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Research Article

Design, Synthesis, Molecular Docking, DNA Binding, Anticancer Antimicrobial Evaluation, of a Novel Thiazolo[5′,4′:5,6]pyrano[2,3-d]pyrimidine Derivative

Fawzia Zakaria El-Ablack[1]*, Samuel Tanas Melek[2] and Esraa Adel Gomaa[1]

[1]Chemistry Department, Faculty of Science, Damietta University, New Damietta, Egypt

[2]Chemical Pathology at EDAC Egyptian Drug Association and Delta University for Science and Technology, Egypt

***Corresponding Author:** Fawzia Zakaria El-Ablack, Chemistry Department, Faculty of Science, Damietta University, New Damietta, Egypt.

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Abstract

Seeking for new effectual anticancer drugs is of great importance. In this study, a newly synthesized and well-characterized pyrimidine derivative (9- (4-chlorophenyl)-8-imino-7-phenyl-5,7,8,9-tetrahydro-2H-thiazolo[5',4':5,6] pyrano[2,3-d]pyrimidine-2,6 (3H)-dithione (ThPP) was prepared, then evaluated in-vitro for its anti-proliferative activity against human hepatocellular carcinoma cell line HePG-2, human breast adenocarcinoma MCF-7, colorectal carcinoma HCT-116, and Human prostate cancer PC-3 cell lines using a colorimetric MTT assay. Further, molecular docking study of data set was carried out by auto docking using CDK-8 (PDB code: 5FGK) and ER-alpha (PDB code: 3ERT) as possible target for anticancer activity. Molecular docking results demonstrated that the pyrimidine scarified compounds displayed good docking score with better interaction within crucial amino acids and corelate to their anticancer results.

Additionally, the synthesized compounds are also tested for their in vitro antioxidant activity by DPPH methods in which compounds exhibited good antioxidant activity.

The interaction of the newly synthesized compound with calf-thymus DNA (CT-DNA) was investigated at pH D 7.2 by using UV–vis absorption and viscosity measurements. also, molecular docking of the tested compounds was carried out to investigate the DNA binding affinity of the tested compound with the prospective target, DNA (PDB-: 1D12). Results indicated that the investigated compound strongly bind to CT-DNA via intercalative mode. Moreover, the prepared compound is screened for their in vitro antibacterial and antifungal activity.

Keywords: Colorectal Cancer; Pyrano[2,3-d]pyrimidine; Anticancer Activity; Docking; CDK-8; ER-alpha; DNA Binding

Introduction

Colorectal cancer (CRC) is a huge international health burden. It is currently ranked as the third most common cancer and the fourth most common cause of cancer-related death globally [1]. The global projections for 2040 of new CRC cases are estimated to reach approximately 3.2 million [2]. Colorectal tumorigenesis is a highly complex process. Although little is known about the exact causes of sporadic cancer (with no family history of genetic predisposition), it is initiated by a number of carcinogenic events, which lead to the accumulation of genetic mutations in oncogenes and tumor suppressor genes, in addition to epigenetic modifications. These events drive the transformation of normal cells into uncontrolled adenomas and eventually to malignant carcinomas [3].

CDKs are a class of enzymes that controls the cell cycle and are novel targets for prospective anticancer drugs [4,5]. CDK8 is a cyclin-dependent kinase that forms part of the Mediator complex, which itself regulates the transcriptional activity of RNA poly-

merase II [6,7]. The progression, transcription and other related functions of cell cycle are regulated by CDK8 that is a heterodimeric kinase protein. The carboxyterminal domain of RNA polymerase II is also phosphorylated by CDK-8. Hence, the inhibition of CDK-8 protein may be essential for regulating tumor [8,9]. CDK8 has been implicated in playing oncogenic roles in numerous cancers, including Wnt/b -catenin dependent colorectal cancer [10,11] a CDK8 has been reported to act as a colon cancer oncogene. The role of CDK8 in both cellular signaling and colon cancer have relied upon RNAi mediated suppression of CDK8 and on the use of a kinase dead mutant CDK8. In this study we aimed to synthesize a potent and selective small molecule inhibitor of CDK8.Otherwise, Pyrimidine ring is one of the mostly used heterocyclic scaffolds in medicinal chemistry. Pyrimidine derivatives have been well recognized for their therapeutic applications i.e. anticancer [12,13], antiviral [14,15], antibacterial [16,17], antifungal [18], anti-inflammatory [19], antioxidant [20], antithyroid [21], anticonvulsant [22], and anti-diabetic [23]. Pyrimidine ring is also an integral part of DNA nucleic acid composition. Various drugs containing pyrimidine nucleus are being used as potent anticancer agents through different mechanisms of action such as. 5-Fluorouracil (5-FU) as thymidylate synthase inhibitor [24].

Merbarone II as DNA topoisomerase II (topoII) catalytic inhibitor [25], Ceritinib (LDK378) III as anaplastic lymphoma. On the other hand, 4H-Pyran s belong to an important class of heterocyclic compounds due to their wide biological and pharmaceutical properties, such as diuretic, spasmolytic, anticancer, anti-coagulant [26], antimicrobial [27], mutagenicity [28], antitumor [29], antiviral [30,31], sex pheromone [32], antiproliferative [33].

The rhodanine derivatives are small compounds with a broad spectrum of biological activities; they are used as antimicrobial [34], antiviral [35], antitubercular [36], HIV-1 integrase inhibitors [37] anti-inflammatory [38], antidiabetic [39], and antitumor agents [40-43]. Rhodanines were found to induce apoptosis through the modulation of the Bcl-2 family proteins [44,45] or through the modulation of other key signaling proteins [46,47]. Moreover, Rhodanines were also reported to reveal their anticancer activity through the inhibition of the phosphatase of regenerating liver (PRL-3) [48].

On the basis of these observations, and in continuation of our previous effort to the synthesis a variety of heterocyclic ring systems for biological and pharmacological evaluation [49-52] we report here the synthesis, molecular docking, DNA binding, in-vitro antimicrobial and cytotoxicity activities of thiazolo[5′,4′:5,6] pyrano[2,3-d]pyrimidine derivatives.

Experimental

Materials and apparatus

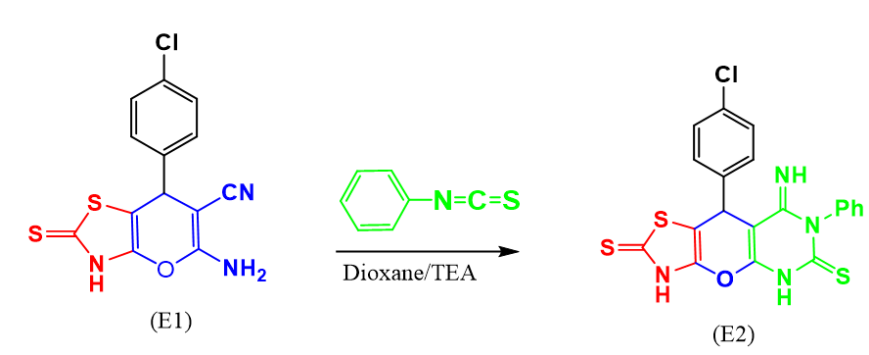
All reagents were purchased from Sigma, Aldrich, and Fluka were used without any further purification. All melting points were determined on an electrothermal apparatus and are uncorrected. Elemental analyses were obtained from Microanalysis unit, Cairo University. Spectroscopic data were obtained using the following instruments: FTIR spectra (KBr discs, 4000-400 cm^{-1}) by Jasco FTIR-4100 spectrophotometer; with maximum resolution 0.9 cm^{-1} ; UV-Visible spectra by Perkin-Elmer AA800 spectrophotometer Model AAS, using a 1.0 cm cell.; the ^{1}H NMR and ^{13}C NMR spectra by JEOL-ECA 500 II NMR Spectrometer at 500 MHz, using DMSO-d_6 as a solvent, The chemical shifts are reported in ppm using tetramethyl silane (TMS) as the internal reference;. Mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX mass spectrometer (Shimadzu) at 70 eV.

