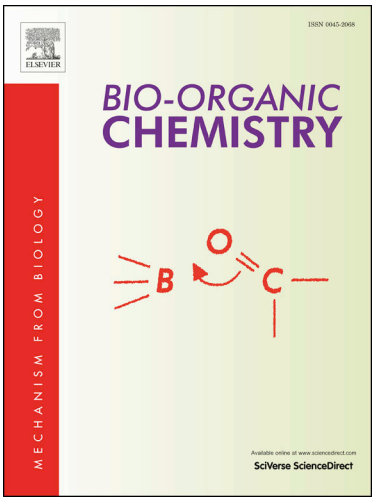
Journal Pre-proofs




# Design, Molecular Modeling and Anticancer Evaluation of Thieno[2,3-d]pyrimidine Derivatives as Inhibitors of Topoisomerase II

Souad A. El-Metwally, Ali K. Khalil, Wael M. El-Sayed

PII: S0045-2068(19)30750-3
DOI: https://doi.org/10.1016/j.bioorg.2019.103492
Reference: YBIOO 103492

To appear in: *Bioorganic Chemistry*

Received Date: 9 May 2019
Revised Date: 7 November 2019
Accepted Date: 1 December 2019

Please cite this article as: S.A. El-Metwally, A.K. Khalil, W.M. El-Sayed, Design, Molecular Modeling and Anticancer Evaluation of Thieno[2,3-d]pyrimidine Derivatives as Inhibitors of Topoisomerase II, *Bioorganic Chemistry* (2019), doi: https://doi.org/10.1016/j.bioorg.2019.103492

# Design, Molecular Modeling and Anticancer Evaluation of Thieno[2,3-d]pyrimidine Derivatives as Inhibitors of Topoisomerase II

Souad A. El-Metwally[a], Ali K. Khalil[b], Wael M. El-Sayed[c,*]

[a] Higher Technological Institute, 10th Ramadan city, Egypt

[b] University of Ain Shams, Faculty of Science, Department of Chemistry, Abbassia 11566, Cairo, Egypt

[c] University of Ain Shams, Faculty of Science, Department of Zoology, Abbassia 11566, Cairo, Egypt

[*] Corresponding author;

Wael M. El-Sayed,

Tel: +202/2482-1633 Fax: +202/2684-2123

Email; waelelhalawany@hotmail.com, wael_farag@sci.asu.edu.eg

Running title: Thienopyrimidine Derivatives as Topoisomerase Inhibitors

**Abstract**

Synthesis of 4-(3,5-Dimethyl-1H-pyrazol-1-yl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine **1** and its functionalized reactions as nucleophile with various electrophilic reagents were performed through facile methods to yield different cyclic and acyclic derivatives (**2-17).** The structures of the newly synthesized compounds were established by their elemental analysis and spectral data. Derivatives **4, 14, 16,** and **17** in addition to the parent compound **1** had $IC_{50}$ at ~ 4-10 μM against HepG2 and MCF7 and were selected for further investigations. All derivatives had high $IC_{50}$ values (> 60 μM) against normal fibroblasts WI38 indicating selectivity against cancer cell lines. Derivatives **4, 14,** and **17** up-regulated the expression of p53 by ~ 3-4 folds. All derivatives caused a significant ~ 3-fold increase in the expression of executive caspase 3 and significant elevation in cleaved caspase 3 activity. The elevation in the expression of caspase 3 by compound **1** and derivative **16** was not accompanied by an increase in p53 expression or cleaved caspase 3 activity. These two thienopyrimidines may act directly on caspase 3. Derivative **17** was unique in reducing the expression of topo II by ~60%. The molecular docking showed that derivatives **4** and **17** with high binding energies could bind and inhibit Topoisomerase II (Topo II). In accordance with the docking modelling, derivatives **4** and **17** reduced the Topo II concentration by 82 and 90%, respectively, compared to the untreated cells. However, the parent compound **1** also caused a significant 34% reduction in the enzyme concentration although it was not predicted as a ligand for the enzyme in the docking study. Taken together, derivatives **4, 14** and **17** showed selective cytotoxicity, could arrest cell cycle, and induce apoptosis. Furthermore, they could serve as cytostatic agents by inhibiting/reducing Topo II.

**Key words:** Thienopyrimidine; Semicarbazide derivative; Cytotoxic; Topoisomerase II; Apoptosis; p53.

## Introduction

Traditional chemotherapeutic agents are cytotoxic to both cancer and normal cells. They usually cause oxidative stress that evokes apoptosis without selectivity leading to sever and sometimes life-threatening effects. On top of that, they may result in secondary cancers or lose their effectiveness due to the emerging of cancer cell resistance. Therefore, the research is focused on targeted therapy and cytostatic agents. Thienopyrimidine derivatives have attracted much attention since they are well known to have a wide range of biological activities. Versatile natural products such as purines, pyrrolopyrimidines, pyridopyrimidines, pteridines, agrochemicals, and veterinary products possess fused pyrimidines [1-3]. Pyrimidine derivatives and heterocyclic pyrimidines showed immense biological activities such as anticancer [4], antiviral [5], antitumor [6], anti-inflammatory [7], and antimicrobial [8, 9] agents. Aromatic and heteroaromatic compounds bearing an *o*-amino nitrile or *o*-amino ester group represent a remarkable class of substrates utilized as precursors for the synthesis of various condensed pyrimidine heterocyclic systems [10]. Thieno[2,3-d]pyrimidine derivatives bearing a phenylamino substituent at the 4-position have shown remarkable anticancer activity as tyrosine kinase inhibitors [11, 12]. Structure-activity relationships studies revealed that various substituted thienopyrimidines, particularly at 4-position with various substituents such as anilino or heterocycles result in enhanced anticancer activities [12-16], Figure 1.

To test the hypothesis and fulfill the aim of the study, 17 thienopyrimidines derivatives were synthesized from the parent compound; 4-hyrazino compound (**1)** [17] by different substitution at the 4-position. Compound **1**, as a strong nucleophile, was allowed to react with diverse electrophilic centers such as diketonic compounds, anhydrides, acid halides, esters, alkyl halides, isocyanate, isothiocyanate derivatives, carboxylic acids, nitrous acid, triethyl

orthoformate, carbon disulphide, acrylonitrile, and aldehydes together with thioglycolic acid. The antiproliferative activity of these new derivatives and the parent compound was tested against epithelial human liver (HepG2) and breast (MCF-7) cell lines in addition to the normal human fibroblasts (WI-38) and compared to the activity of doxorubicin. The most active derivatives were selected for further investigations on the key genes involved in the cell cycle and/or apoptosis in liver cells. Finally, the active derivatives were examined by molecular modeling as inhibitors for topoisomerase II. In addition, the concentration of the later enzyme was also estimated.

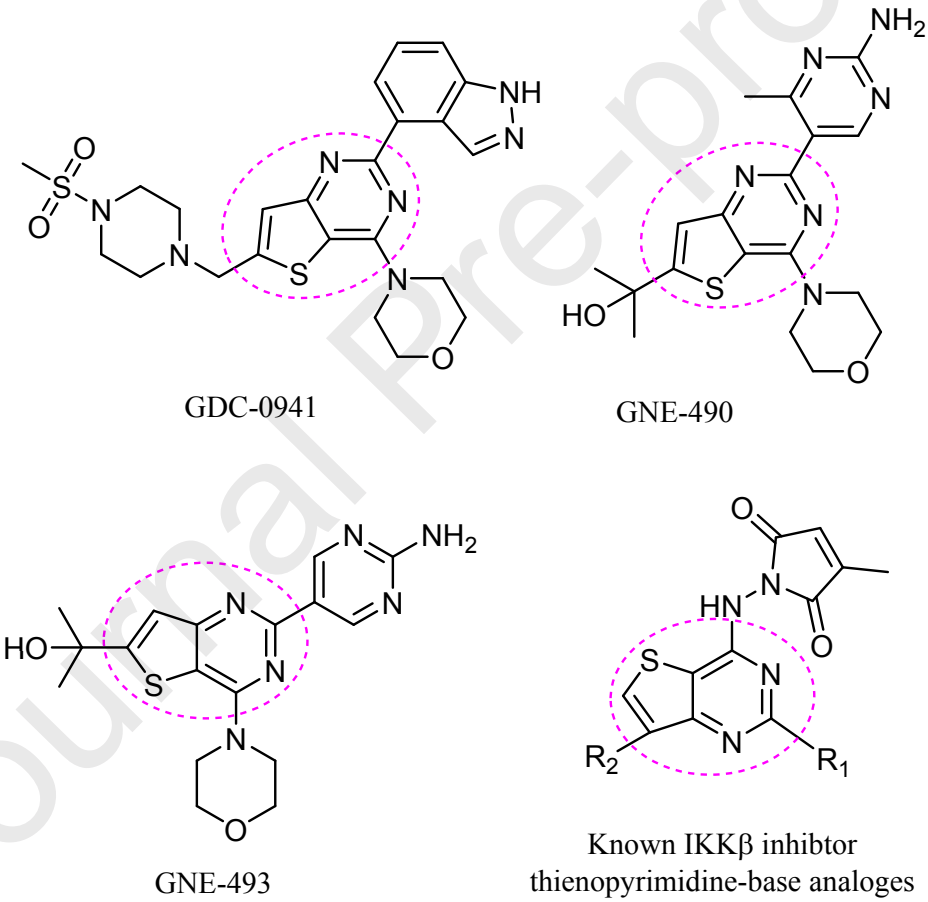



Figure 1: Thienopyrimidines as anticancer agents

Topoisomerases are enzymes that participate in the control of supercoiling and prevention of DNA tangling, therefore, it is essential for the growth of cancer cells. Consequently, topoisomerases

