(1705–1770\) cm\(^{-1}\).

The IR spectrum of thiadiazoline (4) shows two strong bands at 1707 and 1676 cm\(^{-1}\) attributed to C=O of acetyl groups also an absence of absorption bands of NH\(_2\) and C=S group, with the synchronous appearance of new stretching vibration bands assigned to the carbonyl groups at 1707 and 1676 cm\(^{-1}\), can be justified by a strain of thiadiazole ring and clearly displayed the involvement of the azomethine groups in the formation of five-membered rings. Thiadiazoline’s ring closure may be noted by the participation of azomethine (C=N) groups in nitrogen atom cyclization that is predicted to raise electron density in azomethines and to enhance the frequency of absorption (C=N) and the frequency of absorption is shifted about \((1705–1770)\) cm\(^{-1}\) (Toan et al., 2021).

The infrared spectrum of 5,5-diphenyl-1,3,4-thiadiazoline-2-amine (5) which formed by hydrolysis of thiadiazoline displays two strong bands at \(1342–3271\) cm\(^{-1}\) attributed to the NH\(_2\), also the absence of absorption bands of C=O of acetyl groups. Finally, the derivatives of thiadiazolin show a
strong band within range (1608–1631) cm\(^{-1}\) attributed to the azomethine group.

The data of \(^1\)H NMR spectral of the synthesized compounds at room temperature in deuterated DMSO affirm the structure of compounds annotation and the formation of the heterocyclic ring in all compounds (Padmaja et al., 2015), thiosemicarbazone show the NH\(_2\) signal at 8.41, 8.37 ppm as two signals, also shown singlet signal at 8.64 ppm NH attributed to NH, the signals within range (7.34–7.67) ppm attributed to aromatic protons. The Thiazolidine-4-one (2) display signal at 11.9 NH and, singlet signal of –CH\(_3\) protons at 3.86 ppm. All protons in their predicted area are observed. Cyclic ring and –CH\(_2\)- groups. Comparison of thiazolidine-4-one chemical shifts with thiosemicarbazide indicates that the NH2-proton signal in the spectrum is absent. The disappearance of NH2 proton signals from the \(^1\)H NMR thiazolidine-4-one spectrum supports cyclization of the thiosemicarbazone, which is confirmable by the appearance simultaneously of new signals of the five-membered ring. The spectra of (3a-3e) Thiazolidine-4-one derivatives display new signals with range (6.89–7.20) ppm attributed to C—CH of coupled groups with an absence of –CH\(_2\)-protons of cyclic rings. Also, the spectra display signals of aromatic protons and protons signals of coupled groups. Thiadiazolin (4) is produced from cyclization of the thiosemicarbazone, the spectrum shown two signals at 2.03 and 2.20 ppm attributed to OCH\(_3\) also display signal at 11.73 ppm attributed to NH proton, all protons of cyclic, aromatic, and methyl can be seen in the predicted region, the comparison of Thiadiazolin (4) chemical shifts with thiosemicarbazide indicates that the NH\(_2\) proton signal in the spectrum is absent. This confirms the formation of the Thiadiazolin ring. The spectra of compound 5 display a new signal at 6.23 ppm attributed to NH\(_2\) protons with an absence of methyl protons, this supports the acetamide (HN-COCH\(_3\)) is hydrolysis and formed primary amine. The Thiadiazolin derivative (6a-6b) spectra display the signal of azomethine within range (10.3–11.22) ppm and signals of phenolic protons within range (10.3–11.22) ppm as well as signals of aromatic protons.

The \(^13\)C NMR spectra of synthesized compounds, the \(^13\)C NMR spectrum of thiosemicarbazide (1) display signal at \(\delta\) 178.3 ppm attributed to C=S carbon, also exhibits signal from C=N carbon at \(\delta\) 149.5 ppm. Furthermore, the \(^13\)C NMR spectra give good evidence of cyclization of thiosemicarbazide through the absence of C=S from the thiazolidine-4-one spectrum, and a new signal appears at 33.2 ppm attributed to the C=S carbon signal from thiazolidine-4-one. Also, the spectrum of thiaadiazolin (4) displays a signal at 85.6 ppm attributed to C=S, it is from the cyclization of thiosemicarbazide through the absence of C=S from the spectrum, as well as the signals of C=O at 167.9, 170 ppm. The 6a and 6b spectra display azomethine (CH=N) carbon signal 164.6 ppm and 166.5 ppm, respectively. All other carbon assignments were as anticipated (Kashtoh et al., 2014). Mass spectrometry was used to determine the molecular ion peaks of synthesized compounds. The peak intensity provides information about the stability of fragments, especially with the base peak; the molecular ion confirms the proposed structural elucidation.

**Conclusion**

A new class of thiazolidine-4-one and thiadiazol derivatives have been designed and synthesized (1,2, 3a-3e, 4, 5, and 6a-6b). Using the DPPH method, we assessed the scavenger activity of compounds and discovered that compound 6b has the highest antioxidant activity, followed by compound 5. Furthermore, the cytotoxic activity of compounds 5 and 6b against Hepg-2 liver cancer cells revealed that compounds 5 and 6b are more toxic. Compound 5 arrested Hepg-2 cells in the S phase, while compound 6b resulted in a cellular arrest in the G1 phase, and both compounds decreased ROS and apoptosis. Docking studies with proteins Akt1 and CDK4 improved the results of compounds 5 and 6b. Docking studies revealed that both compounds make good contacts with protein binding sites. As a result, these two compounds should be developed as lead compounds for new liver cancer agents that inhibit Akt1 and CDK4.

**Disclosure statement**

All authors declare that there are no conflicts of interest.

**Acknowledgments**

The authors are gratefully to Prof. Dr. Ali Alshawi to review discuss the flow cytometry experiments.

**Funding**

The author(s) reported there is no funding associated with the work featured in this article.

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