Molecular docking studies have been performed using MOE (2014.0901) (Molecular Operating Environment Software The molecules were built with the Perkin Elmer ChemBio Draw and optimized using Perkin Elmer ChemBio3D software. Docking simulation was performed using Auto Dock tools. The molecular docking studies were obtained using the three-dimensional X-ray structure of protein (5FGK), (3ERT) (4CHT), and (3QUM), the cocrystal structures of proteins for colorectal carcinoma (HCT-116), breast cancer cell line (MCF-7), human hepatocellular carcinoma cell line (HepG-2), and Human prostate cancer (PC-3) respectively, using MOE. The selected enzymes were enclosed in a box with number of grid points in x × y × z directions, 20 × 20 × 20.

Synthesis of 5-amino-7- (4-chlorophenyl)-2-thioxo-3,7-dihydro2H-pyrano[2,3-d]thiazole -6-carbonitrile (E1) yellow crystal, recrystallized from ethanol yield 94%m. p232-235 ^{0}C. IR (KBr, νcm-1): 3450, 3430 (NH_2); 3111 (NH), 3009 (CH-aromatic); 2233 (C≡N); 1549 (C=C),1017 (C=S), 1083 (C-N) 1H NMR (DMSO-d_6, δ ppm): 3. 95 (s, 1H, CH); 6.10. (br,1H,NH_2)7.30 (d, J=8,2H, Ar-H,) 8.12 (d,J=8,2HArH), 8.62 (br,2H,NH). ^{13}C-NMR (100MHz, DMSO-d_6): δppm, 188.4, 159.2, 148.3,144.4,130.2, 128.4, 129.0, 126.4, 119.2,71.6,59.9,39.1 (ES- MS, m/z (%): 332.35.

9- (4-chlorophenyl)-8-imino-7-phenyl-5,7,8,9-tetrahydro-2H-thiazolo[5′,4′:5,6]pyrano[2,3-d]pyrimidine-2,6 (3H)-dithione (ThPP) E2

A mixture of 5-amino -7- (4-chlorophenyl)-2-thioxo-3,7-dihydro2H-pyrano[2,3-d]thiazole -6-carbonitrile (E_2) (3.20 g, 10m mol)) with phenyl isothiocyanate (1.35 g, 10 mmol)in 1,4-dioxane or in pyridine (30 ml) containing catalytic amount of TEA (1ml) was refluxed for 8-10 h. After completion of the transformation (TLC control, eluent ethyl acetate–n-hexane), the reaction mixture was cooled to room temperature, then reaction mixture was poured onto iced water. The obtained solid product was crystallized from DMF to give Brown crystals; yield (80%); m.p.: 210-212°C. Anal. IR (KBr, νcm-1): 3353, 3216,3111（three NH), 3009 (CH-aromatic); 1549 (C=C), 1332（C=S); 1017 (C=S),1083 (C-N) ^{1}H NMR (DMSO-d_6, δ ppm): 4. 37 (s, 1H, CH); 7.16 (d,2H, Ar-H)7.21 (d,2H, Ar-H), 7.32 (d, J=8,2H, Ar-H,), 7.56 (d, J=8,2H, Ar-H,), 7.62 (m, J=8,2H, Ar-H,), 9.32 (br,1H,NH) 11.43 (s,1H,NH), 12.72 (br,1H,NH). ^{13}C-NMR (100MHz, DMSO-d_6): δppm,188.4, 182.5,159.2, 148.3, 146.4, 140.3, 137.2, 129.0, 128.4,131.4, 130.2, 71.6, 71.126.1 (ES-MS, m/z (%): 455.99,457.0 (22.5%).



Scheme 1: Synthesis of thiazolo[5',4':5,6]pyrano[2,3-d]pyrimidine derivatives.

DNA binding studies

The newly synthesized compound tends to interact with DNA by docking studies theoretically and by DNA binding experimentally.

Absorption spectral studies

As a diluting solution, we used Tris HCl buffer (5 mM tris (hydroxymethyl) aminomethane (pH = 7.2) and 50 mM sodium chloride). The extinction coefficient at 260 nm (approx. 6,600 M1 cm1) was used to determine the DNA content in Tris HCl buffer, therefore the absorbance values at 260 and 280 nm of DNA were recorded to emphasize its purity. The A260/A280 ratio is normally 1.8–1.9 for pure DNA that is free of protein.[53,54]Original solutions of the substances under investigation were produced in dimethylformamide and diluted in Tris HCl buffer to the required concentration. The titrations were carried out by keeping the complex concentration constant while increasing the DNA concentration. The addition of the same amount of DNA to both synthesized compound and reference solutions account for the absorbance of DNA. The Intrinsic binding constant (Kb) was calculated from the ratio of intercept to slope of a plot of [DNA]/ (εa εf) vs [DNA] of the following equation[55]:

$$\frac{[DNA]}{\varepsilon_a - \varepsilon_f} = \frac{[DNA]}{\varepsilon_b - \varepsilon_f} + \frac{1}{K_b(\varepsilon_{b-\varepsilon_f})} \quad \text{--------- (1)}$$

Where,

- [DNA] is the concentration of CT-DNA in base pairs.
- ϵ_a is the extenction coefficient observed for the complex bound to DNA (A_{obs}/[compound]) at the given DNA concentration.
- ϵ_f is the extenction coefficient of the free compound in solution.
- ϵ_b is the extenction coefficient of the compound when fully bond to DNA.

The binding constants (K_b) for dissimilar different compounds were ascertained by division of the slopes and intercepts Through a plots of [DNA]/ ($\epsilon_a - \epsilon_f$) against [DNA].