have become popular targets for cancer chemotherapeutic agents [18]. Several synthetic chemical analogs have been designed and used as topoisomerase inhibitors that block the action of topoisomerases (I and II) or decrease their expression and/or protein content [19, 20]. This reduction in activity or protein level will affect the topology and stability of DNA leading to single and double stranded breaks that harm the integrity of the genome leading to apoptosis [21]. Drugs poison or inhibit Topo II by two mechanisms. Etoposide, teniposide, doxorubicin, daunorubicin, and amsacrine inhibit the religation of DNA, whereas the ellipticines, azatoxins, and genistein enhance the formation of Topo II cleavage complex. The DNA intercalator doxorubicin poisons Topo II at low concentration and suppresses the enzyme concentration at higher concentration [18-21].

## 2. Results and discussion

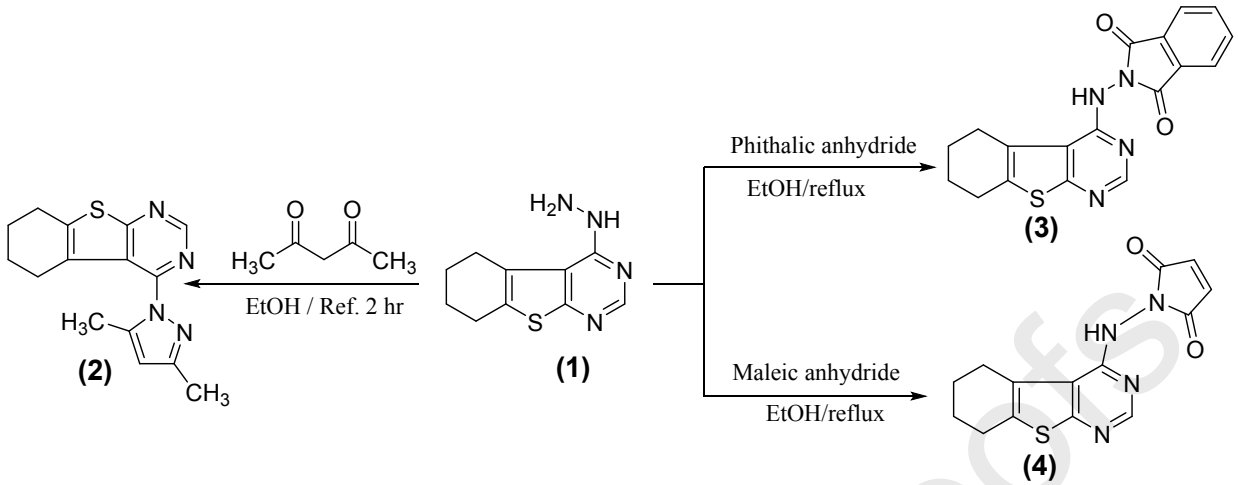
### 2.1 Chemistry

Due to all of the aforementioned facts and as a continuation of our previous work [11, 22-25], the scope of our investigation has been broadened to evaluate the potency of some synthetic analogues of thieno[2,3-d]pyrimidine derivatives as plausible topoisomerase inhibitors. Therefore, a profound assessment of those derivatives as anticancer agents capable of targeting topoisomerase II has been performed. Initially, a series of thieno-pyrimidine with diverse moieties at position 4 has been constructed. The synthetic strategy for constructing them has emanated from allowing the starting compound 4-hyrazino derivative (**1**) [17] as a strong nucleophile to react with diverse electrophilic centers. As electrophilic scafflods, diketonic compounds, anhydrides, acid halides, esters, alkyl halides, isocyanate, isothiocyanate derivatives, carboxylic acids, nitrous acid,

triethyl orthoformate, carbon disulphide, acrylonitrile were utilized. Also aldehydes together with thioglycolic acid have been used as one pot reaction in order to build different thienopyrimidine derivatives.

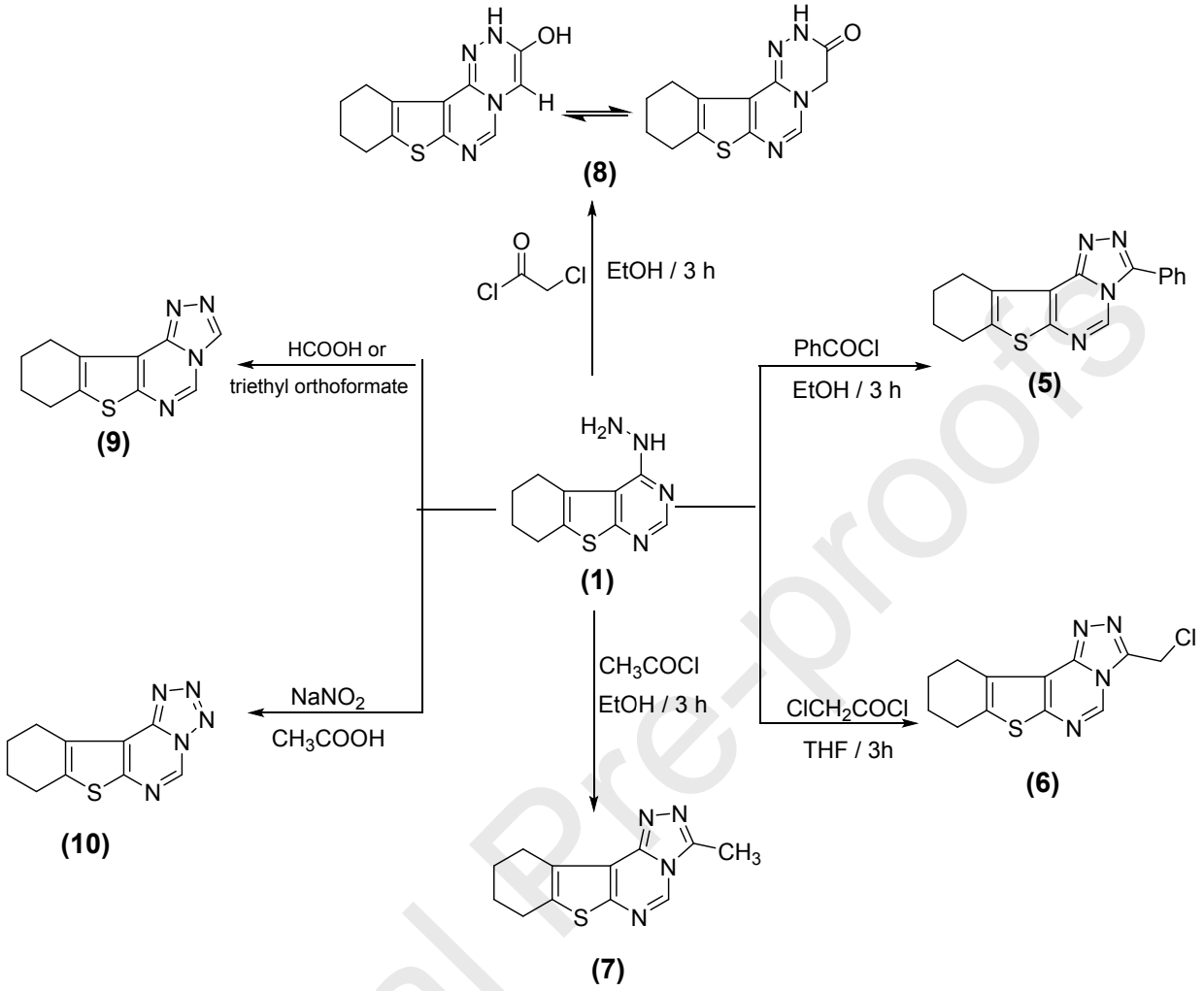
When the starting compound **1** was subjected to react with diketonic compounds such as acetyl acetone, it gave the expected product **2 (Scheme 1)**. $^{1}$H NMR of **2** revealed the presence of two quartet peaks at δ 2.9 & 2.2 ppm assuring the presence of the two methyl groups. In addition, its $^{13}$C NMR showed two peaks at δ 11.8 and 13.6 ppm which confirmed the existence of the methyl groups coinciding with the literature [17].

