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DESIGN, SYNTHESIS, ANTICANCER EVALUATION, DNA BINDING AND MOLECULAR DOCKING OF A NOVEL THIAZOLO[5,4-B]PYRIDINE DERIVATIVES

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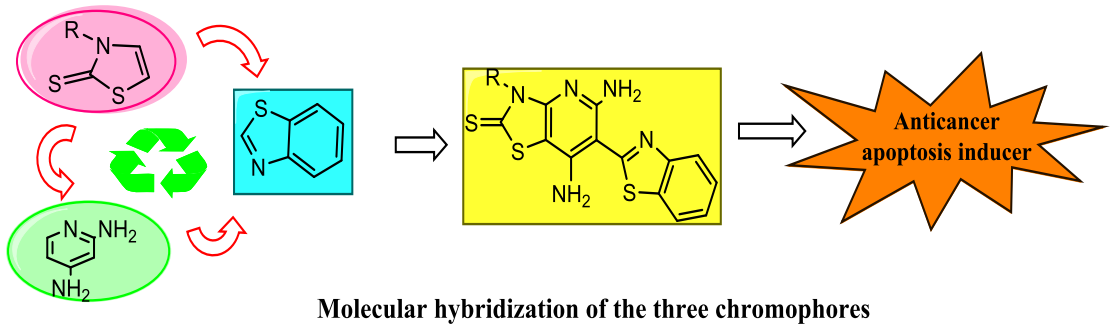
ABSTRACT

In trying to develop new anticancer agents, 5,7-diamino-6-(benzo[d]thiazol-2-yl)-3-phenylthiazolo[4,5-b] pyridine-2(3H)-thione derivative was designed, synthesized. The design was based on a molecular hybridization approach, and evaluated in-vitro for their 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. Additionally, the synthesized compounds are also tested for their in vitro antioxidant activity by DPPH methods in which compounds exhibited good antioxidant activity. The calf thymus DNA binding activity of enaminonitrile thiazole compound and benzo[d]thiazol-2-yl pyridine derivative were studied by UV-Vis absorption titration 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-: 1BNA;6BNA).The results suggest that these compounds bind to DNA in an intercalative mode and the intrinsic binding constants (K_b) of diamino-benzo[d]thiazol-2-yl-thiazoe-and enaminonitrile thiazole with CT-DNA were found to be (7.24×10^5 and 5.59×10^5 M^{-1}) . The antimicrobial activities of thiazole derivative were tested against gram negative bacteria (Escherichia coli, Klebsilla pneumonia and Pseudomonas sp), gram positive bacteria (Staphylococcus aureus, Bacillus cereus) and fungal (Aspergillus niger, Fusarium oxysporum and Candida albicans). Docking calculations were carried out using Docking Server (Bikadi, Hazai, 2009). Besides, the docking results for synthesized derivatives were in agreement with the in vitro antitumor results.

KEYWORDS: Anticancer; thiazole; DNA binding; anti-proliferative activity; molecular docking; thiazolo[5,4-b]pyridine; Cytotoxicity and antimicrobial activity.

GRAPHICAL ABSTRACT



Molecular hybridization of the three chromophores

1.INTRODUCTION

Cancer is an uncontrolled proliferation of cells results due to the disorder in cell division and proliferation mechanism. It usually occurs due to the abnormal activity of some enzymes and due to some genetic mutations.[1]

Thiazole is a core structural motif in a variety of natural products, such as vitamin B1 (thiamine) and penicillin. Thiazoles are promising scaffolds in the pharmaceutical chemistry and many of their derivatives were reported to exhibit a wide variety of biological properties including antifungal,[2] antimicrobial,[3–5] anti-inflammatory.[6,7] analgesic,[8] anti-cancer,[9,10] anti-HIV activities.[11]

anticonvulsant activities,[12] antimalarial.[13] and anti-hypolipidemic activities.[14,15] thiazoles are involved in the development of pain therapy drugs.[16] They act as fibrinogenic receptor antagonists with antithrombotic activity.[17] and as new bacterial DNA gyrase B inhibitors.[18]

Furthermore, Pyridines are an important class of heterocyclic compounds because they occur in many natural compounds that have biological activity such as vitamin B3 (niacin) and vitamin B6 (pyridoxin) and natural alkaloids.[19] Compounds containing the pyridine ring have a wide range of biological profiles including anticancer,[20–22] anti-inflammatory,[23,24] antioxidant,[25] antimicrobial,[26–31] anti-viral,[32,33] antidiabetic,[34] agents.

Moreover, the benzothiazole ring is the key motif of a wide range of biologically active compounds, including antitumor,[35-53] antimicrobial,[54–62] antiviral,[63,64] antibacterial,[65,66] antifungal.[67,68] antiparasitic.[69,70] antioxidant,[71] antidiabetic,[72] immunomodulating,[73] and anti-inflammatory agents.[74–76] Besides, many benzothiazole derivatives were found to be responsible for inhibition of topoisomerase II,[77] and tyrosine kinase histone deacetylase enzyme.[78,79] Nowadays, in the design of new drugs, the concept of molecular hybridization is actively used. This concept means combining two or more moieties of different biologically active compounds, each of which is known to possess pharmacological activity, in new hybrid molecules, resulting in the enhancement of biological effects and overcoming drug resistance.[80]

In view of the abovementioned findings and in continuation of our previous work aimed at the synthesis of a variety of heterocyclic ring systems for biological and pharmacological evaluation,[81– 83] we report here an efficient method for the synthesis of thiazole derivatives attached to benzothiazole and pyridine moieties in which benzothiazole -2-acetonitrile was an excellent synthon for this synthesis and were designed to fulfill the objectives of the target anticancer activity.

2. Experimental

2.1. Materials and apparatus

All reagents were purchased from Sigma, Aldrich, Fluka and Merck and 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 (4k9g), (4dk7), (3h5b) and (PC-3), the cocrystal structures of proteins for colon, human liver, breast cancer and prostate cancer cells, respectively, using MOE The selected enzymes were enclosed in a box with number of grid points in $x \times y \times z$ directions, 20 × 20 × 20.

2.2.1. Synthesis of (cyanomethyl benzothiazole) Benzothiazol-2-yl acetonitrile (4)

Equimolar mixture of 2-aminothiopheol(10mmol) and malononitrile (10 mmol) in acetic acid (10mmol) ad ethanol (10ml) was magnetically stirred at room temperature until no further precipitate is produced, then was allowed to stand overnight. The resultant pale-yellow precipitate is isolated by suction and recrystallization from ethanol; yield 1.4 g (80%) m.p.101- 102).

2.2.2. Synthesis of 4-Amino-3-phenyl-2-thioxo-2,3-dihydrothiazole-5-carbonitril (7)

To a solution of malononitrile (10 m mol) and phenyl isothiocyanate (10mmol) in DMF (30ml) containing catalytic amount of TEA(1ml) elemental Sulphur (10.0 m mol) was added. The reaction mixture was heated under reflux for 3 hours, cool at room temperature, then dilution with ice-cold water. The obtained solid product was collected by filtration. Brown crystals; yield (80%); m.p.: 185-188° C. Anal. Calcd. (%) for $C_{10}H_7N_3S_2$ (233.31): C, 51.50; H, 3.0; N, 18.0, S,27.5. Found (%): C, 51.13; H, 3.1; N, 18.04, S, 27.1. IR (KBr, νcm-1): 3450, 3430 (NH_2); 3050 (CH-aromatic); 2220 (CN); 1625 (C=C),1200 (C=S). 1H NMR (DMSO-d_6, δ ppm): 4.179 (s, 2H, NH_2); 7.32-7. 43 (m, 5H, C_6H_5). ^{13}C-NMR (100MHz, DMSO-d_6): δppm,186.4, 154.6, 133.9, 129.6. 129.0, 128.4, (Ar-C), 117.4, ,58.2 MS, m/z (%): 233 (M+.,100), 156 (15) EI-MS (m/z): calcd. 233.31 (found 233.01): $[M + H]^+$.