Viscosity measurements

At room temperature of 27 ^{0}C, the viscosity of DNA was measured using an Ubbelohde viscometer. The DNA concentration was held constant (1x10^{-4} mol/L) throughout each measurement, whereas the chemical concentration was varied between 0 and 2 – 8 x10^{-5} mol/L). A digital timer was used to record the flow time. Flow time was measured with a digital stop watch, three times for each sample and an average flow time was calculated (Sudeepa K., *et al*. 2018) [56]. The relative viscosities η were calculated using equation 2[57]: The sample flow time was recorded three times and the average time was used to calculate the results. $(\eta/\eta_0)^{1/3}$ against [complex]/[DNA], where ηis the specific viscosity of DNA in the presence of thiazolo pyrano pyrimidine compound (E2) and η_o is the viscosity of DNA alone. The values of η and η_0 were calculated using

$\eta = (t\text{-}t_o) / t_o$ ------- (2)

where,

- t is the observed flow time of DNA containing solution.
- t_o is the flow time of buffer alone

The data were presented as $(\eta/\eta_o)^{1/3}$ vs. [compound]/ [DNA] ratio of the concentration of the compound to DNA[58].

$$\frac{\eta}{\eta_0} = \frac{t_{\text{complex}} - t_0}{(t_{\text{DNA}} - t_0)/t_0}$$

Where,

- η is the viscosity of the DNA in the presence of compound
- η_o is the viscosity of DNA in the absence of compound (the viscosity of DNA alone).

Molecular docking study

Molecular docking technique can be used as a tool to predict the drug-DNA interactions for the rationale design as well as in the mechanistic study by placing a small molecule into the binding site of the target specific region of the DNA mainly in a noncovalent fashion[59]. calculations were carried out on 1d12 - DNA protein model. The crystal structure of DNA was obtained from the Protein Data Bank (PDB ID: 1d12). Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of Auto Dock tools[60] (Morris, Goodsell., *et al*. 1998). Affinity (grid) maps of 20×20×20 Å grid points and 0.375 Å spacing were generated using the Auto grid program [60] (Morris, Goodsell., *et al*. 1998). Auto Dock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively.

In vitro antiproliferative activity

Cell line

Mammalian cell line HCT-116 (colorectal carcinoma), HepG-2 cells (human Hepatocellular carcinoma), mammary gland breast cancer cell line (MCF-7) and Human prostate cancer (PC-3) were obtained from VACSERA Tissue Culture Unit Cairo, Egypt.

Chemical used

Chemical reagents used Dimethyl sulfoxide (DMSO), crystal violet and trypan blue dye were purchased from Sigma (St. Louis, Mo., USA). Fetal Bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA were purchased from Lonza. Crystal violet stain (1%): It composed of 0.5% (w/v) crystal violet and 50% methanol then made up to volume with ddH_2O and filtered through a Whatmann No.1 filter paper.

Cytotoxicity evaluation using viability assay

The *in vitro* growth inhibitory activity of the newly synthesized pyrimidine compound was investigated using the standard colorimetric method (MTT assay) against four human tumor cell lines including mammary gland breast cancer cell line (MCF-7), human hepatocellular carcinoma cell line (HepG-2), colorectal carcinoma (HCT-116, and Human prostate cancer (PC-3) obtained from vaccines VACSERA-Cell Culture Unit, Cairo, Egypt. Doxorubicin was used as a standard anticancer drug for comparison. The cells were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics were added (100 units/mL penicillin and 100 µg/mL streptomycin) at 37°C in a 5% CO_2 incubator.

The cells were seeded in 96-well plate at a cell concentration of 1×10^4 cells per well in 100µl of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37ºC in a humidified incubator with 5% CO_2 for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells

(maximal 0.1%) was found not to affect the experiment. After incubation of the cells for at 37°C, various concentrations of sample were added, and the incubation was continued for 24 h and viable cells yield was determined by a colorimetric method (Mosmann T., 1983) [61,62].

In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated. The optical density was measured with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as:

% Cell viability = [(OD_t / OD_c)]x100% -----(3)

Where,

- OD_t is the mean optical density of wells treated with the tested sample
- OD_c is the mean optical density of untreated cells.

The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound, and % Cell inhibition = 100 - cell viability. The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using GraphPad Prism software (San Diego, CA. USA).

Antioxidant assay

Radical scavenging assay of DPPH (1,1-diphenyl-2picrylhydrazyl) is used to evaluate the antioxidant activity of the synthesized compounds. In this assay, DPPH as a stable radical due to its paramagnetic nature aries from its odd electron.

DPPH radical scavenging activity

The antioxidant activity of synthesized derivatives E1 and E2was monitored using DPPH free radical scavenging assay based on standard procedures. Freshly prepared (0.004%w/v) methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was prepared and stored at 10C in the dark. A methanol solution of the test compound was prepared. A 40 mL aliquot of the methanol solution was added to 3ml of DPPH solution. Absorbance measurements were recorded immediately with a UV-visible spectrophotometer. The decrease in absorbance at 515 nm was determined continuously, with data being recorded at 1 min intervals until the absorbance stabilized (16 min). The absorbance of the DPPH radical without antioxidant (control) and the reference compound ascorbic acid were also measured. All the determinations were performed in three replicates and averaged. The percentage inhibition (PI) of the DPPH radical was calculated according to equation (4) (Yen GC., *et al*. 1994) [63]:

PI = [{ (AC- AT)/ AC} x 100]--------- (4)

Where,

- AC Absorbance of the control at t = 0 min.
- AT absorbance of the sample + DPPH at t = 16 min.

Molecular docking in silico analysis

Molecular docking study was carried out to analyses the binding mode of the synthesized compound against human colorectal carcinoma and breast adenocarcinoma cancer cell lines (human Hepatocellular carcinoma)and human prostate cancer. molecular docking studies have been performed using MOE (2014.0901) (Molecular Operating Environment Software The molecules were built with the Perkin Elmer ChemBio Draw and optimized. The 3D structure of newly synthesized compounds were obtained Similarly, the 3D structures of different used proteins were retrieved from Protein data bank (www.rcsb.org; Table 2). The Molecular Docking Server [64] was used to perform docking calculations. The ligand atoms were given Gasteiger partial charges. Rotatable bonds were defined after nonpolar hydrogen atoms were combined. The fundamental hydrogen atoms, Kollman unified atom type charges, and solvation parameters were all added using Auto Dock tools [60]. The Lamarckian genetic algorithm (LGA) and the Solis and Wets local search approach were used to simulate docking [65]. The ligand molecules' initial positions, orientations, and torsions were established at random. The docking process released all rotatable torsions. Each docking experiment was built from two separate runs, each of which was limited to 250,000 energy evaluations. A population of 150 was set. A translational step of 0.2 was used during the search, as well as quaternion and torsion steps of 5 [66].