As anticipated, when compound **1** was treated with acid anhydride namely phthalic anhydride, it gave derivative **3**. The structure of derivative **3** was confirmed by the appearance of two carbonyl peaks at 1790 and 1733 cm$^{-1}$ in IR and in addition to two peaks at 166.47, 166.75 ppm in $^{13}$C NMR. As an additional proof, $^{1}$H NMR revealed the presence of two doublets at 8.0 and 8.4 ppm corresponding to four aromatic hydrogens of the phenyl group. On the other hand, the reaction of compound **1** with maleic anhydride proceeded to completion to afford derivative **4**. In both reactions with the anhydrides, the reaction seemed to commence with the amino group attack on one of the two carbonyl groups followed by a simple condensation reaction. Unambiguously, the $^{13}$C NMR spectrum proved the structure by exhibiting peaks at 168.5, 168.5 ppm for the two carbonyl groups whereas the $^{1}$H NMR showed a doublet peak at δ 6.4 ppm representing the two methine hydrogens of the pyrrrolidinone moiety. Meanwhile, IR spectrum showed characteristic peaks for two carbonyl groups at 1725, 1765 cm$^{-1}$ **(Scheme 1)**.




**(Scheme 1)**

Utilizing another type of electrophilic reagents such as benzoyl chloride, chloro acetyl chloride, and acetyl chloride was investigated by subjecting compound **1** to react with them. The mechanistic pathway for the synthesis of the expected products **5**, **6** and **7** commenced with the loss of hydrogen halide followed by the attack of N-3 of the pyrimidine ring on the carbonyl group to give the expected cyclic products. The structure of derivative **5** was confirmed by the appearance of the five aromatic protons as two doublet peaks at δ 8.5 ppm for 2H and one triplet peak at δ 7.5 ppm for 3H in the $^{1}$H NMR spectra. In case of chloro methyl derivative **6**, a singlet peak at δ 5.0 ppm appeared for the methylene group while in case of the methyl derivative **7**, a singlet peak for the methyl group at δ 2.7 ppm was found**.** IR and $^{1}$H NMR spectra of all the three revealed the absence of the NH and $NH_2$ peaks. However, in case of the methyl derivative **7**, its $^{13}$C NMR showed a peak at 14.7 ppm for the methyl group. Furthermore, the structure of derivative **7** was authentically proved from the reaction of compound **1** with glacial acetic acid and/or acetic anhydride which showed coincidental accordance with the proposed structure of **7 (Scheme 2)**.




**(Scheme 2)**

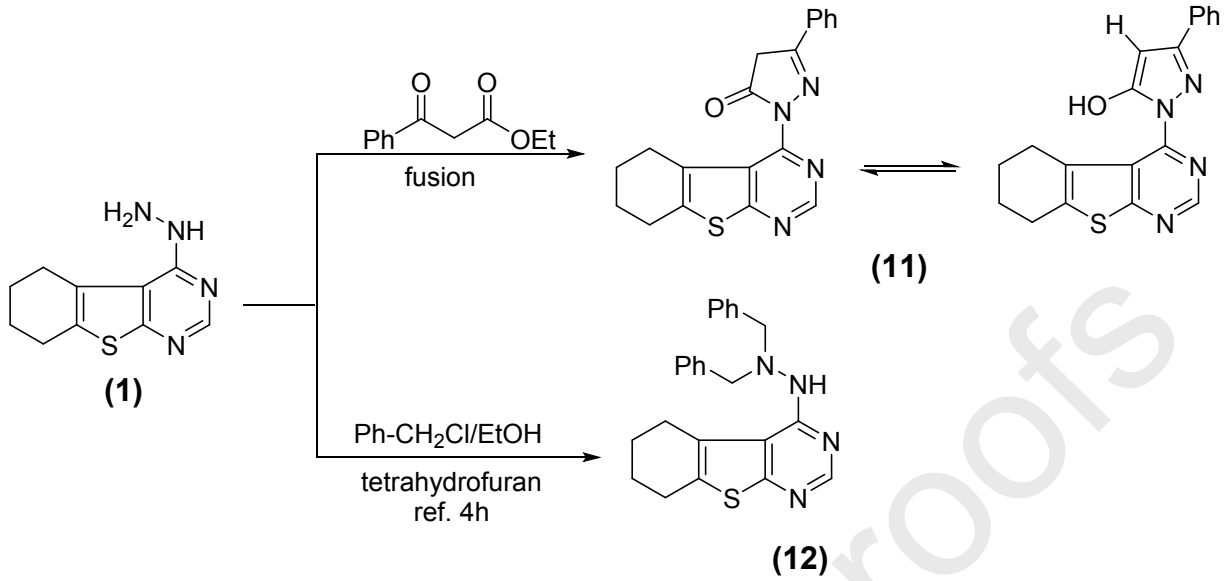
Surprisingly, when the reaction with chloroacetyl chloride was performed in ethanol as a solvent, the reaction proceeded *via* a different route which was initiated with the removal of two moles of HCl to afford the product **8**. The structure of this compound was proved by the appearance of both the NH and C=O of lactam ring peaks in the IR spectrum at 3241 and 1661cm$^{-1}$, respectively. Compound **8** is believed to be existing in a tautomeric equilibrium **(Scheme 2)**, since it has two singlet peaks in its $^{1}$H NMR spectrum at δ 8.4 and 9.2 ppm corresponding to N-C=H

proton pyrimidine hydrogen, respectively accompanied with the appearance of two weak peaks at δ 1.9 and 3.2 ppm for both the OH and NH protons.

When the hydrazino compound **1** was refluxed with formic acid, the reaction resulted in the formation of triazolo derivative **9** where a singlet peak appeared at δ 8.5 for the one proton of the thriazole ring and lacked any peaks for NH or $NH_2$ functional group in both IR and $^1$H NMR spectra. As an additional proof, compound **9** was also authentically prepared by refluxing the starting compound **1** with triethyl orthoformate in a neat reaction **(Scheme 2)**.

To achieve the target of constructing the desired tetrazine derivative **10**, a freshly prepared ice cold solution of nitrous acid was added to compound **1**. Structural elucidation of derivative **10** was ascertained by the absence of the NH and $NH_2$ peaks in either the IR or $^1$H NMR spectrum. Meanwhile, a singlet peak at δ 9.1 ppm corresponding to the pyridine aromatic proton has been shown in the $^1$H NMR spectrum **(Scheme 2)**.

Fusion of compound **1** with ethyl benzoyl acetate at 180$^o$C afforded the pyrazolone derivative **11**. The structure of derivative **11** was confirmed by the absence of both NH and $NH_2$ absorption peaks in IR and the presence of a carbonyl absorption band at 1665 cm$^{-1}$ while in the $^1$H NMR spectrum a broad peak for 5H phenyl protons at δ 7.0-7.5 ppm and a peak at δ 7.9 ppm for the pyrazolone proton, a CH proton peak at δ 8.4 ppm for pyrimidine proton, and one OH proton at δ 10.4 ppm. The appearance of the OH and NH peaks could be interpreted by the existence of the pyrazolone derivative **11** in different tautomeric forms as shown in **(Scheme 3)** The nucleophilic attitude of hydrazine compound **1** was further evaluated when it was allowed to react with benzyl chloride as an alkylating reagent. The reaction proceeded to afford the N-dialkyl product **12** where two moles of the alkylating agent were consumed **(Scheme 3)**.

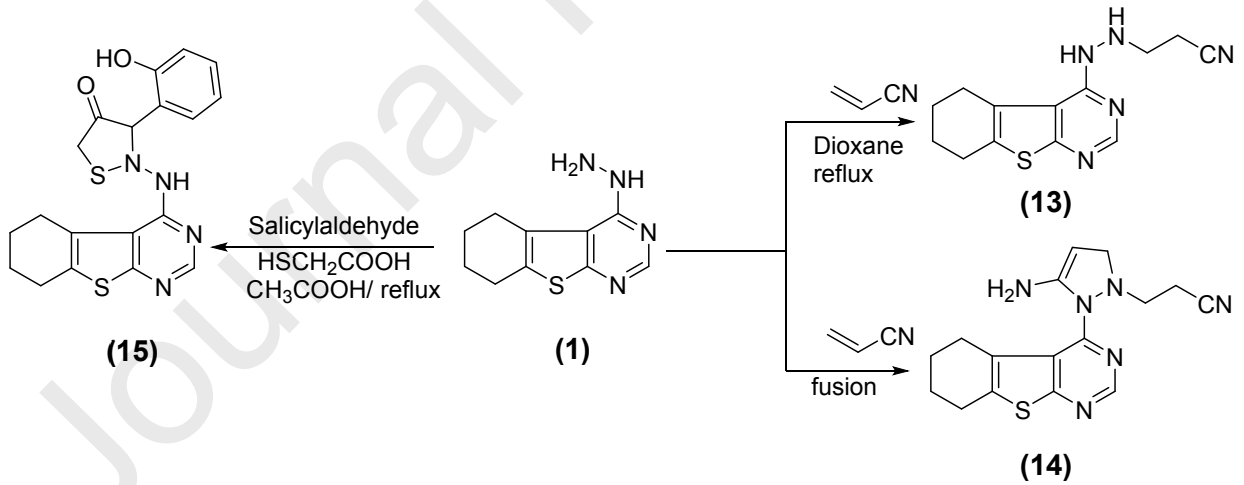



**(Scheme 3)**

The structure of the alkylated product **12** was confirmed by the absence of the $NH_2$ absorption band from the IR spectrum. $^1H$ NMR revealed the existence of doublet peak at δ 2.1 ppm corresponding to one methylene group and another peak for the other methylene group at δ 2.1 ppm besides absorption peaks at 7.2-10.0 ppm corresponding to the twelve protons; 10 aromatic hydrogen appear as (m, 1H) at δ 7.43 ppm, (m, 5H) at δ 7.8 ppm, (d, 3H) at δ 8.4 ppm (d, 1H) at δ 8.9 ppm, pyrimidine one hydrogen at δ 8.54 ppm, and one NH proton as a singlet peak at δ 9.6 ppm.