2.2.3. Synthesis of (DBTHP): 5,7-diamino-6-(benzo[d]thiazol-2-yl)-3-phenylthiazolo[4,5-b]pyridine-2(3H)-thione(8)

A mixture of enaminonitrile derivative (TH) 10 mmole and 2-(benzothiazol-2-yl) acetonitrile (10mmol) were taken in a round bottom flask in ethanol (30ml) containing a catalytic amount of pepridine as catalyst. The reaction mixture was refluxed for 8 hrs, after completion of reaction, ethanol was evaporated under vacuum and the residue was triturated with methanol as brown crystals; yield (3. 5g,85%); m.p.: 205-208° C; UV/Vis (DMSO) λmax = 386 nm. Anal. Calcd. (%) for $C_{19}H_{13}N_5S_3$ (407.54): C, 56.00; H, 3.22; N, 17.18,

S,23.60. Found (%): C, 55.13; H, 3.1; N, 17.04, S, 22.71. IR (KBr, vcm-1): 3433, 3420 (NH_2); 3091, 3055 (CH–Ar); 1625 (C=C), 1591 (C=N);1200 (C=S); 1070 (C– S–C). 1H NMR (DMSO-d_6, δ ppm): 6.27 (s, 2H, NH_2); 7.74(2H, NH2), 8.148-7.75 (m,4H, C_6H_4, benzothiazole), 7.35-7.55(m,5H, C_6H_5), ^{13}C-NMR 100MHz, DMSO-d_6): δppm,184.32,162.24, 153.4,150. 12.147.3,133.1,128.6, 126.91, 126.34, 122.65102. 153.12,51.44,43. MS, m/z (%): 407.03 (M+.,100), 408.04 ([M + H] $^+$ 21.3) EI-MS (m/z): calcd. 407.54 (found 407.03): [M + H] $^+$.

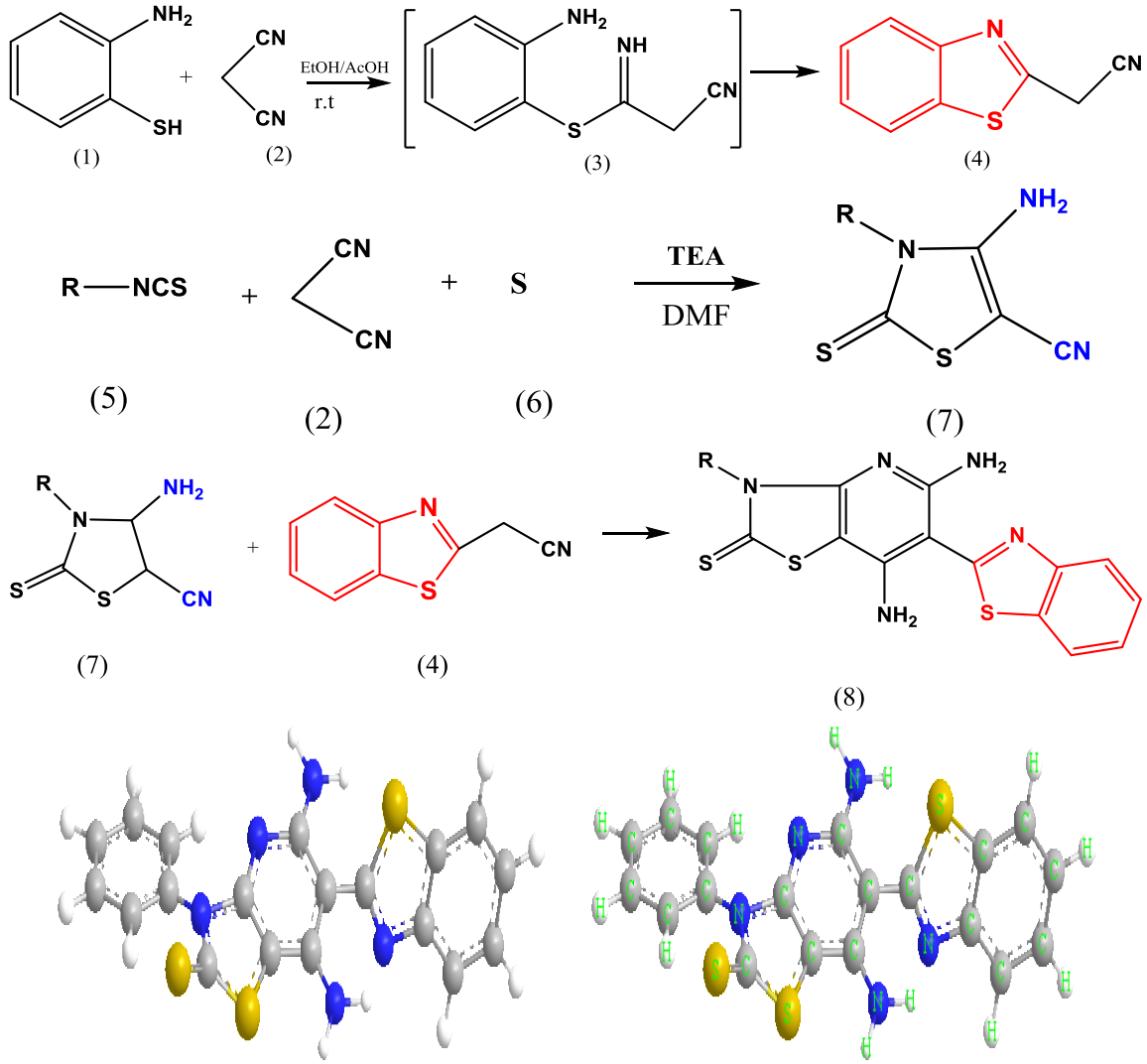


Fig. (1): Geometry optimized structures of the investigated phenylthiazolo[4,5-b] pyridine derivative (DBTHP).

2.3. DNA binding experiments

The binding properties of thiazole (TH) and thiazolo[4,5-b] pyridine (DBTHP) derivatives to Calf Thymus DNA (CT-DNA) have been studied using electronic absorption spectroscopy. The stock solution of CT-DNA ($1x10^{-3}$) was prepared in 5mM Tris-HCl/50mM NaCl buffer at physiological (pH=7.2), which a ratio of UV absorbance at 260 and 280 nm (A_{260}/A_{280}) indicating that the DNA was sufficiently free of protein[84], and the concentration was determined by UV absorbance at 260 nm (Ɛ=6600 $M^{-1}.cm^{-1}$) (Reichmann MF et al., 1954) [85].

Electronic absorption spectra (200-600nm) were carried out using 1cm quartz cuvettes at 25^{o}C by fixing the concentration of (TH and DBTHP) ($8x10^{-5,}$ $4x10^{-5}$) respectively while gradually increasing the concentration of CT-DNA (0.00– $5.4x10^{-5}$M, 0.00–$3.33x10^{-5}$M) about (TH, DBTHP) respectively. An equal amount of CT-DNA was added to both the compound solutions and the references buffer solution to eliminate the absorbance of CT-DNA itself. For every addition, the mixture was shaken and allowed to keep for 10 min at room temperature, and then the absorption spectra were recorded. With help of Wolfe–Shimer equation (1), The intrinsic binding constant (K_b) was determined[86]:

$$[DNA] / (\epsilon_a - \epsilon_f) = [DNA] / (\epsilon_b - \epsilon_f) + 1 / K_b(\epsilon_a - \epsilon_f) \quad (1)$$

Where,

- [DNA] is the concentration of CT-DNA in base pairs.
- ϵ_a is the extinction coefficient observed for the complex bound to DNA (A_{obs}/[compound]) at the given DNA concentration.

- ϵ_f is the extinction coefficient of the free compound in solution.
- ϵ_b is the extinction coefficient of the compound when fully bond to DNA.

The binding constants (K_b) for different compounds were ascertained by slopes and intercepts Through a plot of [DNA]/(ϵ_a–ϵ_f) *against* [DNA].

2.4. Viscosity measurements

To investigate the mode of interaction between binding of TH and **DBTHP** with CT-DNA. Viscosity measurements were performed at compound concentration within the range of ($2 – 8 \times 10^{-5}$ mol/L) and each compound was added into a DNA solution (1×10^{-4} mol/L) present in the viscometer. 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)**. The relative viscosities η were calculated using equation (2[88]):

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

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.[89]
- 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).