Antimicrobial investigation

The antimicrobial evaluation of synthesized derivative was performed using agar well diffusion method [67]. at concentrations of 50, 100, and 150 µg/mL in dimethyl sulphoxide (DMSO), which was also applied alone as a control. The antibacterial activities against two local gram negative bacteria (*Escherichia coli*, RCMB 010052, ATCC25955) Pseudomonas aeruginosa (ATCC and also against *Bacillus cereus*, *Staphylococcus aureus* (ATCC 35556), as a Gram-positive bacteria, The antibacterial screening capability of samples measured by the diameter of the inhibition zone (in mm) on bacterial culture and compared with standard drug Penicillin G [32]. The antifungal activities were also tested against filamentous fungus *Aspergillus niger* (ATCC 16404), *Fusarium oxysporium* and *Candida albicans* (IMRU 3669), using DOX agar medium. The agar medium was inoculated with the tested microorganisms and poured into sterile petri dishes then leaved to solidify. Wells (10 mm) were made by a sterile cork borer. In each well, 100 µL of the tested compound were added and then plates were incubated at 37°C or 30°C for bacteria and fungi, respectively. Penicillin and miconazole were used as standard antibacterial and antifungal, respectively. The antimicrobial activities were assayed in terms of zone of inhibition diameters after 24 hours and 7 days for both bacteria and fungi, respectively. The antimicrobial activities of the compounds were established by calculating their activity index by using the following expression [15].

$$\%\ \text{Activity Index} = \frac{\text{Zone of inhibition by test compound (diameter)}}{\text{Zone of inhibition by standard (diameter)}} \times 100 \quad \text{--- (5)}$$

Results and Discussion

Chemistry

9- (4-chlorophenyl)-8-imino-7-phenyl-5,7,8,9-tetrahydro-2H-thiazolo[5′,4′:5,6]pyrano[2,3-d]pyrimidine-2,6 (3H)-dithione (ThPP) E2was synthesized in two steps (Scheme 1). Initially, 5-amino-2H-Pyran[2,3-d] thiazole derivative (APTh) (E1) was synthesized according to a multicomponent method with a three-component reaction using one-pot, tandem cascade Knoevenagel condensation/Michael addition and cyclization reactions of diverse aryl aldehydes, malononitrile and 2-thioxothiazolidin-4-one in non-solvent, the synthesis of thiazolo pyrano[2,3-d]pyrimidine derivatives E2was efficiently achieved under thermal heating via the treatment with phenyl isothiocyanate. The structures of all synthesized compounds were confirmed by their spectroscopic and analytical data, Thus, the IR spectra of ThPP derivative showed the characteristic stretching absorption bands in the frequency range of ν max (KBr)/cm-1) 3353, 3216,3111 (three NH), 3009 (CH-aromatic); 1HNMRa multiplet at δ 7.16–7.62. ppm assignable to the aromatic protons, beside three broad singlets (D2O exchangeable) at δ ~ 9.32,11.58 and 12.72 ppm due to the three NH protons.

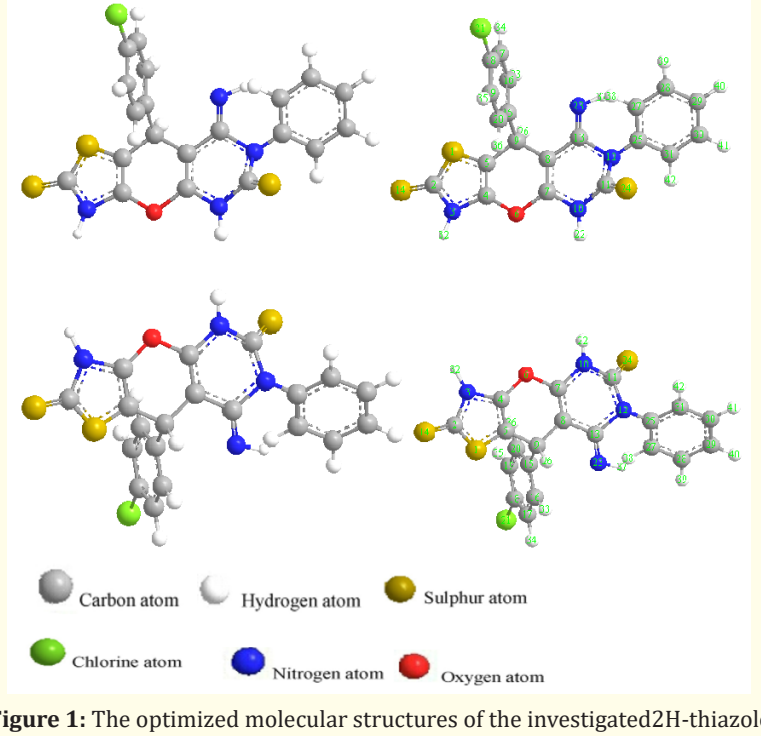


Figure 1: The optimized molecular structures of the investigated2H-thiazolo [5',4':5,6]pyrano[2,3-d]pyrimidine-2,6(3H)-dithione (ThPP) E2.

DNA binding studies

The interaction of newly synthesized compounds with DNA is one of the crucial steps for the functioning of many drugs as most of the drugs specifically target DNA. To obtain more selective agents, exhaustive knowledge of DNA binding mechanism is required [21].

Electronic absorption titrations

Electronic absorption spectroscopy is reported to be an effective method in examining the binding modes and binding extent of DNA with the synthesized compounds. In the current study, quantification of CT-DNA binding affinity with synthesized compound (ThPP) was studied using the electronic spectral technique by measure the change in absorbance and shift in wavelength upon increasing concentrations of CT-DNA solution in a fixed concentration of ThPP (E2).

Figure 2 depicts the absorption spectra of the newly synthesized compound E2 in the absence and presence of calf thymus DNA.

We have determine the intrinsic binding constant (Kb) to CT-DNA by monitoring the absorption intensity of the charge transfer spectral bands at (λ380 nm) Figure (2) indicates the electronic absorption spectra for E with and without adding of CT-DNA. The values of Kb were calculated using the plots of [DNA]/ (εa-εf)versus [DNA] (Figure 3). The calculated values is 5.79×10^{4} M^{-1}.

Viscosity measurements

Viscosity measurements, consider as one of the hydrodynamic methods, accurately identify any change in DNA length and are

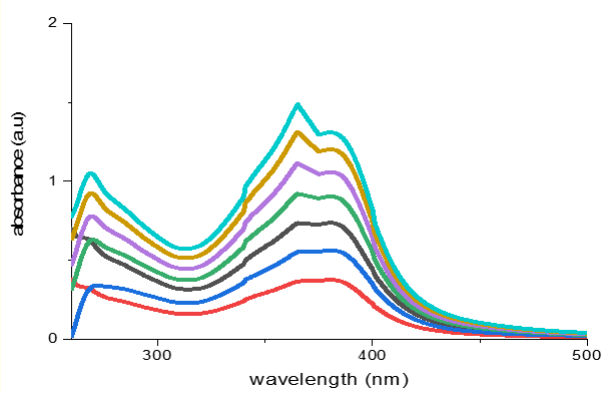


Figure 2: Absorption spectra of (THPP) in buffer pH 7.2 at 25°C in the presence of increasing amount of CT-DNA.