As the last electrophilic reagent of those which have been utilized, acrylonitrile has been exploited. It is worth mention that the nature of the product was found to be dependent on the reaction conditions under which the reaction was performed. For example, under refluxing an ethanolic solution of compound **1** with acrylonitrile, a simple nucleophilic attack on the highly deficient acrylonitrile carbon (C-3) took place to give product **13** IR spectrum of such compound

revealed the presence of CN absorption peak at 2252 $cm^{-1}$ and two absorption peaks for two NH at 3382, 3289 $cm^{-1}$ whereas $^{13}C$ NMR spectrum showed peaks for the two methylene groups $CH_2$-CN, $CH_2$-N at δ 14.7, 22.1 ppm, respectively and an absorption peak for the cyano group at δ 120.11 ppm. Meanwhile, $^{1}H$ NMR showed peaks at δ 1.7, 2.7, 1.9, 3.3 ppm corresponding to the two methylene groups $CH_2$-CN, $CH_2$-N, respectively. However, under fusion conditions, the same reaction was initially preceded by attacking of the amino group as a nucleophile on the β carbon of one mole of acrylonitrile following Michael reaction to give derivative **13** as an intermediate which in turn its NH as a nucleophile further attacked the other cyano group to give the cyclic product **14**. The structure of derivative **14** was verified by the presence of absorption peaks at 2246 and 3378 $cm^{-1}$ in the IR spectrum corresponding to CN and $NH_2$ groups, respectively. In addition, $^{13}C$ NMR spectrum showed a peak at δ 119.3 ppm corresponding to the cyano group. $^{1}H$ NMR revealed a singlet peak at δ 3.6 ppm ($CH_2$, pyrazole) **(Scheme 4)**.

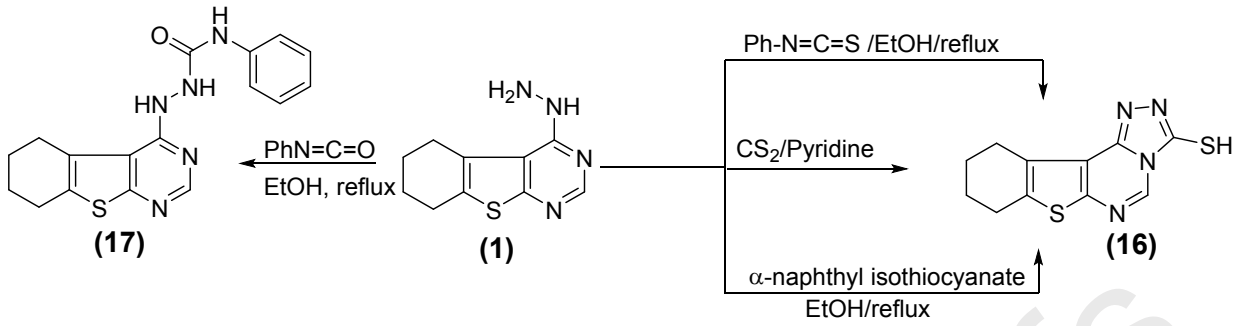



**(Scheme 4)**

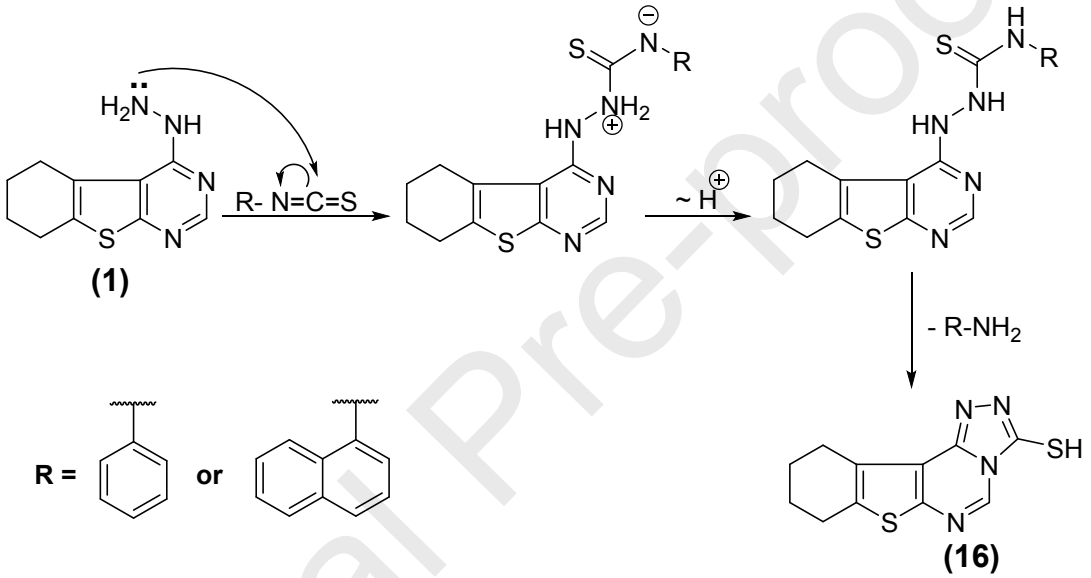
Condensation of compound **1** with salicyaldehyde in the presence of thioglycolic acid in acetic acid under reflux condition gave the isothiazolidine derivative **15 (Scheme 4)**. Its structure

was confirmed by the presence of absorption peaks in IR spectrum at 3470, 1650 $cm^{-1}$ corresponding to the hydroxyl group and carbonyl group, respectively and lacking of absorption peaks for the $NH_2$ group. Also, the mass spectrum of derivative **15** gave a molecular ion peak at m/z 398.9 (4.93 %).

The reaction of compound **1** with carbon disulphide in pyridine gave the expected triazolo-thione derivative **16 (Scheme 5)** with no absorption band for the $NH_2$ group in the IR spectrum and a singlet peak at δ 14.6 corresponding to SH proton in the $^1H$ NMR spectrum. Also, its mass spectrum assured the structure by the presence of $M^+$ peak at m/z 262.0 (100%). When compound **1** was treated with carbon disulphide or α- naphthyl isothiocyanate or phenyl isothiocyanate gave the same product **16.** The plausible mechanism of formation of derivative **16** is illustrated in **(Scheme 6**). This was confirmed by the absence of the aromatic hydrogens and appearance of a singlet broad absorption peak for the SH proton at δ 14.6 ppm in the $^1H$ NMR spectra of both compounds which means the release of both the α-naphthyl amine and aniline fragments during the attack upon the C=S bond followed by cyclization and releasing of the amine moiety. However, this was not the behavior in the case of phenyl isocyanate where the reaction was halted at the step of forming the Semicarbazide derivative **17** with no further cyclization **(Scheme 5)**. This can be attributed due to the lower reactivity of isocyanates derivatives compared with their corresponding isothiocyanates. The structure of **17** was proved by the presence of the carbonyl absorption peak at 1653 $cm^{-1}$ in the IR spectrum and the appearance of five protons of the phenyl group at δ 7.1-7.5 ppm in its $^1H$ NMR spectrum.




**(Scheme 5)**




**Scheme 6:** Suggested mechanistic pathway for the release of amino moiety.

## 2.2. Biology

Doxorubicin was cytotoxic to both liver and breast cell lines in addition to normal fibroblasts with $IC_{50}$ at ~ 4-5 µM **(Table 1).** Thienopyrimidine derivatives having $IC_{50}$ equal to or less than ~ 10 µM were considered active. Derivatives **4, 14, 16,** and **17** in addition to the parent compound **1** had $IC_{50}$ at ~ 4-10 µM against HepG2 and MCF7 and were selected for further investigations. All derivatives had high $IC_{50}$ values (> 60 µM) against normal fibroblasts

WI38 indicating selectivity against cancer cell lines. All new derivatives were less cytotoxic to the normal fibroblasts than the parent compound **1** which had an $IC_{50}$ at ~ 40 μM in normal cells.