2.5. Molecular docking

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.[90],26] Calculations were carried out on 6bna - DNA protein model. The crystal structure of DNA was obtained from the Protein Data Bank (PDB ID: 6BNA).

Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of Auto Dock tools.[91] Affinity (grid) maps of 20×20×20 Å grid points and 0.375 Å spacing were generated using the Autogrid program Auto Dock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively.

2.6. In vitro antiproliferative activity

2.6.1. 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.

2.6.2. 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.

2.6.3. Cytotoxicity evaluation using viability assay

The in vitro growth inhibitory activity of the newly synthesized compounds was investigated using the colorimetric MTT assay against the four cell lines, 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) were obtained from 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 (100units/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).[92]

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:

$$\% \text{ Cell viability} = [(OD_t / OD_c)] \times 100\% \quad (3)$$

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).

2.7. Antioxidant Assay

The antioxidant activity of synthesized compounds was determined by the DPPH free radical scavenging assay in triplicate and average values were considered.

DPPH radical scavenging activity

The antioxidant activity of synthesized derivatives TH and DBTHP was 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)[94]**

$$PI = [\{(AC - AT)/ AC\} \times 100] \quad (4)$$

Where,

- *A*C Absorbance of the control at t = 0 min.
- *A*T absorbance of the sample + DPPH at t = 16 min.

2.8. Molecular Docking

The 3D structure of newly synthesized compounds was obtained Similarly, the 3D structures of different apoptotic proteins were retrieved from Protein data bank (www.rcsb.org; Table 2). Docking calculations were carried out using Docking Server (https://www.dockingserver.com/web) (Bikadi and Hazai, 2009)[95] Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools (Morris and Goodsell, 1998).[91] Affinity (grid) maps of 20× 20×20 Å grid points and 0.375 Å spacing were generated using the Auto grid program91. Auto Dock parameter set- and distance dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method (Solis and Wets, 1981)[96] Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. The overall binding potential was assessed by the interaction profile and binding energy of synthesized thiazoles derivatives (see Table 3). Each docking experiment was derived from 2 different runs (ga_run) that were set to terminate after a maximum of 250000 energy evaluations (ga_num_evals). The population size was set to 150 (ga_- pop size). During the search, a translational step of 0.2 Å (tstep), and quaternion and torsion steps of 5 (qstep) were applied.

2.9. Antimicrobial investigation

The agar well diffusion method,[97] was applied to investigate the antimicrobial activity of the synthesized analogs compound 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 three local gram-negative bacteria (Escherichia coli, RCMB 010052, ATCC25955) Pseudomonas aeruginosa (ATCC 10145) and Klebsiella pneumonia and also against Bacillus cereus, Staphylococcus aureus (ATCC 35556), as a Gram-positive, at concentrations of 50, 100, and 150 µg/mL in DMSO. 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.

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

3-RESULTS AND DISCUSSION

3.1. Chemistry

Enaminonitriles are important intermediates for the preparation of heterocyclic compounds possessing diverse biological activities. They are of particular interest as very promising reagents for cascade heterocyclization which will undoubtedly become one of the main approaches to the targeted synthesis of heterocycles.[60-36] Chemistry of cyclic enaminonitriles has been reviewed in 1992 by Wamhoff.[101] and due to the pronounced biological and pharmacological activities of thiazolo[5,4-b]pyridine.[102-106] We report here the synthesis of 4-Amino-3-phenyl-2-thioxo-2,3-dihydrothiazole-5-carbonitril as the key precursor for the synthesis of 5,7-diamino-6-(benzo[d]thiazol-2-yl)-3-phenylthiazolo[4,5-b]pyridine-2(3H)-thione(DBTHP).

The key starting material, 4-Amino-3-phenyl-2-thioxo-2,3-dihydrothiazole-5-carbonitril (TH), has been obtained via refluxing equimolar mixture of isothiocyanate, malononitrile and sulfur powder in DMF containing catalytic amount of TEA. On the other hand, cyanomethyl benzothiazole can be prepared by the reaction of 2-mercaptoaniline and malononitrile in EtOH/AcOH mixture at room temperature. The treatment of 4-Amino-3-phenyl-2-thioxo-2, 3-dihydrothiazole-5-carbonitril with 2-(benzothiazol-2-yl) acetonitrile afforded the target compound (DBTHP). The structure of thiazolo [4,5-b]pyridine derivative was confirmed on the basis of their elemental analysis and spectral data. Hence, IR which showed the disappearance of cyano group and 1H-NMR spectrum in DMSO-d6 revealed the presence of multiple at the range δ:6.39-6.33 ppm attributed to the two NH_2 group of pyridine ring.

3.2. DNA binding studies

DNA is the classical pharmacological target as anticancer agents, therefore, the study of the potential interaction of **TH** and **DBTHP** derivatives with DNA is of paramount importance for the development of molecules with potential medicinal applications.

3.2.1. 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 present study, quantification of CT-DNA binding affinity with heterocyclic compound (TH and ABTHP) 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 TH and ABTHP Compounds.

Thus, we have determined the intrinsic binding constant (*K*b) to CT-DNA by monitoring the absorption intensity of the charge transfer spectral bands at (λ365 nm) for TH and (λ312nm) for ABTHP. Moreover, Figure (2) indicates the electronic absorption spectra for TH and ABTHP with and without adding of CT-DNA. The binding constant K_b values obtained from the absorption spectral technique for TH and ABTHP were calculated as 5.59×10^5 and 7.24×10^5 M^{-1}, respectively.

The obtained results revealed that the compounds have a strong association with DNA base pairs through intercalation mode.[117,118] in hypochromic and bathochromic shift.

The hypochromic feature is due a decrease in transition energies resulted from the overlapping π* orbitals of the intercalated compounds with π orbitals of DNA base pairs. Based on obtained binding constant values for the tested compounds, The binding constant of (ABTHP) are comparatively higher than that of (TH).

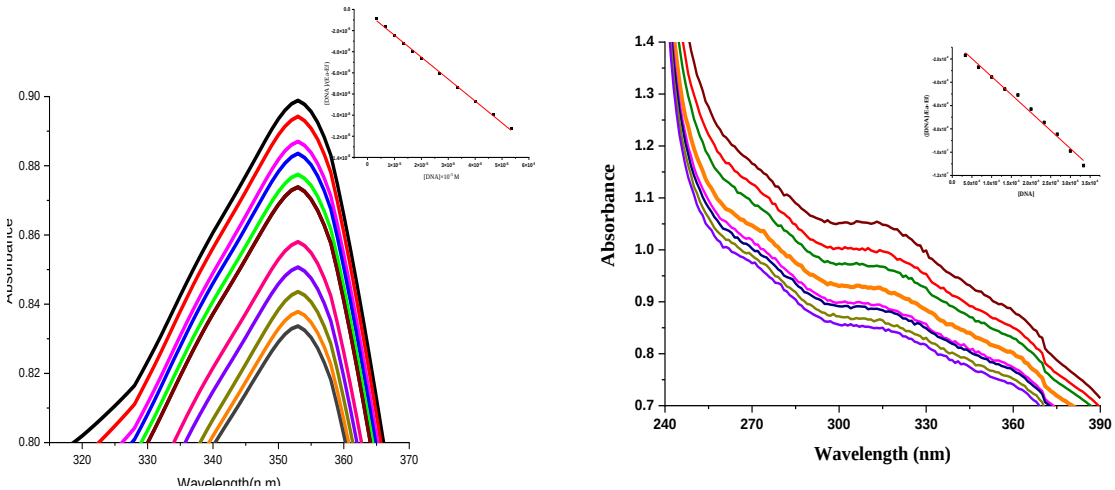


Fig. (2): Absorption spectra of (TH) & (BTH) in buffer pH 7.2 at 25°C in the presence of increasing amount of CT-DNA. Arrows indicate the changes in absorbance upon increasing the CT-DNA concentration. Inset: plot of [DNA]/ ($\varepsilon_a - \varepsilon_f$) *vs*. [DNA] for titration of DNA with compounds.