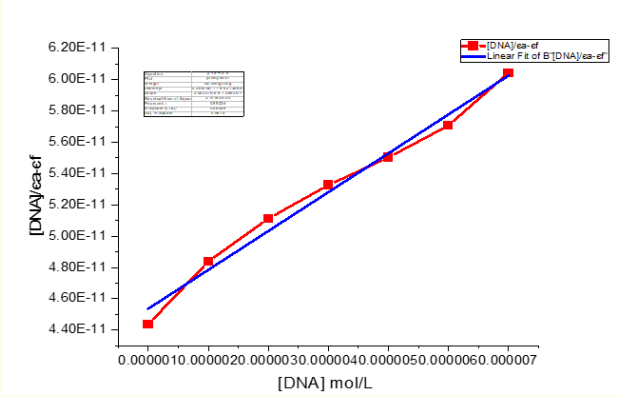


Figure 3: Plots of [DNA]/(εa εf)versus [DNA].

thus considered an effective instrument for evaluating the binding mechanism of chemicals with DNA [68]. In the case of traditional intercalative binding, an increase in the overall DNA length caused by an increase in the distance between the intercalating site base pairs in DNA causes a significant increase in the viscosity of the DNA solution. Compounds that partially bind to DNA grooves and/or nonclassical intercalation under similar conditions, on the other hand, frequently cause less or no difference in DNA viscosity [69]. The effect of increasing the concentration of thiazolopyrano pyrimidine E2 on DNA relative viscosity is seen in Figure 4 in this study. The fact that the relative viscosity of DNA increases as the quantity of thiazolopyrano pyrimidine derivative E2 increase reveals suggests that the thiazolopyrano pyrimidine derivativeE2 and DNA bond mostly intercalatively. This observation can be explained on the fact that, classical intercalation model demands that the DNA helix must lengthen as base pairs are separated to accommodate the binding complexes, leading to the increase of DNA viscosity, as for the behaviors of the known DNA intercalators

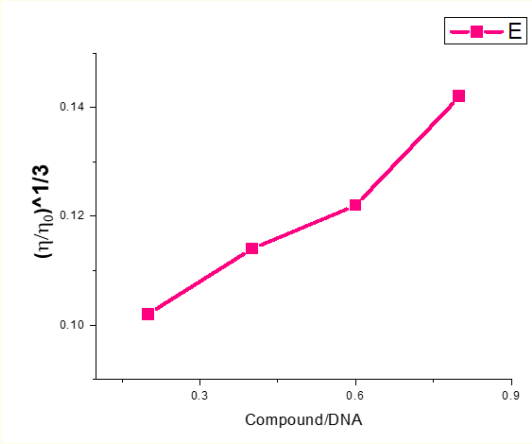


Figure 4: Effect of increasing amounts of thiazolo pyrano pyrimidine derivative (THPP) on the relative viscosity of CT-DNA ($1x10^{-4}$M) at 25°C.

Molecular docking study

The binding to DNA structure is generally hypothesized to be essential for the cytotoxic activity of the compound. Docking of the synthesized thiazolo pyrano pyrimidine derivative E2 was carried out with the co-crystal structure of DOX-DNA sequence d (CGATCG) complex (PDB: 1D12) [70] To predict and understand the possible binding mode and the respective interactions of the designed compound with the cell DNA structure. The docked results explained that the synthesized Compound (E2) interact with DNA helix at regions of DC5, DG6 with binding energy -5.09 as shown in figure 5.

Anti-proliferative activity

The newly synthesized compound E2 have been *in vitro* evaluated for their anticancer activities against four different cancer cell lines namely, hepatocellular carcinoma (HepG-2), human breast adenocarcinoma (MCF-7) and human colon cancer (HCT-116) and PC-3 (human prostate carcinoma) cells using MTT assay ,the results were expressed as growth inhibitory concentration (IC_{50}, in µg/ml) values, which represent the compound concentrations required to produce a 50% inhibition of cell growth after 24 h of incubation compared to untreated controls as shown in Figure 6 and Table 1.

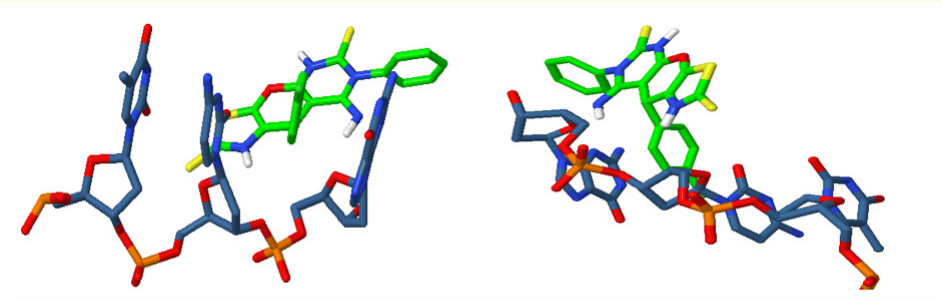
The results which were listed in (Table 1) showed that the thiazolo pyrano pyrimidine derivative exhibited good anti-proliferative activities against all the tested cell lines. As for activity against Hepatocellular carcinoma cells HepG2 cell line the Inhibitory activity with IC_{50} = 14.9 ± 1.5 µg/ml. while the result showed high percentage viability with IC_{50} = 15.5 ± 0.3 µg/ml against human colon cancer (HCT-116). The obtained results showed strong activity of thiazolo pyran pyrimidine compound THPP against human prostate carcinoma cells PC-3 was detected under these experimental conditions with IC_{50} = 8.28 ± 0.7 µg/ml. The obtained results also reveals strong activity of synthesized compound THPP against Breast carcinoma cells MCF-7cell line with IC_{50} = 20.17 ± 1.6 µg/ml (Table 1). *In vitro* cytotoxicity against hepatocellular carcinoma (HepG-2), human breast adenocarcinoma (MCF-7) human colon cancer (HCT-116) and prostate cancer activity of new synthesized compounds.

		HePG2	HCT-116	MCF-7	PC-3
**	DOX	4.50 ± 0.2	5.23 ± 0.3	4.17 ± 0.2	6.09 ± 0.3
2	THPP(E2)	14.9 ± 1.5	15.5 ± 0.3	3.9 ± 22.8	8.28 ± 0.7

Table 1

* IC50 (µg/ml): 1 - 10 (very strong). 11 - 20 (strong). 21 - 50 (moderate). 51 - 100 (weak) and above 100 (non-cytotoxic)

** DOX: Doxorubicin.



Results Table

Rank	Est. Free Energy of Binding	Est. Inhibition Constant, Ki	vdW + Hbond + desolv Energy	Electrostatic Energy	Total Intermolec. Energy	Frequency	Interact. Surface
1.	-5.09 kcal/mol	185.31 uM	-5.51 kcal/mol	-0.15 kcal/mol	-5.66 kcal/mol	100%	450.403

Figure 5: Molecular docking of (ThPP) with the co-crystal structure of DOX-DNA sequence d(CGATCG) complex and energy values obtained in docking calculations.

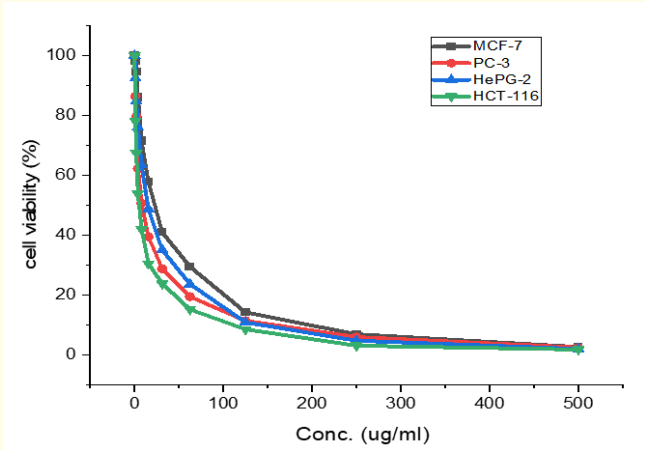


Figure 6: Comparative anticancer activities (in terms of IC50) of (THPP) on MCF-7, HCT-116, HePG-2 and PC-3.