**Table 1: Influence of the thienopyrimidine derivatives on the viability of HepG2, MCF7 and WI-38 cells.**

| Cpd | $IC_{50}$ (μM) | | |
|---|---|---|---|
| | **HepG2** | **MCF7** | **WI-38** |
| DOX | 4.50±0.22 | 4.17±0.21 | 5.23±0.87 |
| 1 | 7.84±0.81 | 10.32±1.10 | 39.91±7.67 |
| 2 | 49.47±3.13 | 56.62±3.40 | 81.81±9.71 |
| 3 | 30.02±2.25 | 17.28±1.53 | 68.67±4.13 |
| 4 | 6.71±0.77 | 5.71±0.67 | 60.67±3.18 |
| 5 | 38.13±2.67 | 32.09±2.31 | 68.31±14.35 |
| 6 | 53.23±3.37 | 45.38±3.10 | 59.01±4.57 |
| 7 | 73.16±4.01 | 81.57±4.70 | >100 |
| 8 | 65.48±3.80 | 69.20±3.91 | 66.33±9.33 |
| 9 | 76.85±4.21 | 92.73±5.20 | >100 |
| 10 | 22.90±1.83 | 27.16±1.90 | 32.54±5.33 |
| 11 | 84.37±4.83 | >100 | >100 |
| 12 | 16.96±1.47 | 14.27±1.21 | 64.00±5.37 |
| 13 | >100 | >100 | >100 |
| 14 | 4.38±0.51 | 3.96±0.31 | 74.71±11.23 |
| 15 | 41.85±2.90 | 37.45±2.83 | 45.25±3.17 |

| | | | |
|---|---|---|---|
| 16 | 5.51±0.4 | 9.19±0.9 | 73.33±8.11 |
| 17 | 6.17±0.93 | 5.89±0.87 | 89.13±9.93 |

After 48h of incubation, the viability of cells was measured with 5 different concentrations (three wells per every concentration) of the investigated compounds by MTT assay. The $IC_{50}$ was determined from the dose-response curves as the mean of two parallel experiments. Doxorubicin (Dox) was used as a positive control.

To explore the molecular mechanism(s) through which these active derivatives exerted their antiproliferative activity, the effect of the most active derivatives (**4, 14, 16** and **17)** and the parent compound **1** on the expression of key genes involved in cell cycle and apoptosis (cdk1, p53, caspase 3, and topo II) was investigated in HepG2 cells and compared to that of doxorubicin **(Table 2)**. Doxorubicin was the only treatment that affected the expression of cdk1 causing ~ 70% reduction compared to the untreated cells in agreement with previous reports [26, 27]. The ability of the cell to progress to S phase depends on the cdk1 [28]. The downregulation of cdk1 by doxorubicin along with the elevation in p53 expression suggest cell cycle arrest. p53, the guardian of the genome, is a tumor suppressor gene. It is mutated in more than 50% of human cancer types [29]. It plays a central role in the regulation of cell cycle progression, DNA stability, and apoptosis. DNA damage induces the elevation in p53 expression which arrests the cell cycle at the G1/S checkpoint, stimulates the DNA repair mechanisms, and if the damage is sever, p53 initiates apoptosis [30]. Derivatives **4, 14,** and **17** upregulated the expression of p53 by ~ 3-4 folds compared to ~ 2-folds elevation caused by doxorubicin. Similar effects were previously reported for doxorubicin [31]. The elevations in p53 caused by the parent compound and derivative **16** did not achieve a statistical significance.

All derivatives investigated as well as doxorubicin caused a significant ~ 3-fold increase in the expression of executive caspase 3 **(Table 2)**. The elevation of p53 in the current study could be responsible for the parallel elevation in the expression of caspase 3. Caspase 3 is the main execution member responsible for apoptosis induced by both extrinsic and intrinsic pathways [32]. Procaspase 3 is cleaved and activated by other initiator caspases in a well-controlled cascade. However, the elevation in the expression level of caspase 3 by compound **1** and derivative **16** was not accompanied by an increase in p53 expression. These two Thienopyrimidines may act directly on caspase 3. Currently, there is no clinically approved drug that specifically activates effector caspase 3 directly without going through the regular activation by initiator caspases and p53. Therefore, these derivatives could be chemically modified to maximize their specific caspase targeting potential. Since many tumor cells overexpress procaspase 3 [33], having specific targeted drugs that bypass all upstream apoptotic events and directly hit effector caspase 3 will be a substantial advance and this will significantly reduce the resistance of tumor cells to chemotherapeutic agents. In this study, we did not measure the protein level of enzyme activity of the cleaved caspase 3. Since a linear correlation between gene expression and enzyme level or activity is not always present due to post-transcriptional and translational events, therefore, these derivatives deserve further investigations. Many FDA-approved chemotherapeutic agents were found to elevate p53 expression [34], and induce caspases [35] in tumor cells

Derivative **17** was the only treatment that significantly affected the expression of topo II resulting in ~60% reduction compared to the untreated cells. Doxorubicin also significantly reduced the expression of topo II but to a lesser extent; ~40%. Topo II prevents the DNA tangling

by double stranded breaks and is an essential enzyme for DNA replication and cell proliferation [36]. Inhibitors of Topo II are extensively examined as antineoplastic agents [37].

**Table 2: Effect of thienopyrimidine derivatives on the expression of p53, cdk1, caspase 3 and topo II in HepG2 after 8 h incubation.**

| Treatment | p53 | Cdk1 | Caspase 3 | Topo II |
|---|---|---|---|---|
| | | (fold induction from untreated cells) | | |
| DOX | 2.37 ± 0.31[a] | 0.31 ± 0.02[a] | 3.37 ± 0.57[a] | 0.57 ± 0.09[a] |
| 1 | 1.27 ± 0.24 | 1.24 ± 0.17 | 2.97 ± 0.21[a] | 0.90 ± 0.11 |
| 4 | 3.01 ± 0.42[a] | 0.88 ± 0.21 | 2.78 ± 0.05[a] | 0.92 ± 0.16 |
| 14 | 2.67 ± 0.10[a] | 1.15 ± 0.18 | 2.51 ± 0.06[a] | 1.11 ± 0.04 |
| 16 | 1.43 ± 0.09 | 1.00 ± 0.35 | 2.97 ± 0.12[a] | 0.93 ± 0.09 |
| 17 | 3.83 ± 0.11[a] | 0.92 ± 0.24 | 3.10 ± 0.21[a] | 0.37 ± 0.04[a] |

[a] Significant difference ($p< 0.05$) as compared to untreated cells. The data are expressed as mean ± SEM for two independent experiments.

**Molecular docking**

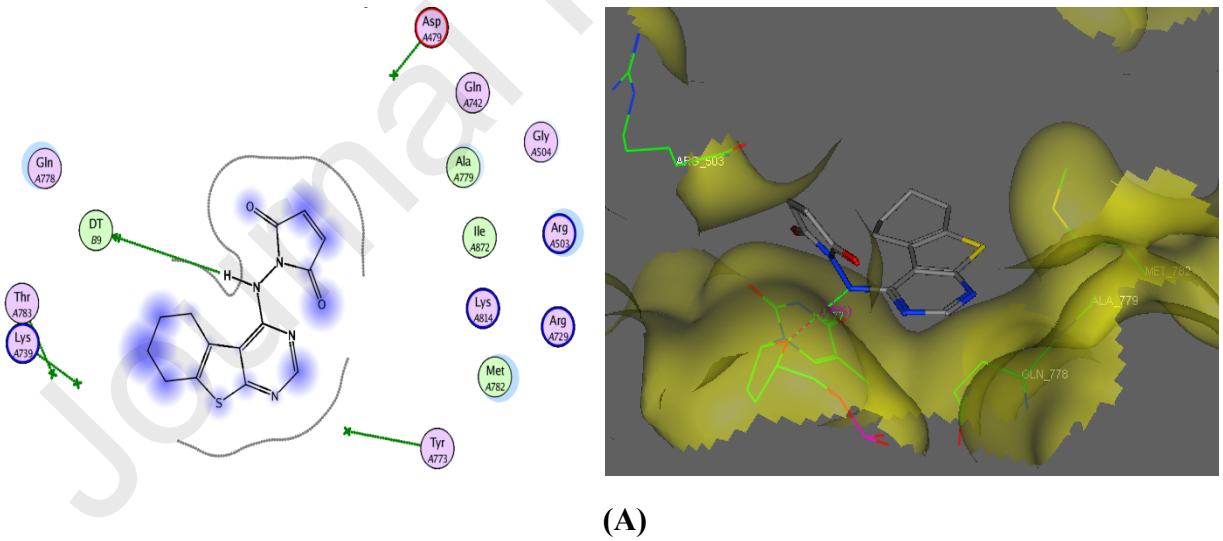
A molecular docking study was performed using MOE 2008.10 software for the most active synthesized compounds reported in Table **1** in addition to doxorubicin as a reference DNA intercalator, into the DNA binding site of topoisomerase II (ID: 3qx3). According to the interaction results presented in Table **3,** the most active compounds **4** and **17** showed high binding energies with DNA-Topo II target, with energies of -27.78 and -36.74 kcal/mol, respectively, compared to doxorubicin (-34.80 kcal/mol). All compounds formed a network of molecular interactions (H-bonds, vdw, and π-aromatic) with the active site residues of Topo II when analyzed in 2D plots as shown in Figure **2**.