3.2.2. Viscosity measurements

Viscosity measurement considers one of the most effective means to study the binding mode of a compound to DNA due to its sensitivity to the change of length of DNA. A significant increase in the viscosity of DNA on the addition of a compound indicates the classical intercalative mode of binding to DNA. In contrast, a complex that binds in the DNA grooves by partial and/or non-classical intercalation causes less pronounced (positive or negative) or no change in DNA solution viscosity.[107]

Classical intercalators fit between the two strands of DNA, causing the helix to unwind and the base pairs to separate, and this leads to a significant increase in the length of the DNA molecule and in DNA viscosity. In contrast, a partial or non-classical intercalator can cause a bend (or kink) in the DNA helix, leading to its shortening and concomitantly reducing the viscosity of the DNA molecule. DNA groove binding molecules cause no change in DNA chain length and also have no effect on the viscosity of the DNA solution.[108]

The effects of the TH and DBTHP on the viscosities of the CT-DNA solution are shown in **Figure (3)** where it was clarified by measuring the relative specific viscosity of DNA after the addition of varying concentration of the TH and DBTHP. To further investigate the interaction mode of the binding mode of TH and DBTHP with DNA. Viscosity experimental results increases of relative viscosity of CT-DNA gradually by increasing concentration of the both synthetic derivatives TH and DBTHP. This observation can be explained in the fact that, classical intercalation model demands that the DNA helix must lengthen as base pairs are separated to accommodate the binding compounds, leading to the increase of DNA viscosity, as for the behaviors of the known DNA intercalators.

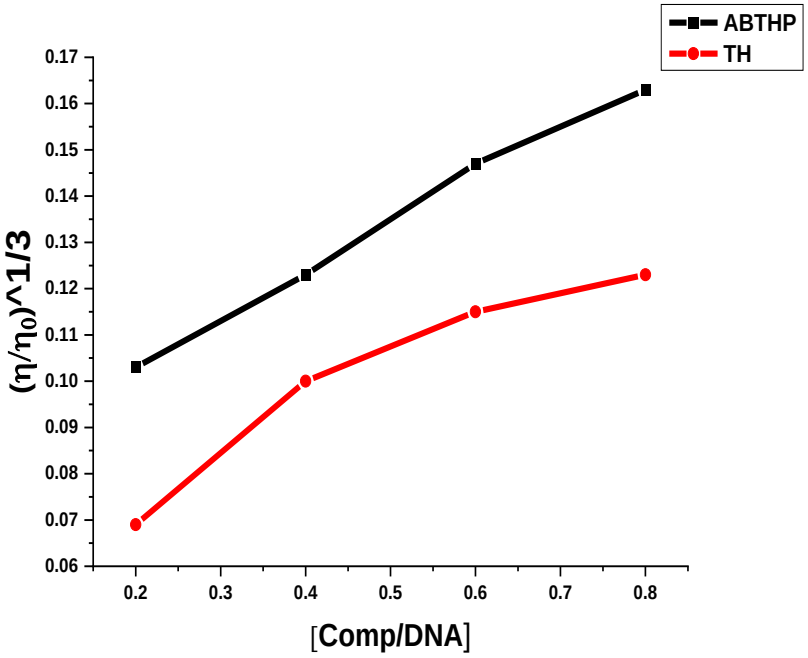


Fig. (3): Effect of increasing amounts of (TH, DBTHP) on the relative viscosity of CT-DNA ($1x10^{-4}$M) at 25°C.

3.2.3. Molecular Docking Study

Molecular docking is an attractive tool to investigate the interactions of compound and DNA for the planning and development of new drugs. Dodecamer sequence (B-DNA) is very abundant in natural DNA. Docking of the synthesized derivatives was carried out with DNA duplex of the sequence d(CGCGAATTCGCG)2 dodecamer (PDB ID: 1BNA) to predict the preferred orientation of the compounds inside the DNA. The docked results clearly explained that there is strong interaction between the thiazolo[4,5-b] pyridine and, thiazole derivative TH with 1BNA by binding energy of −5.02 and -3.97 Kcal/mol respectively.

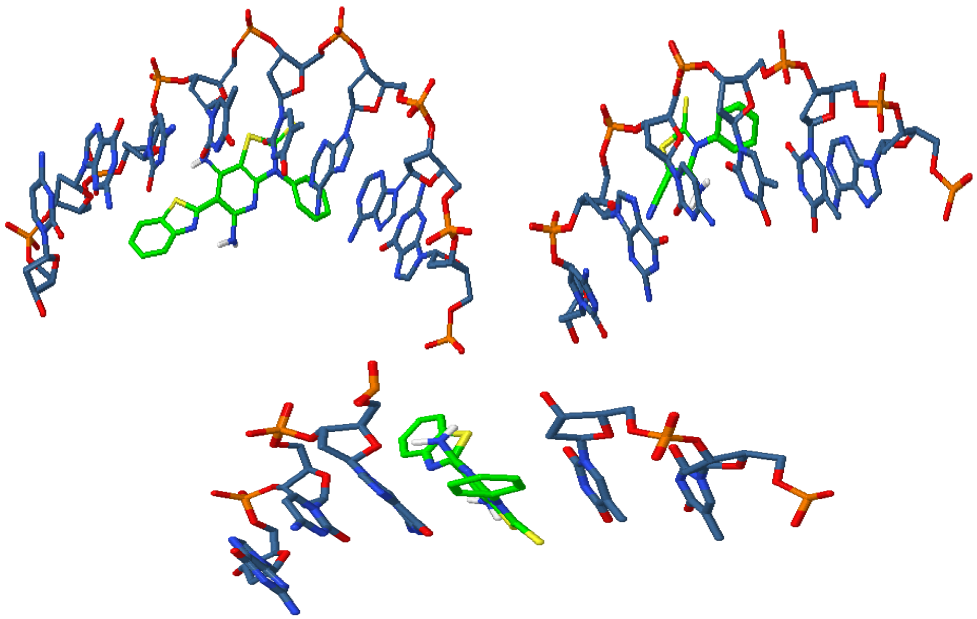


Fig. (4): Molecular docking of (TH, ABTHP) on the1BNA-DNA.

3.3. Cytotoxic activity

This study presents the synthesis and antiproliferative activity of compounds having pyridine, thiazole, and benzothiazole pharmacophores. The in vitro cytotoxicity of enamino thiazole **TH** and t**hiazolo[4,5-b] pyridine** derivative **DBTHP** were investigated against HepG2(human hepatocellular carcinoma), MCF-7(human breast carcinoma), PC-3(human prostate carcinoma) and HCT-116 (human colon carcinoma) cell lines. The cell viability was determined by MTT assay the results were expressed as growth inhibitory concentration (IC50, 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 Fig. 4 and Table 1, which reveal that the tested compounds exhibited good to moderate anti-proliferative activities against the tested cell lines. As for activity against HepG2 cell lineIC_{50} of TH = 37.21±2.7µg/ml while thiazolo -pyridine derivative showed the percentage viability IC_{50} at 24.7 ± 9.5 µg/ml. The obtained results showed strong activity of thiazole compound TH against MCF-7cell line with $IC_{50=}$20.17±1.6 µg/ml whereas, thiazolo pyridine compound displayed moderate cytotoxic activities with IC_{50} 42.6 ± 31.8µg/ml.

The data also reveal that thiazole and thiazolo pyridine are strong active agents (IC_{50} = 15.87±1.3µg/ml against HCT-116 cell line and lower toxicity against PC-3 IC_{50} of TPBTH = 168.9 ± 4.57 µg/ml as shown in Figure (5-7).