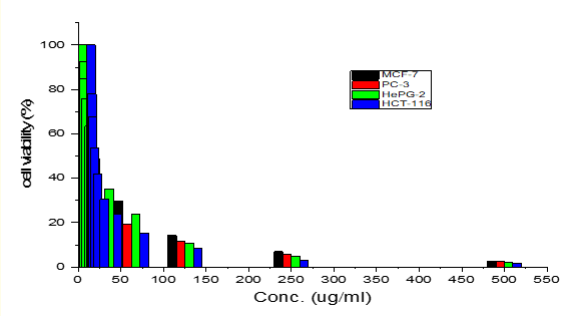


Figure 7: Anticancer screening graph of synthesized compound.

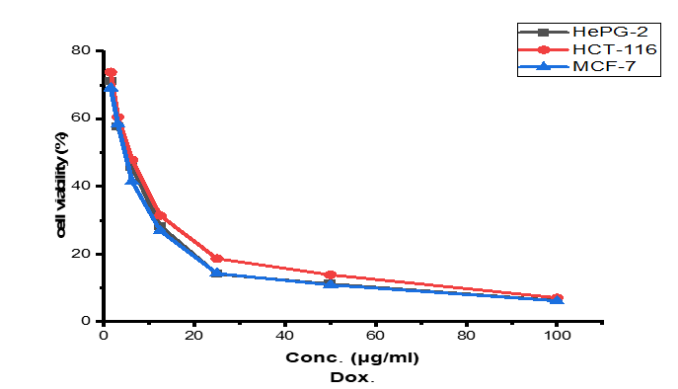


Figure 8: Comparative anticancer activities (in terms of IC50) of DOX stander on MCF-7, HCT-116 and HePG-2.

Antioxidant activity

Radical scavenging assay of DPPH (1,1-diphenyl-2picrylhydrazyl) is used to evaluate the antioxidant activity of the THP and ATHPP.

The antioxidant activity of synthesized compounds ThPP (E2), PTH (E1)was determined by the DPPH free radical scavenging assay in triplicate and average values were considered.

2,2-Diphenyl-1-picryl-hydrazyl is a free radical which was used for the determination of antioxidant activity. It can accept electron or hydrogen from the compound and get reduced. After the incubation of one hour, colour was changed and absorbance was decreased. The absorbance at 517 nm was measured and IC_{50} values were calculated from the graph shown in Figure 8. The sample showed an antioxidant activity under these experimental conditions IC_{50} of ThPP = 82.46 ± 0.53µg/ml and for PTH = 26.55 ± 0.19, where The Ascorbic acid Reference standard showed an antioxidant activity under these experimental conditions IC_{50} = 16.81 ± 0.10µg/ml. as shown in Figure 9.

Molecular docking

CDK8 has been reported to act as a colon cancer oncogene. The role of CDK8 in both cellular signaling and colon cancer have relied upon RNAi mediated suppression of CDK8 and on the use of a kinase dead mutant CDK8. In order to more fully investigate the role of CDK8 in colon cancer, we aimed to develop a potent and selective small molecule inhibitor of CDK8. The crystal structure of cyclin dependent kinase 8 (PDB Id: 5FGK) which has a good resolution of about 2.36 Å was used for docking study. The binding site of the target was generated using co-crystallized ligand (5XG) as reference (X=-4.307, Y=-22.82, Z=152.14). Further, the newly synthesized compound was docked with human estrogen ER alpha receptor of MCF7 (PDB id: 3ERT) which have good resolution about 1.9 Å, co-crystallized ligand (OHT) was selected for docking study.

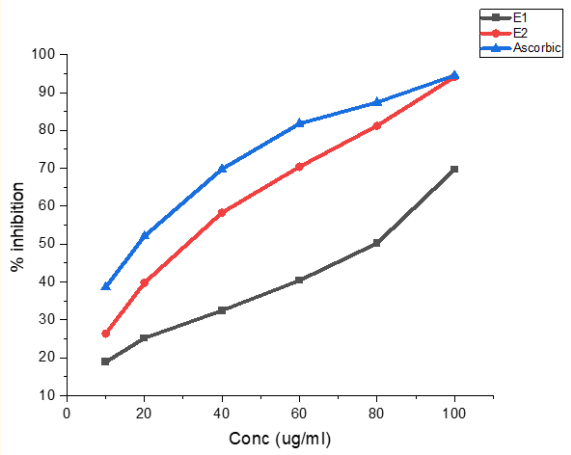


Figure 9: Free radical scavenging studies of Pyrano [2,3-d]thiazole PTH, thiazolo[5',4':5,6]pyrano[2,3-d]pyrimidine THPP and ascorbic acid.

The molecular docking results demonstrated in terms of negative energy value that the lower the binding energy value, best would be the binding affinity with the receptor [71]. The results are presented in Tables 3 and Figures 10–12.

Docking results with cdk-8 protein (HCT-116), showed that the synthesized compound exhibits good docking score with estimated free energy of binding (-8.17 kcal/mol) at target site of protein and revealed the formation of three H.B with ASP- 173 and TYR -32; halogen bond with Arg-150 and polar interaction with THR-31 This docking results correlate to their anticancer activity results which displayed good anticancer activity against cancer cell line (HCT116).

Docking results with 3-ert showed that the synthesized compound exhibits good docking score (-8.09 kcal/mol). The interaction of synthesized compound with 3-ert exhibited five HB with MET-343 THR-347 and LEU525 ; pi-pi and cation-pi with TR-P383residue ; halogen-bond with, GLY420, GLY521.

The interaction between the ligand and the protein topoisomerase II (data bank code of 4CHT) was investigated using molecular docking. With a decent binding score of -7.11 kcal mol^{-1} and an estimated inhibition constant (Ki) of 6.17 μM, the results showed that the ligand has the ability to interact with the target protein. The polar interaction with HIS538, (-0.4088) and interaction with GLU528, THR534 (-1.2348), ASP535 (-0.9922), GLU366 (-0.9846), LYS593 (-0.4756), ASP411 (-0.4608), VAL330 (-0.343), ALA536 (-0.1496) and THR329 (-0.0472). Furthermore, the ligand formed hydrophobic contacts with the target protein (Figures 11 - 12).

The docking between thiazolopyrano pyrimidine derivative and prostate cancer 3QUM exhibited four polar interaction with Arg-60 and ASN -61 amino acid in addition of hydrophobic interaction with ILE-59. Molecular docking results suggest that the thiazolo pyrano pyrimidine compound (E) can act as of great interest in successful chemotherapy.