The proposed binding mode of derivative **4** is the formation of various interactions in the binding pocket of the enzyme. A hydrogen bond between the NH and DTB9 was formed with a distance of 2.77 A° and the planar aromatic system was involved in a hydrophobic interaction with LysA739, ThrA783 and TyrA773 residues (Figure **2A**). The same behaviour was exhibited by derivative **17** which showed two hydrogen bonds interaction between the two amidic NH groups and the C=O group of AspA479 with a distance of 2.95 and 2.43 A°. In addition, the planar aromatic system (thieno-pyrimidine) showed hydrophobic interactions with LysA739, ThrA783 and TyrA773 residues. Moreover, the benzene ring moiety was oriented to form aromatic stacking interaction with LysA456 and ArgA503 (Figure **2B**). Camptothecin (CPT) binds to topoisomerase and DNA by hydrogen bonds forming in a ternary complex and thereby stabilizing it. This prevents the re-ligation of DNA and therefore causes DNA damage and apoptosis [38]. CPT showed

promising clinical results, and two analogues (topotecan and irinotecan) of CPT were approved by FDA as effective cancer therapeutic agents [39]. Another analogue of CPT; a lurtotecan with a pyrimidine moiety was clinically used in treating resistant ovarian cancer [40].

**Table 3. The docking binding free energies of thienopyrimidine derivatives against topoisomerase II**

| Compound | Binding free energy (kcal/mol) |
|---|---|
| 1 | -19.01 |
| 4 | **-27.78** |
| 14 | -18.11 |
| 16 | -16.18 |
| 17 | **-36.74** |
| Doxorubicin | **-34.80** |




**(A)**

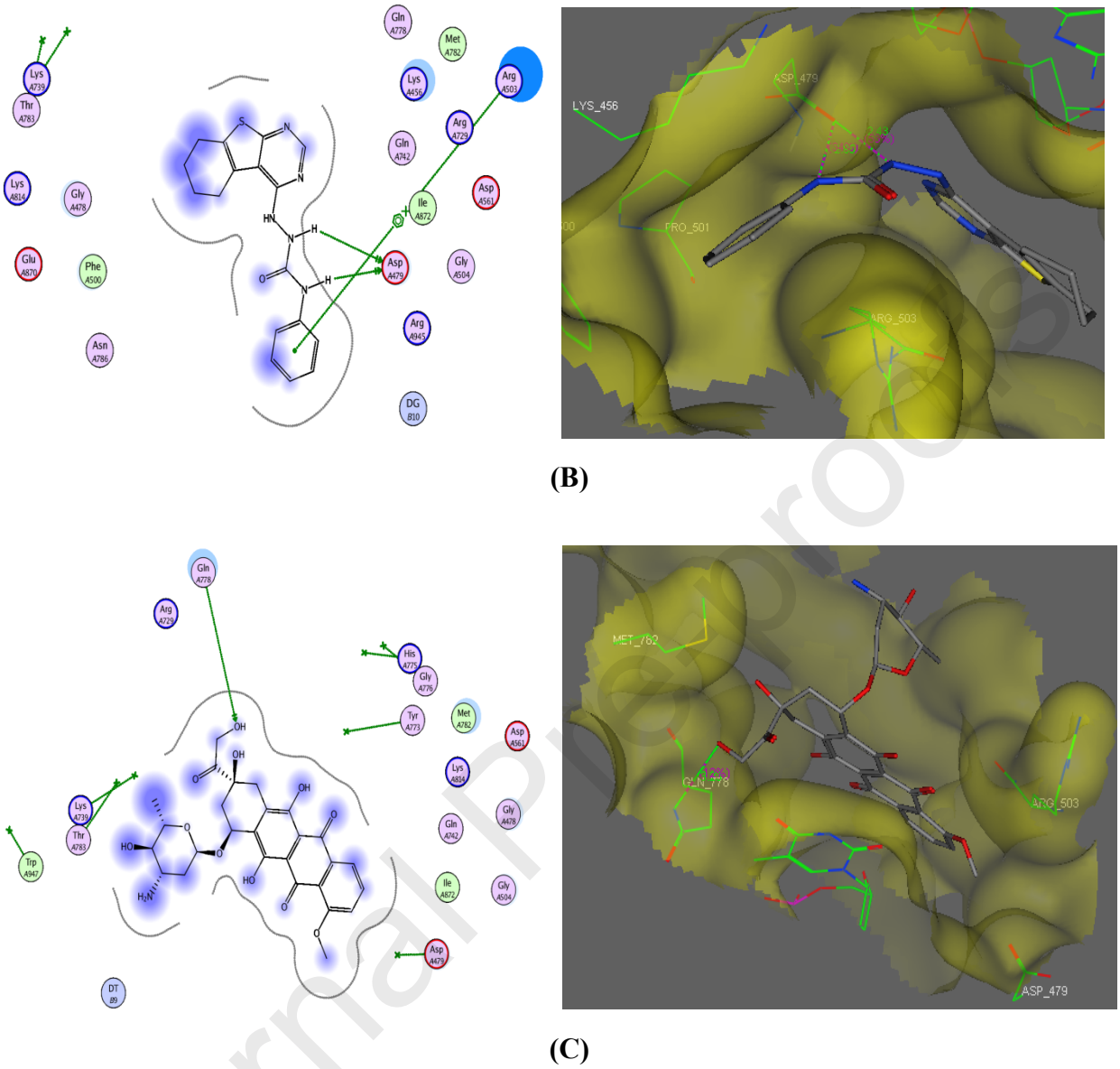



Figure 2: Calculated binding mode of derivatives 4 (A), 17 (B), and doxorubicin (C) (grey sticks) within the binding pocket of topo II receptor. The active pocket has been represented as yellow surface. Other residues have been hidden for sake of clarity.

From the gene expression profile, it was clear that only derivative **17** inhibited the expression of topo II but the docking study showed that derivatives **4** and **17** are projected to bind and inhibit the enzyme activity. The molecular modeling is directed to the protein and not to the genome level. Therefore, the topo II protein levels were measured in HepG2 after treatment with

the most active antiproliferative derivatives at their $IC_{50}$ values **(Figure 3)**. The results were somewhat parallel to the docking results. Derivatives **14** and **16** were devoid of any significant effect (15 and 17% decrease, respectively) compared to the untreated cells. In accordance with the docking modelling, derivatives **4** and **16** reduced the enzyme concentration by 82 and 90%, respectively, compared to the untreated cells. However, the parent compound **1** also caused a significant 34% reduction in the enzyme concentration although it was not shown to bind the enzyme in the docking study. This could be due to its effect on the post-transcriptional processing of the mRNA or translation of the protein; a hypothesis that needs further investigation. Doxorubicin caused ~ 87% decrease in the enzyme concentration as compared with the untreated cells. The antitumor activity of doxorubicin is mainly due to intercalation of DNA and reduction in the concentration and activity of Topo II [41, 42].

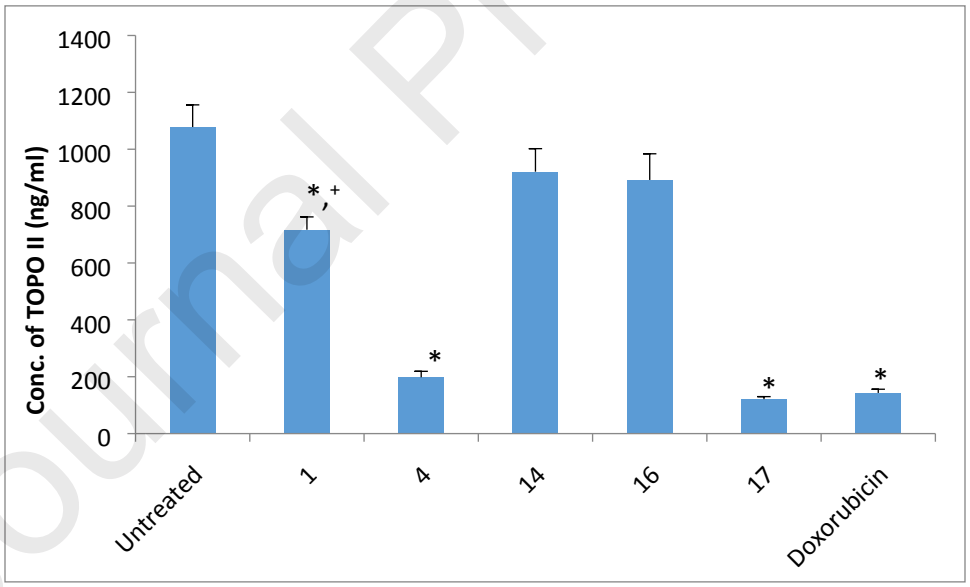



Figure 3: The inhibitory activity of the novel thienopyrimidine derivatives against topoisomerase II (Topo II) in HepG2 cells. Each compound has been assigned three wells and the experiment was performed twice. Data are expressed as mean ± SEM. * Significant difference compared to untreated cells. $^{+}$ Significant difference compared to doxorubicin-treated cells