(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

No.	Comp.	In vitro Cytotoxicity IC_{50} (µg/ml)*			
		HePG2	HCT-116	MCF-7	PC-3
**	DOX	4.50±0.2	5.23±0.3	4.17±0.2	6.09 ± 0.3
1	TH	37.21±2.7	15.87±1.3	20.17±1.6	69.90±7.8
2	DBTHP	24.7 ± 9.5	15.87±1.3	42.6 ± 31.8	NA

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

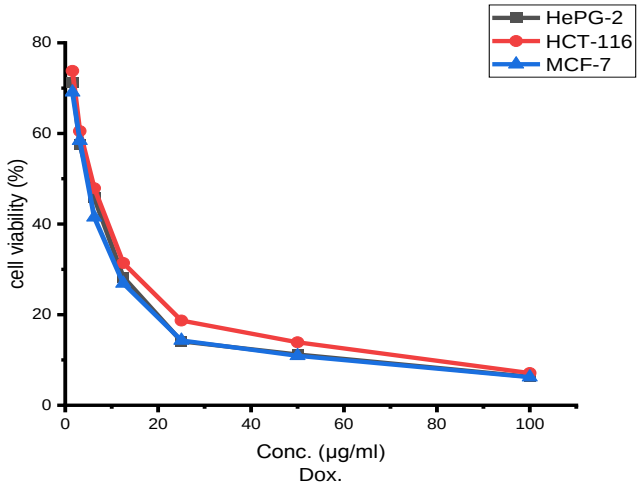


Fig. (5). Comparative anticancer activities (in terms of IC50) of DOX stander on MCF-7, HCT-116, HePG-2and PC-3.

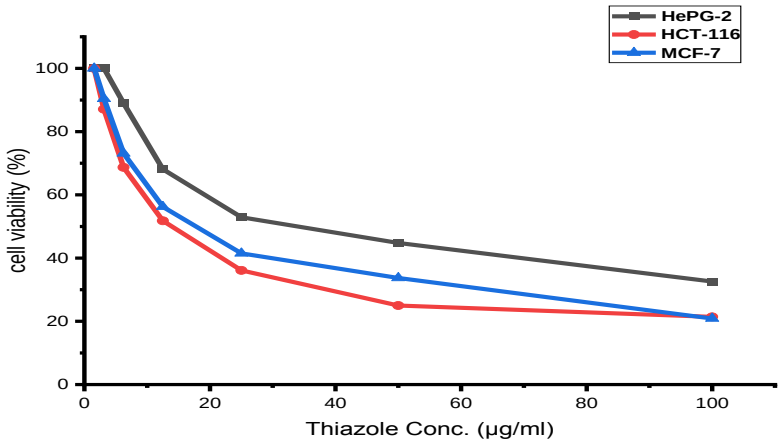


Fig. (6): Comparative anticancer activities (in terms of IC50) of (TH,) on MCF-7,HCT-116,HePG-2and PC-3.

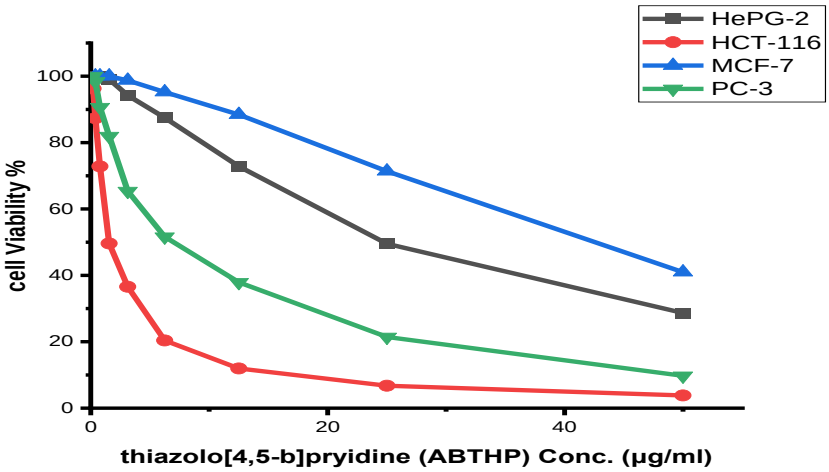


Fig. (7): Comparative anticancer activities (in terms of IC50) of (DBTHP) on MCF-7, HCT-116, HePG-2and PC-3.

3.4. Antioxidant activity

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

The reaction between the DPPH· radical scavenger and the prepared compounds TH, DBTHP was monitored spectrophotometrically at λ 515 nm based on ascorbic acid as a standard antioxidant under the same conditions. The sample showed an antioxidant activity under these experimental conditions IC50 of ABTHP = 106.5 ± 8.1 µg/ml and for TH =47.88±0.25, where The Ascorbic acid Reference standard showed an antioxidant activity under these experimental conditions IC50 = 14.1 ± 0.5 µg/ml. as shown in **Figure (5).**

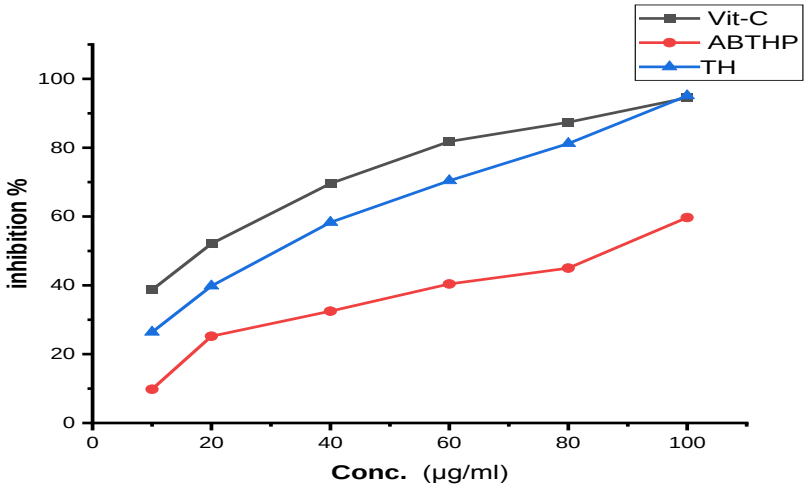


Fig. (8): Free radical scavenging studies of thiazole, thiazolo[4,5-b]pyridine and ascorbic acid.

3.5. Antimicrobial activity

The Antimicrobial screening of the synthesized compound DBTP was done by agar well diffusion technique against Gram positive and Gram negative bacterial and fungal strains. we used more than one test organism to increase the chance of detecting its antimicrobial activities. 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*). Antimicrobial activity results indicated (Table 2),have shown that the synthesized compound promising antimicrobial activity as compared to standard drug penicillin G (antibacterial) .In the case of Gram negative bacterial study, compound displayed appreciable antibacterial activity against Escherichia coli in all concentration (in conc.50 µg/mL inhibition zone = 3 mm with activity index 25.0 % for 100 µg/mL inhibition zone = 8mm with activity index 20.6.0 % for 150 inhibition zone = 15 mm with activity index 35.7 %). On the other hand, the synthesized compound exhibited excellent activity against *Klebsiella- pneumonia* with>100.0 % activity index in high and low concentration but not exhibit any 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 = 2.0mm with activity index 50.0 % for **50** µg/mL, while for 150 µg/mL inhibition zone = 12 mm with activity index 70.6 %.

The compound did not show any antifungal activity in All concentration used against Aspergillus niger, *Candida albicans* ad Fusarium *oxysporum*

Table (2): Antibacterial activities data TH and DBTHP derivatives.