Antimicrobial activity

The Antimicrobial screening of the synthesized compound thiazolo pyrano pyrimidine derivative E was performed using agar well diffusion technique against Gram positive and Gram negative bacterial and fungal strains. The used organisms in the present investigation included gram negative (*Escherichia coli, Klebsiella pneumonia* and *Pseudomonas* sp. bacteria), gram positive bacteria, (*Bacillus cereus* and *Staphylococcus aureus*). The synthesized compound's ability to restrict the growth of microorganisms was examined using inhibition zone diameter; the reported results in (Table 3) revealed that the synthesized compound have a promising antimicrobial activity as compared to standard drug penicillin G (antibacterial)and miconazol**e** (antifungal). compound displayed appreciable antibacterial activity against *Escherichia coli* in all concentration (in conc.50 μg/mL inhibition zone = 7 mm with activity

Compound	Enzyme receptor	Gibbs. free energy of binding ΔGb (kcal/mol)	Inhibition constant Ki (nM)	Electro-static energy	Interacting Residues	
Thiazolo-pyrano pyrimidine	5FGK	-8.17	4o8.7.21	-0.10	LYS-153, TYR-32, ILE-64, Phe-176, THR31, SER62, GLU-66, Arg150, ASP151, ASP173,	ALA-100, ASP-173, TYR-32
	3ERT	-8.09	1.17	-0.00	HIS-524, Phe-404, TRP383, LEU 391, LEU384, LEU-346, GLY-521 GLY-521, ALA-350, ALA 420, Met 528	ET343, THR347 LEU 525
	4CHT	-7.11	6.17	-0.04	THR329, VAL330, GLU366, LYS-409, ASP 411, GLU528, THR534, ASP535, ALA536, LYS593	HIS-538
	3QUM	-4.99	218.46	-0.01	ASN-61, ILE-59,ARG36,ARG60SER88	ASN-61
5-FU	5FGK	-3.58 kcal/mol	2.39	-0.11	GLU66, LYS54, LEU70, ILE79 ALA172, Phe176, LYS52,TYR32 172: ALA 176: PH ARG217	GLU66, ASP173, MET174
	3ERT	-4.49 kcal/mol	511.16 uM	-0.15	ARG394, LEU387, LEU391, GLU353, LEU346, LEU391	GLU353

Table 2: The best binding free energies (ΔGb) and inhibition constants (Ki) among the docked poses of thiazolo pyrano pyrimidine derivative.

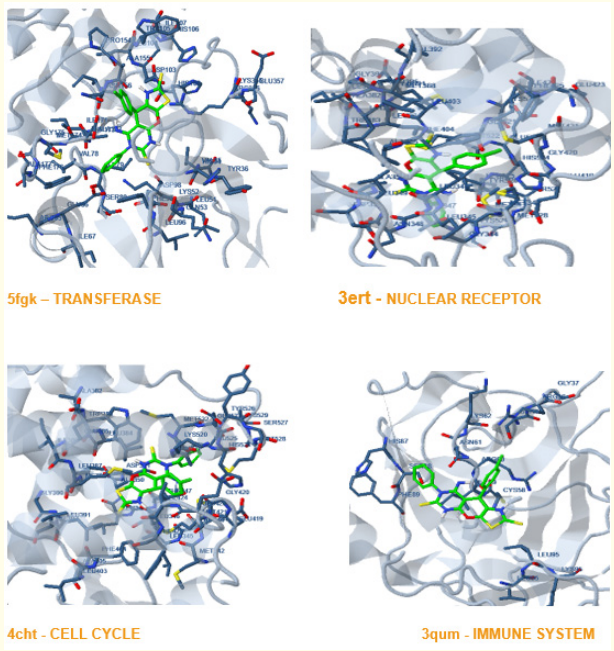


Figure 10: 3D representation the interaction of the synthesized compound (E) (green colour) with the active site residues of 5FGK; 3ERT; 4CHT and 3QUM proteins.

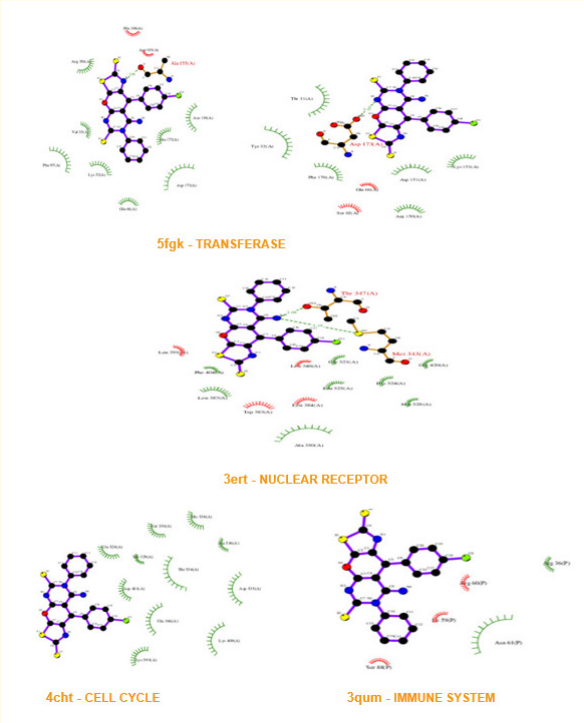


Figure 11: 2D docked model of synthesized compound into the active site of 5fgk, 3ert, 4CHT and 3QUM.

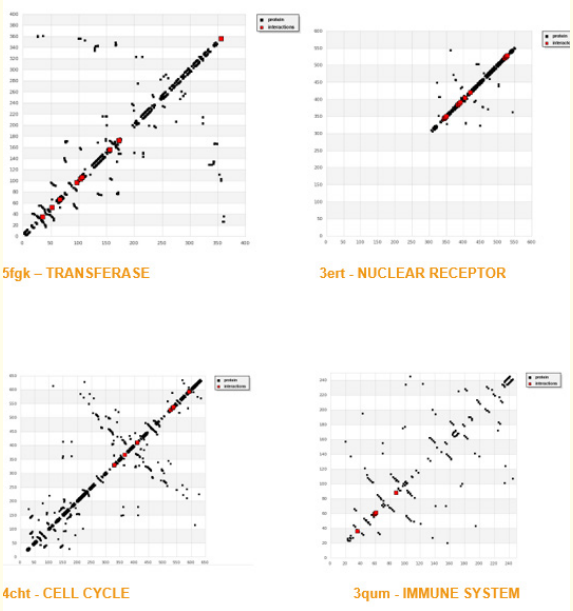


Figure 12: HP plot showed the interaction of (E) ligand with specific amino acids in target proteins.

index 58.33%; for 100 µg/mL inhibition zone = 13mm with activity index 33.33% and for 150 inhibition zone = 21mm with activity index 50.0%). On the other hand, the synthesized compound exhibited excellent activity against Klebsiella- pneumonia with inhibition zone = 9 mm in conc. 50 µg/mL; for 100 µg/mL inhibition zone = 15mm and for 150 inhibition zone = 22mm). and exhibit >100.0% activity index in high and low concentration activity against *Pseudomonas* sp. bacteria.

For gram positive the synthesized compound exhibit, activity index >100.0% in high and low concentration against Staphylococcus aureus, and showed good antibacterial activity against *Bacillus Cereus*, (inhibition zone = 4.0mm with activity index 100.0% for 50 µg/mL, and 100.0% activity index for 150 µg/mL inhibition zone = 18 mm while the activity index of 46.57% for 100 µg/with inhibition zone 7.0 mm.