**Effect of the active compounds (1, 4, 14, 16 and 17) on the cleaved caspase 3 activity in HepG2**

Since a linear correlation between gene expression and protein expression does not always exists and since caspase 3 exists as inactive procaspase 3, measuring the cleaved caspase 3 activity is considered the most accurate way to examine the effect of the most active compounds from the previous investigations **(1, 4, 14, 16** and **17)** on caspase 3 and link it to apoptosis. Compounds **4**, **14**, and **17** caused a significant elevation in the enzyme activity comparable to that caused by doxorubicin (Figure 4). These results do not correlate well with those reported for genes in table 2, where all compounds significantly elevated the gene expression. Therefore, we believe that measuring qPCR alone is not a sensitive measure due to the complexity of translation and post-translational processing. This clearly indicates that compounds **4, 14,** and **17** induce apoptosis through caspase 3 while compounds **1** and **17** work through different pathway(s).

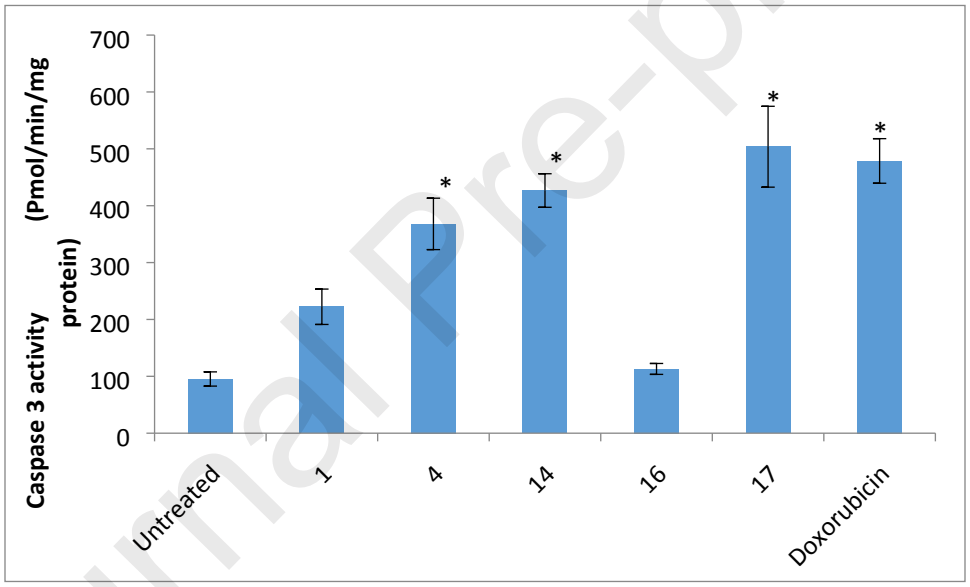



Figure 4: The effect of derivatives 1, 4, 14, 16 and 17 on the cleaved caspase 3 activity in HepG2 cells. Data are expressed as mean ± SEM, n=3. * significant inhibition compared to untreated cells.

**Conclusions**

Previous studies revealed that various substituted thienopyrimidines at 4-position with various substituents enhanced their anticancer activities. Consequently, 17 thienopyrimidines derivatives were synthesized from the parent compound; 4-hyrazino compound (**1)** by different substitution at

the 4-position. These new derivatives were examined for their antiproliferative activity, their effects on key genes involved in cell cycle and apoptosis. Finally they were evaluated as inhibitors for TopoII by molecular docking and by measuring the enzyme concentration in liver cells. Derivatives **4, 14, 16,** and **17** in addition to the parent compound **1** had $IC_{50}$ at ~ 4-10 μM against HepG2 and MCF7 and were selected for further investigations. All derivatives had high $IC_{50}$ values (> 60 μM) against normal fibroblasts WI38 indicating selectivity against cancer cell lines. Derivatives **4, 14,** and **17** up-regulated the expression of p53 by ~ 3-4 folds which functions to arrest cell cycle, induce DNA repair, or induce apoptosis. These same derivatives significantly elevated the cleaved caspase 3 activity indicating the occurrence of apoptosis. All derivatives caused a significant ~ 3-fold increase in the expression of caspase 3, the final and most important member of execution phase of apoptosis. Compound **1** and derivative **16** induced caspase 3 expression without affecting p53 expression but did not affect the cleaved caspase 3 activity. Derivative **16** was unique in reducing the expression of topo II by ~60%. The molecular docking showed that derivatives **4** and **17** with high binding energies could bind and inhibit Topo II. In accordance with the docking modelling, derivatives **4** and **17** reduced the Topo II concentration by 82 and 90%, respectively, compared to the untreated cells. Taken together, derivatives **4, 14,** and **17** showed selective cytotoxic, could arrest cell cycle, and induce apoptosis. Furthermore, they could serve as cytostatic agents by inhibiting/reducing Topo II and therefore, they deserve further investigations as small molecules targeted therapies.

## 3. Experimental

### 3.1 Chemistry

### 3.1.1 General

Melting points were determined in an open capillary in a Gallenkamp melting point apparatus (Sanyo Gallenkamp, UK) and are uncorrected. Pre-coated silica gel plates (Kieselgel 0.25 mm, 60 F254, Merck, Germany) were used for thin layer chromatography. IR spectra (KBr disc) were recorded using an FT-IR spectrophotometer (Perkin Elmer, USA). $^{1}$H NMR spectra were scanned on a NMR spectrophotometer (Bruker AXS Inc., Switzerland), operating at 400 MHz for $^{1}$H and $^{13}$C NMR. Chemical shifts are expressed in δ values (ppm) relative to TMS as an internal standard, using DMSO-$d_6$ or $CDCl_3$ as a solvent. Elemental analyses were done on a model 2400 CHNSO analyzer (Perkin Elmer, USA). All values were within ±0.4 % of the theoretical values. All reagents used were obtained from Sigma (St. Louise, USA) and was directly used for the preparation of the target compounds.

### 3.1.2 Synthesis

**Formation of4-( 3,5-dimethyl-1H-pyrazolyl) and 3,4-dimethyl-triazin-4-ol. thienopyrimidine derivatives (2):**

An amount of 0.005 moles of some diketonic compound ; acetyl acetone was added to a solution of compound **1** (1.1 g, 0.005 mole) in 30 mL ethanol and refluxed for 2 h. The formed precipitate was filtered off and crystalized from the suitable solvent.

**4-(3,5-Dimethyl-1H-pyrazol-1-yl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine (2)**

$C_{15}H_{16}N_4S$: Pale buff crystals (ethanol); m.p. (260$^{o}$C); yield (80 %); IR (KBr ν$cm^{-1}$) 2939, 2861, 1566; $^{1}$H NMR ($CDCl_3$) $\delta$ ppm: 1.60 (m, 2H, $CH_{2cyclohexane}$), 1.76 (m, 2H, $CH_{2cyclohexane}$), 2.1 (s, 3H, $CH_3$), 2.2 (s, 3H, $CH_3$), 2.8 (m, 4H, $2CH_{2cyclohexane}$), 6.1 (s, 1H, =CH), 8.9 (s, 1H $_{pyrimidine}$);

$^{13}$C NMR ($CDCl_3$): 11.8, 13.3, 22.3, 22.5, 24.9, 26.1, 77.3, 107.5, 126.4, 127.1, 139.6, 141.5, 150.0, 151.4; Anal. Calcd: C, 63.35; H, 5.67; N, 19.70; Anal. Found: C, 63.26; H, 5.55; N, 19.63.

**Formation of isoindoline-1,3-dione&furan-2(5H)-one derivatives 3 and 4:**

A quantity of 0.005 moles of some acid anhydrides; phthalic or maleic anhydride was added to a solution of compound **1** (1.1 g 0.005 mole) in 30 mL ethanol and refluxed for 3h where a precipitate was formed during the reaction. After completion of the reaction, the precipitate was separated by filtration and crystalized from the appropriate solvent.