Compound concentration	***E. coli pneumoniae***	***Klebsiella***	***Pseudomonas sp.***	***Bacillus Cereus***	***Staphylococcus* aureus**
	Diameter of inhibition zone (mm)	**Diameter of inhibition zone (mm)**	**Diameter of inhibition zone (mm)**	**Diameter of inhibition zone (mm)**	**Diameter of inhibition zone (mm)**
Imidazolyl thiazole 50	3 ± 0	5±0	-ve	2 ± 0	5±.0
100	8± 0.03	12±0	-ve	6± 0.	13±0
150	15±0	20±0	-ve	12±0.06	21±0
Penicillin					
50	12	-ve	14 ± 0.03	4 ± 0.	2±0.06
100	39	-ve	13 ± 0	15 ± 0.03	13±0
150	42	-ve	20 ± 0.06	17 ± 0.06	15 ± 0.03

3.6. Molecular docking studies

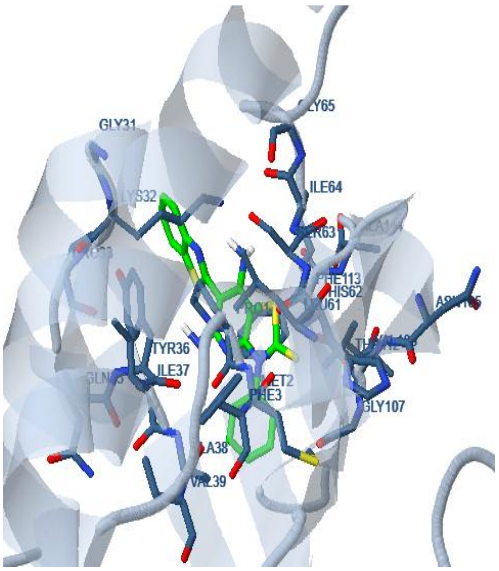
Molecular docking has become a widespread tool for drug discovery.[109,110] To illustrate the potential binding mode of the synthesized derivative thiazolopyridine into the active sites of the four types of tumor cells we investigated (HCT-116, HepG-2 MCF-7 and PC-3), a docking study of the synthesized thiazolo pyridine derivative **DBTHP** against each cell type using MOE (2014.0901) (Molecular Operating Environment software). The molecular docking studies were performed using (**4k9g),** (**4dk7), (5ha9,3hb5**), and **(3QUM)** the cocrystal structures of proteins for colon, human liver, and breast, and prostate cancer cells respectively. 4k9g is the macrophage migration inhibitory factor, a pleiotropic pro-inflammatory cytokine, which has a contributing role in cancer progression and metastasis and thus is now considered a promising anticancer drug target. 4dk7 is a structural modification of a series of dual LXRα/β agonists, which led to the identification of a new class of LXRβ partial agonists. **5ha9** a novel PARP1 antagonist (BL-PA10) that induces apoptosis and inhibits metastasis in triple negative breast cancer.

The results are presented in Tables 3 and Figures 9−11. From the results of docking study, we noticed that the highest binding interactions were found between the thiazolo pyridine derivatives and **4k9g** of colon cancer (resolution 1.55 Å) with binding score (−9.15kcal/mol) which is compatible with experimental results of the highest activity against colon cancer HCT-116 (Table 1). It is found that it interacts with LYS-32 and PHE-113 via arene cation interaction and arene-arene interaction and hydrophobic interaction with the residues MET2, ALA 38, ILE 64 andVAL106. In the case of **4dk7** for human liver, (**2.45Å** resolution) with binding score (−4.62kcal/mol) 1by forming two strong hydrogen bonds with the active site residuesHIS-217(length: 2.54Å) and PRO-412(length: 1.12Å), and arene cation interaction with the active site residuesHIS-217, beside the hydrophobic interaction with active site residues ILE -409, Pro-412, Leu-214. On the other hand, docking simulation of synthesized compounds was performed against active site of human breast cancer**5ha9** that induces apoptosis and inhibits metastasis in triple negative breast cancer (PDB ID: **5ha9**, resolution 4.01 Å) with binding score (−9.34kcal/mol) and exhibited four hydrogen bonds with the active site residues of ASP105(length: 3.18, 3.25,3.38,3.78Å);two hydrogen bod with TYR 228 (length:3.48)and hydrogen bond with GLU(3.72) in addition to π-π interaction with HIS-201,TYR236,TYR246 and cation -π interaction with HIS201 residue .Also the docking study with breast cancer 3hp5 (2Å resolution), the binding affinity exhibited two hydrogen bonds with the active site residuesGLY-9(length:2.84 Å) ,GLY-92(length: 2.88Å) beside the hydrophobic interaction with active site residues Val -66 ,ALA-91-and pi-pi interaction with PHE-192 .The docking between thiazolo pyridine derivative and prostate cancer exhibited four hydrogen bond with ASN -61 amino acid in addition of hydrophobic interaction with ILE-59.

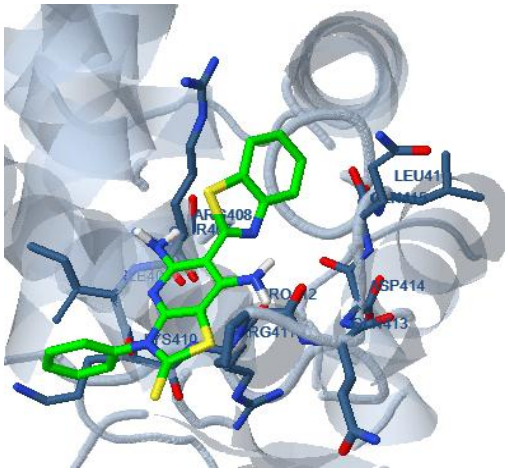
The interaction between the ligand and the protein topoisomerase II (data bank code of 4CHT; 3.25Å resolution) was also investigated which revealed a decent binding score of $-6.49\ \text{kcal mol}^{-1}$ and an estimated inhibition constant (Ki) of 17.53μM, the results showed that the synthesized derivative has the ability to interact with the target protein via forming seven hydrogenation bond with GLU-528, HIS-538, VAL330 are responsible for this interaction. Furthermore, the ligand formed hydrophobic contacts with the target protein.

Table (3): The best binding free energies (ΔGb) and inhibition constants (Ki) among the docked poses of thiazole compounds.

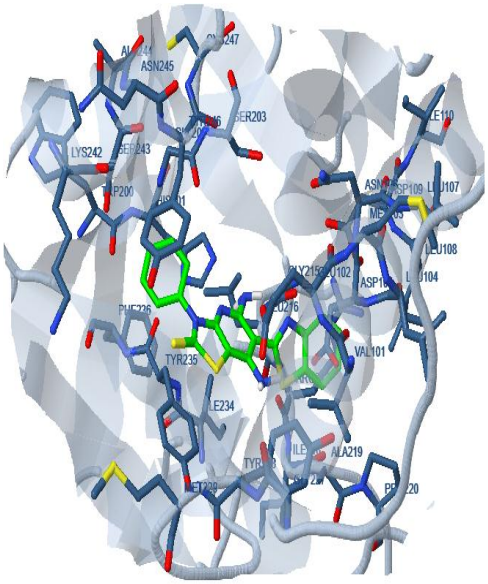
Compound	Enzyme receptor	Gibbs. free energy of binding ΔGb(kcal/mol)	Inhibition constant Ki (nM)	Electrostatic energy	Interacting Residues
Thiazolo pyridine	**4k9g**	-9.15	4o8.7.21	-0.10	LYS-32,TYR-36,ILE-64,Phe-113 ,MET2,VAL-106,ALA-38,HIS-62
	4dk7	-4.26	747.76	-0.50	HIS-217, PRO-412, ILE -409 Leu-214, GLN-415, ARG-408
	5ha9	-9.34	114.31	-0.12	ASP105,HIS201,GLU102,TYR-228,TYR 235, TYR246, Leu108, ARG217,Leu216,SER243,ASP109
	,**3hb5**	-7.78	1.79	-0.04	GLY-9, GLY92, SER11, ARG37, VAL66, ALA91, PHE192, THR -8, SER-12 ARG217
	3QUM	-4.74	336.01	-0.16	ASN-61, ILE-59,HIS-87
	4CHT	−6.49	17.53	-0.06	GLU-528,HIS-538,VAL330,LYS-409,ARG151,ASP535,ALA536. THR534
TH	**4dk7**	-3.46	2.90	-0.05	Pro228,Asn250,Leu211,Leu 214,Tyr2,Lys208, Lys227, Ile230