The synthesized compound did not show any antifungal activity in all concentration used against *Aspergillus niger* and *Candida albicans* but exhibited good activity against *Fusarium oxysporum* for 100,150 µg/mL concentration used.

Synthetic compound	Concentration, µg/mL	Gram-positive bacteria		Gram-negative bacteria		
		Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas sp.*
E2	50	4 ± 0	8 ± 0	7 ± 0	9 ± 0	13 ± 0
	100	7 ± 0	13 ± 0	13 ± 0	15 ± 0	15 ± 0
	150	18 ± 0	21 ± 0	21 ± 0	26 ± 0	17 ± 0
Penicillin	50	4 ± 0	2 ± 0.06	12 ± 0.06	-ve	14 ± 0.03
	100	15 ± 0.03	13 ± 0	39 ± 0.03	-ve	13 ± 0
	150	17 ± 0.06	15 ± 0.03	42 ± 0.06	-ve	20 ± 0.06

Table 3: Antibacterial activities of the synthesize compound E2 in comparison with benzylpenicillin (penicillin G) as a standard drug.

*The diameter of zones of inhibition (mm).

Synthetic compound	Concentration, µg/mL	Fungi		
		Aspergillus niger	*Fusarium oxysporum*	*Candida albicans*
E2	50	-ve	-ve	-ve
	100	-ve	10 ± 0.06	-ve
	150	-ve	12 ± 0.03	-ve
	150	-ve	11 ± 0.03	-ve
Miconazole	50	10 ± 0.03	11 ± 0.06	10 ± 0.03
	100	13 ± 0.06	13 ± 0.06	12 ± 0.03
	150	18 ± 0.03	16 ± 0.14	16 ± 0.06

Table 4: Antifungal activities of the thiazolo pyrano pyrimidine (E2) in comparison with miconazole as a standard drug.

*The diameter of zones of inhibition (mm).

Frontier molecular orbitals

The highest occupied molecular orbitals (HOMO) and lowest unoccupied molecular orbitals (LUMO) are a pair of orbitals in the compound allowing them to interact more strongly. These orbitals are entitled as Frontier molecular orbitals (FMO,s). From the FM, values of the quantum chemical parameters of compounds {such as the energy of the highest occupied molecular orbital, EHOMO, energy of the lowest unoccupied molecular orbital, ELUMO, energy gap (ΔE) electronegativity (χ), chemical potentials (Pi), dipole moment (µ), hardness (η), softness (σ), additional electronic charge (ΔNmax)} have been calculated using the equations listed in the literature [72-76]. The data are listed in Table 4. Figure S8 shows the HOMO and LUMO molecular orbitals of the synthesized compound The energy components are listed in Table 5. The energies of the HOMO and LUMO are negative which indicate that the com-

pounds under investigation are stable [77]. The lower values of ELUMO indicate the more ability of the molecule to accept electrons [78]. A molecule with small Frontier orbital gap is generally associated with chemical reactivity and kinetic stability [79]. Also Molecules with low orbital energy gap are known as soft molecules [80].

Global electrophilicity (ω) serves as an important marker of reactivity and may be used to compare molecules on their electron-donating ability [81]. High global electrophilicity implies that the molecule behaves as an electrophile. High global electrophilicity implies that the molecule behaves as an electrophile.

The result reveals that chemical hardness and ΔE values of the synthesized compound is low, whereas the values for absolute softness are high, which confirms that the compounds is reactive and thus, there is flexibility in their use for biological cases [45,46]. The

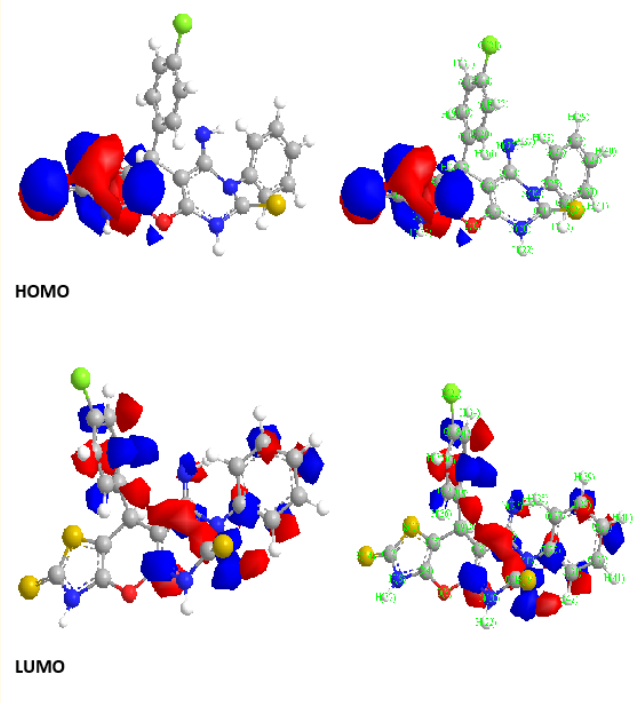


Figure 13: The highest occupied Molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of (ThPP) thiazolo Pyrano pyrimidine derivatives.

Compound	$-E_{HOMO}$ (a.u.)	$-E_{LUMO}$ (a.u.)	ΔE (a.u.)	χ (a.u.)	η (a.u.)	σ $(a.u.)^{-1}$	- Pi (a.u.)	S $(a.u.)^{-1}$	ω (a.u.)	ΔN_{max} (a.u.)
E2	6.823	1.640	5.183	4.2315	2.5915	0.3859	4.2315	0.18294	3.4548	1.6328
E1	6.006	0.803	5.203	3.4045	2.6015	0.3844	3.4045	0.1922	2.2277	1.308

Table 5: The calculated quantum chemical parameters of ThPP (E2) derivative.

results validate the agreement with the anti-cancer study results.

Conclusion

In our present work, 9- (4-chlorophenyl)-8-imino-7-phenyl-5,7,8,9-tetrahydro-2H-thiazolo[5′,4′:5,6]pyrano[2,3-d]pyrimidine-2,6 (3H)-dithione (ThPP) E2was synthesized The DNA binding mode of the synthesized compound ThPP with CT-DNA has been evaluated using absorption, spectra study and viscosity measurements. UV absorption spectral titrations of the synthesized compounds with DNA reveal that the compounds bind to CT-DNA through intercalation mode (Kb 5.79×10^{4} M^{-1}) The

thiazolopyrano pyrimidine hybrid molecule displayed significant radical scavenging activity. A comparative antimicrobial screening and anticancer activities of prepared thiazolo[5′,4′:5,6]pyrano[2,3-d]pyrimidine have been studied. Against HCT-116 (human colon carcinoma) HepG2 (human hepatocellular carcinoma), MCF-7 (human breast carcinoma), and PC-3 (human prostate and Further, molecular docking study demonstrated that compound E2 showed best docked score with strong to moderate anticancer potency toward allthe enzyme used The docking results correlate to their anticancer activity results which displayed good anticancer activity against cancer cell line (HCT116HepG2, MCF-7, and PC-3.

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