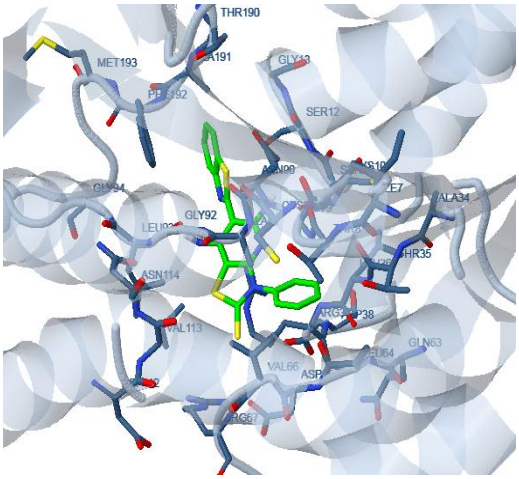
A) **4k9g**



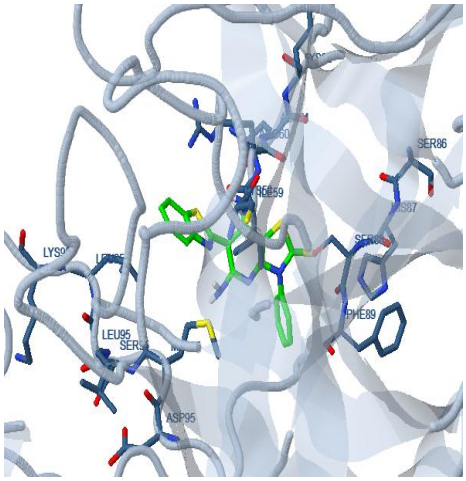
B) **4dk7**



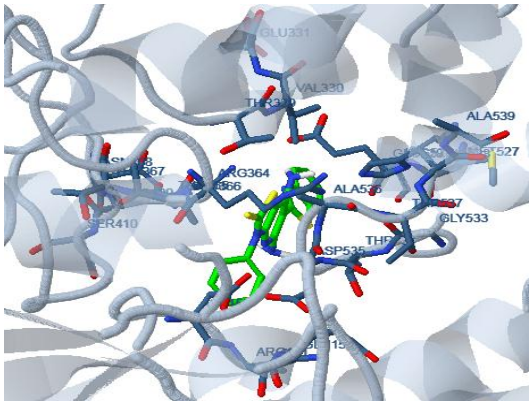
C) **5ha9**



D) **3hb5**



E) **3QUM**



F) 4CHT

Fig (9) 3D representation of compound ABTHP) (green colour) with active site residues of showing 4k9g, 4dk7, 5ha9, 3hb5 3QUM and 4CHT protein.

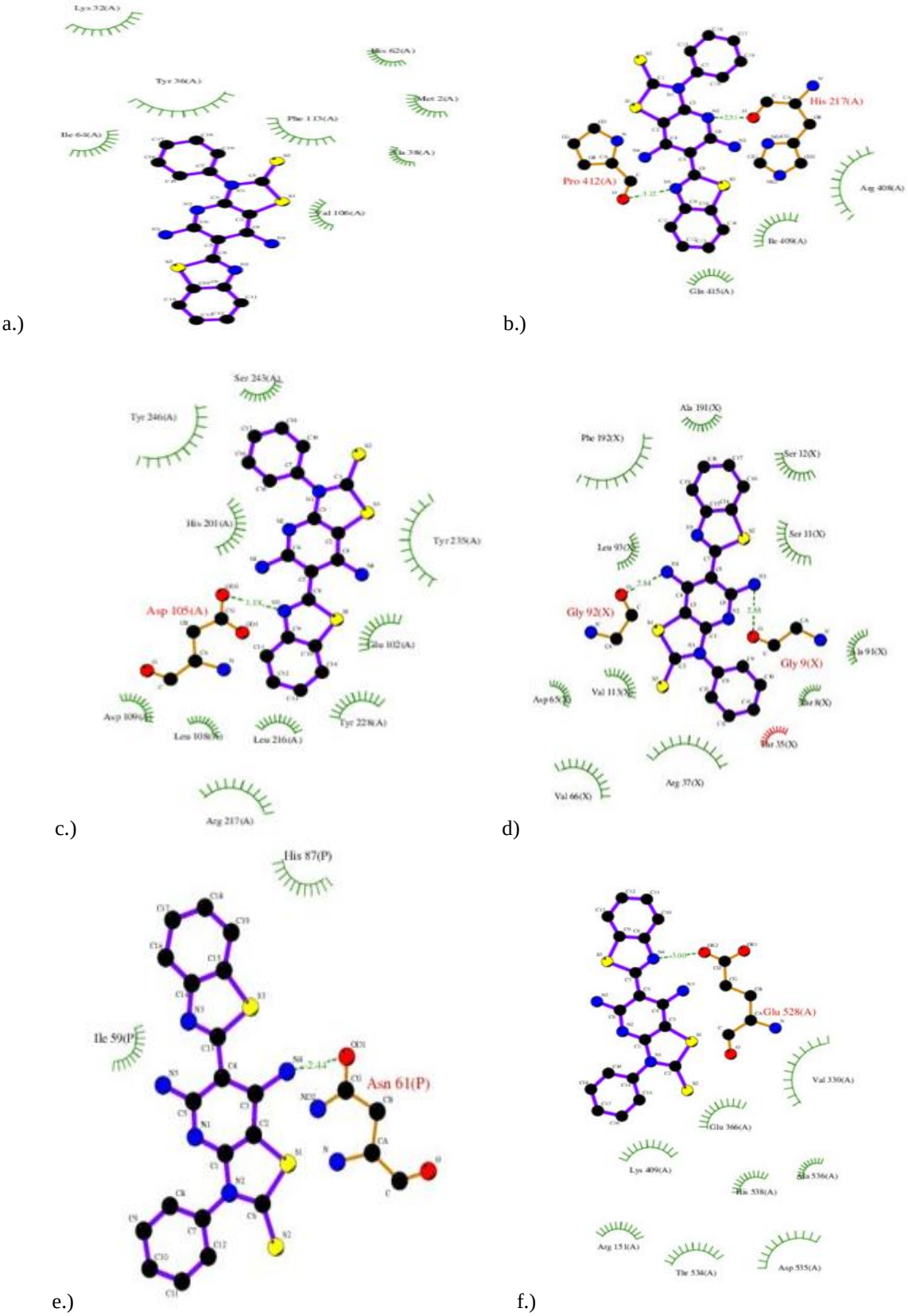


Figure (10): 2D docked model of synthesized compounds into the active site of 4k9g , 4dk7 ,3hb5 ,3QUM and 4CHT.

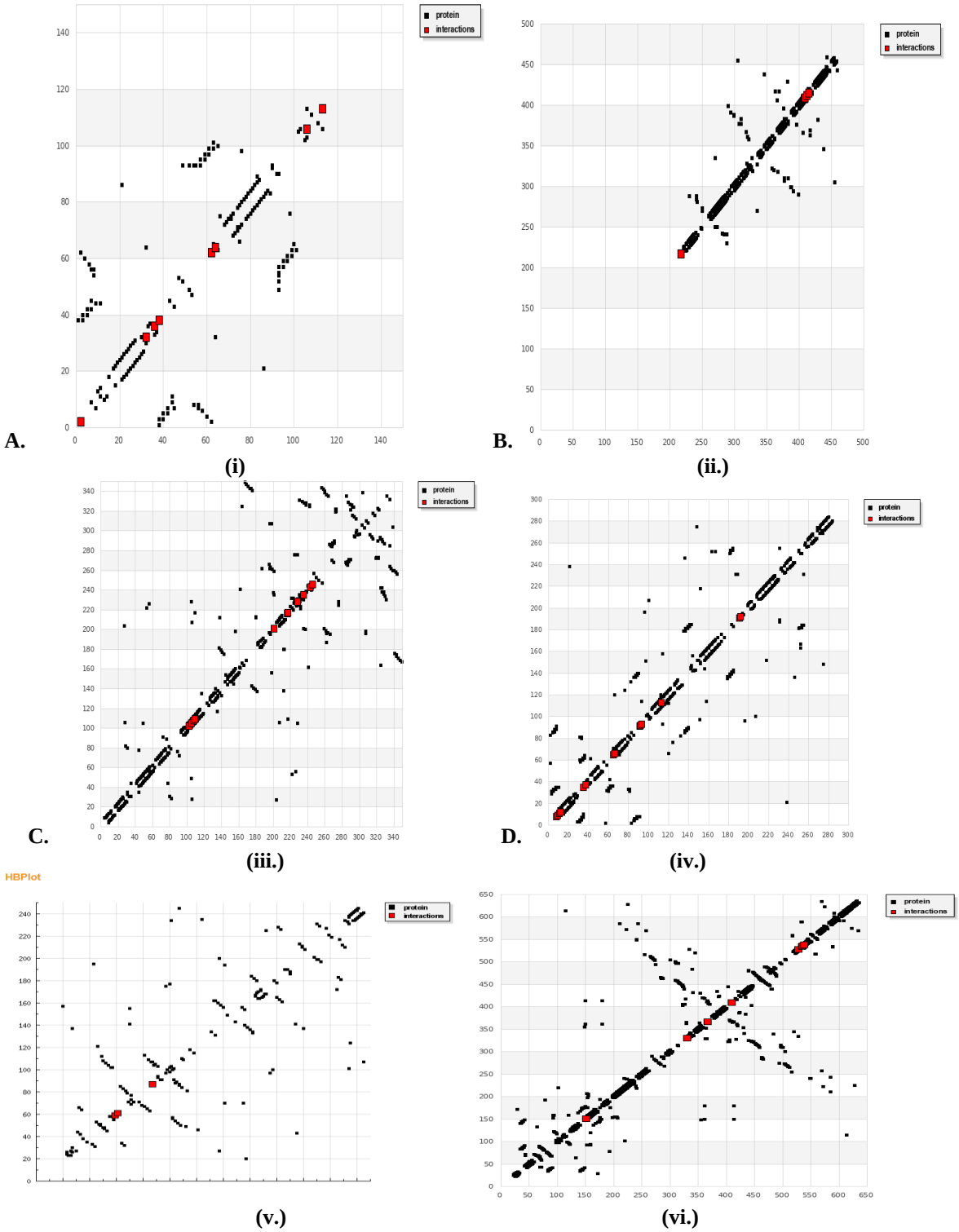


Figure (11): HP plot showed the interaction of thiazolo[4,5-b]-pyridine with specific amino acids in 4dk7 and 4k9g, 5ha9 .3h5b, pc-3 and 4cht target protein.

3.7. Molecular Structure

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), absolute electronegativity (χ), chemical potentials (Pi), absolute hardness (η), absolute softness (σ), global electrophilicity (ω), global softness(S) additional electronic charge (ΔN_{max})} have been calculated using the equations listed in the literature.[111–114] The data are listed in Table 4.

Figure S8 shows the HOMO and LUMO molecular orbitals of the thiazolopyridine derivative.

The energies of the HOMO and LUMO are negative which indicate that the synthesized compounds are stable.[115,116] The lower values of ELUMO indicate the more ability of the molecule to accept electrons.[119] A molecule with small Frontier orbital gap is generally associated with chemical reactivity and kinetic stability.[120]

Table (4): The calculated quantum chemical parameters of ABTHP and TH 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.)
DBTHP	5.496	1.386	4.110	3.441	2.055	0.4866	3.441	0.2433	2.8809	1.6745
TH	6.006	0.803	5.203	3.4045	2.6015	0.3844	3.4045	0.1922	2.2277	1.308

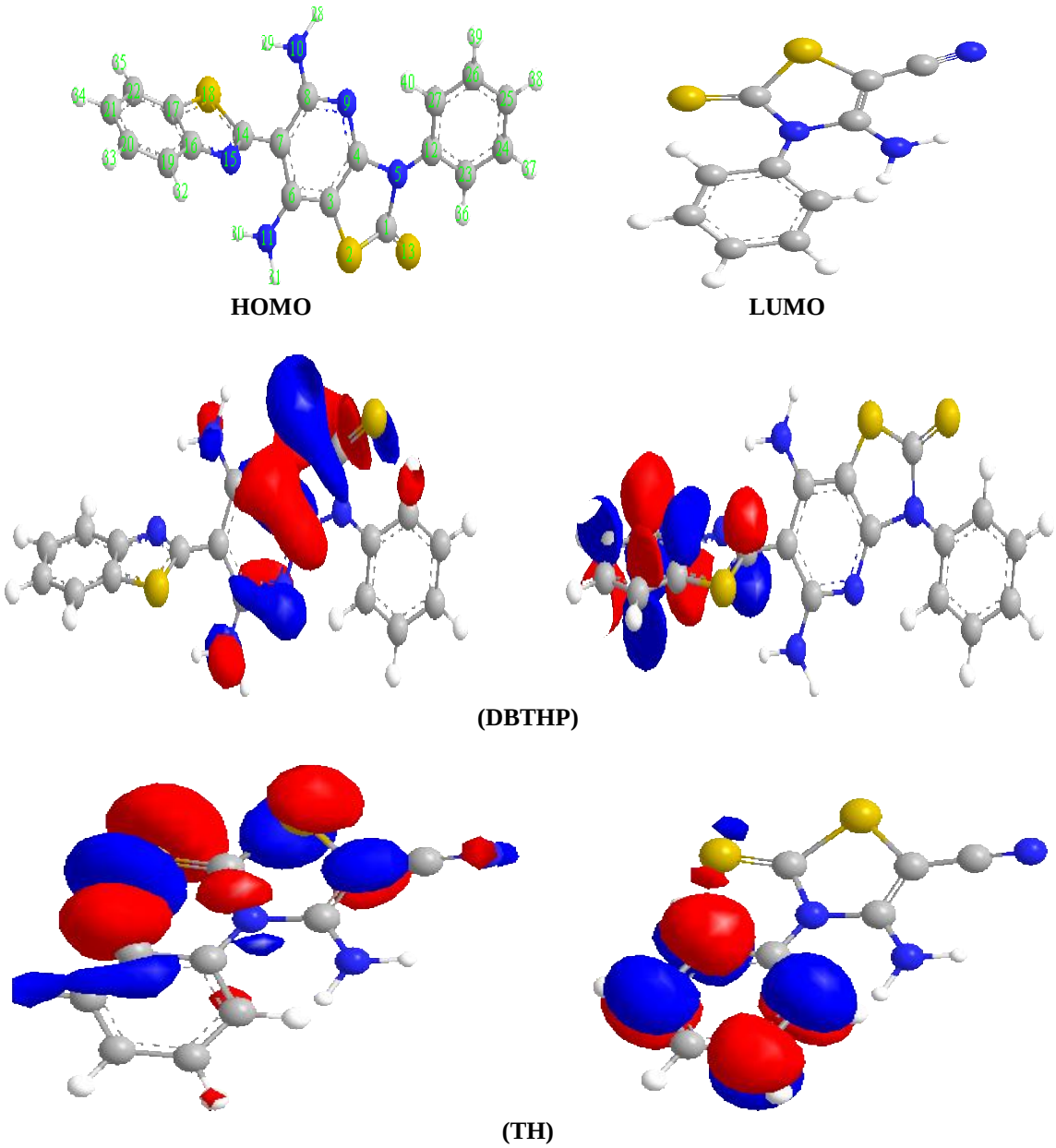


Fig. (11): The highest occupied Molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of (TH), (DBTHP) thiazole derivatives.

CONCLUSIONS

In summary, new thiazole-based derivatives **5**,7-diamino-6-(benzo[d]thiazol-2-yl)-3-phenylthiazolo[4,5-b] pyridine-2(3H)-thione have been synthesized by the reactions of 4-Amino-3-phenyl-2-thioxo-2,3-dihydrothiazole-5-carbonitril with benzothiazol-2-yl acetonitrile. The DNA binding mode of the synthesized compounds TH and DBTHP with CT-DNA have 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 $=5.59\times10^5$ and 7.24×10^5 M^{-1}) respectively. The thiazole derivatives displayed significant radical scavenging activity. A comparative antimicrobial screening and anticancer activities of prepared thiazolo[4,5-b] pyridine have been studied. Against HCT-116 (human colon carcinoma) HepG2(human hepatocellular carcinoma), MCF-7(human breast carcinoma), and PC-3(human prostate and PC-3 and also compared with that of the standard anticancer drug doxorubicin. Furthermore, the docking behavior of the compounds had been carried out against the cocrystal structures of proteins for colon, human liver, and breast, and prostate cancer cells. From molecular docking interaction, DBTHP demonstrate interactions with the active site residues with good dock score.

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