**2-((5,6,7,8-Tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)amino)isoindoline-1,3-dione (3)**

$C_{18}H_{14}N_4O_2S$: White crystals (Ethanol / DMF); m.p. (268$^o$C); yield (80 %); IR (KBr ν cm$^{-1}$) 3402, 3354, 2939, 1790, 1733; $^1$H NMR ($CDCl_3$) $\delta$ ppm: 1.7 (m, 4H, $2CH_{2cyclohexane}$), 2.7-2.9 (m, 4H, $2CH_{2cyclohexane}$), 7.7 (d, $2H_{aromatic}$), 8.0 (d, $2H_{aromatic}$), 8.1(s, $1H_{Pyrimidine}$), 8.4 ( s,1H ,NH, $D_2O$ exchangeable ); $^{13}$C NMR ($CDCl_3$): 20.8, 22.2, 25.4, 25.8, 116.3, 124.2 ,125.1, 130.0, 134.8, 136.0, 151.9, 155.1, 166.4, 166.7, 176.3; Anal. Calcd: C, 61.35; H, 4.00; N, 15.99; Anal. Found: C, 61.25; H, 3.95; N, 15.88.

**5-(2-(5,6,7,8-Tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)hydrazono)furan -2(5H)-one (4)**

$C_{14}H_{12}N_4O_2S$: Yellow crystals (DMF); m.p. (205$^o$C); yield (60 %); IR (KBr ν/ cm$^{-1}$) 3337, 3049, 2949, 1645;$^1$H NMR(DMSO-$d_6$) $\delta$ ppm; 1.8 (m, 4H, $2CH_{2cyclohexane}$), 2.7 (d, 2 H, $CH_{2cyclohexane}$), 3.0 (d ,2H, $CH_{2cyclohexane}$), 6.4 (d, $2H_{olefinic}$), 8.3 (s, $1H_{pyrimidine}$), 8.7 (s ,1H ,NH, $D_2O$

exchangeable); $^{13}$C NMR (DMSO-$d_6$): 22.07, 22.47, 24.89, 25.63, 115.22, 126.69, 129.12, 129.97 ,133.17, 133.67, 152.01, 163.11 ,166.75, 168.57; MS m/z: 300 [M+1, 71.76%], 204.01 (100%), 81.92 (62.46 %), 53.84 (61.06 %); Anal. Calcd: C, 55.99; H, 4.03; N, 18.65; Anal. Found: C, 55.79; H, 3.936; N, 18.46.

**Formation of 3-alkyl-8,9,10,11-tetrahydrobenzo[4,5]thieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine derivatives 5, 6 and 7:**

A 0.005 mole of different acid halides; benzoyl chloride, chloro acetyl chloride and acetyl chloride was added to a solution of compound **1** (1.1 g, 0.005 mole) in 30 mL ethanol for **6** and **8** (tetrahydrofuran for **7**) and refluxed for 3 h. After completion, the precipitated solid was filtered off and crystallize from the appropriate solvent.

**3-Phenyl-8,9,10,11-tetrahydrobenzo[4,5]thieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine(5):**

$C_{17}H_{14}N_4S$: Creamy crystals (ethanol/ DMF); m.p. (211$^o$C); yield (70%); IR (KBr ν cm$^{-1}$), 3054, 2976, 2934, 1622; $^1$H NMR ($CDCl_3$) $\delta$ ppm: 2.1 (m, 4H, $2CH_{2\ cyclohexane}$), 3.2 (m, 2H, $CH_{2cyclohexane}$), 3.3 (m, 2H, $CH_{2cyclohexane}$), 7.5 (t, 3 $H_{aromatic}$), 8.5 (d, 2 $H_{aromatic}$), 9.2 (s, 1 $H_{pyrimidine}$); $^{13}$C NMR ($CDCl_3$): 22.2, 23.0, 25.3, 25.5, 120.4, 127.6, 128.5, 129.3, 130.3,130.5, 135.3, 138.8, 149.6, 153.4, 165.0; Anal. Calcd.: C, 66.64; H, 4.61; N, 18.29; Anal. Found: C, 66.54; H, 4.51; N, 18.40.

**3-(Chloromethyl)-8,9,10,11-tetrahydrobenzo[4,5]thieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine (6)**

$C_{12}H_{11}ClN_4S$, White crystals (ethanol /DMF); m.p. (170$^o$C); yield (80 %); IR (KBr ν cm$^{-1}$) 3060, 3015, 2935, 1612; $^1$H NMR (DMSO- $d_6$) $\delta$ ppm: 1.9 (m, $4H_{cyclohexane}$), 2.9 (d, 2H, $CH_{2cyclohexane}$), 3.0 (d, 2H, $CH_{2cyclohexane}$), 5.0 (s, 2H, $CH_2$), 9.6 (s ,$1H_{pyrimidine}$); $^{13}$C NMR ($CDCl_3$):

22.1, 23.0, 25.4, 25.6, 35.1, 127.1, 129.0, 136.8, 139.9, 144.7, 149.8, 152.6; Anal. Calcd: C, 51.70; H, 3.98; N, 20.10; Anal. Found: C, 51.66 H, 3.78; N, 19.95.

**3-Methyl-8,9,10,11-tetrahydrobenzo[4,5]thieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimi-dine (7):**

$C_{12}H_{12}N_4S$: White crystals (Ethanol-DMF); m.p. (255$^oC$); yield (80 %); IR (KBr ν $cm^{-1}$) 3055, 2974, 2931, 1790, 1618; $^1H$ NMR ($CDCl_3$) $\delta$ ppm, 2.0 (m, 4H, $2CH_{2cyclohexane}$) ,2.7 (s, 3H, $CH_3$) 3.0 (m, 2H, $CH_{2cyclohexane}$), 3.2 (m, 2H, $CH_{2cyclohexane}$), 9.1 (s, $1H_{Pyrimidine}$), $^{13}C$ NMR: 14.7, 22.1, 23.0, 24.4, 25.5, 120.1, 129.0, 135.1, 138.8, 149.3, 153.6, 165.0; Anal. Calcd: C, 58.99; H, 4.59; N, 22.93; Anal. Found: C, 58.87; H, 4.53; N, 22.89.

**Formation of 9,10,11,12-tetrahydro-2H-benzo[4',5']thieno[2',3':4,5]pyrimido[6,1-c][1,2,4]triazin-3-ol (8):**

A 0.005 mole of chloroacetyl chloride (7 mL) was added to a solution of compound **1** in 30 mL ethanol and refluxed for 3 h. After the reaction being completed the precipitated solid was filtered off and crystallize from the appropriate solvent. $C_{12}H_{12}N_4OS$: White crystals (ethyl acetate); m.p. (137$^oC$); yield (75%); IR KBr ν $cm^{-1}$) 3241 (NH), 3027, 2921, 1661 (C=O); $^1H$ NMR ($CDCl_3$) $\delta$ ppm: 1.9 (broad, 1H, NH, $D_2O$ exchangeable), 2.0 (m, 4H, $2CH_{2cyclohexane}$), 3.0 (d, 2H, $CH_{2cyclohexane}$ and 1H, OH, $D_2O$ exchangeable), 3.2 (d, 2H, $CH_{2cyclohexane}$), 8.4 (s, 1H, N-C=H), 9.3 (s, $1H_{pyrimidine}$); $^{13}C$ NMR ($CDCl_3$): 22.1, 22.9, 25.2, 25.6, 76.2, 120.6, 129.0, 135.5, 139.5, 148.6, 153.5, 154.6; Anal. Calcd.: C, 55.37; H, 4.65; N, 21.52; Anal. Found: C, 55.35; H, 4.55; N, 21.42.

**Formation of 8,9,10,11-tetrahydrobenzo[4,5]thieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine derivatives (9)**

A 0.005 mole of formic or neat triethyl orthoformate was added to a solution of compound **1** (1.1g; 0.005 mole) in 30 mL ethanol and refluxed for 4 h. The precipitated solid was filtered off and crystalize from the suitable solvent. $C_{11}H_{10}N_4S$: white crystals (Ethanol); m.p. (130-132 $^oC$ ); yield ( 55 % ); IR (KBr ν $cm^{-1}$ ) 3060, 3018, 2935, 1613; $^1H$ NMR ($CDCl_3$) $\delta$ ppm: 2.0 ( m, 4 H, $2CH_{2cyclohexane}$), 3.0- 3.1 (m, 4 H, $2CH_{2cyclohexane}$), 8.4 (s, $1H_{triazolo}$), 9.1 (s, $1H_{pyrimidine}$); $^{13}C$ NMR ($CDCl_3$): 22.1, 22.9, 25.4, 26.5, 120.4, 129.0, 135.4, 139.4, 140.3, 153.4, 154.5; Anal. Calcd.: C, 57.37; H, 4.38; N, 24.33; Anal. Found: C, 57.27; H, 4.30; N, 24.20.

**Formation of 8,9,10,11-tetrahydrobenzo [4,5] thieno[3,2-e]tetrazolo[1,5-c]pyrimidine (10)**

To a solution of compound **1** (1.1 g, 0.005 mole) in 20 mL acetic acid in an ice bath, a solution of sodium nitrite (0.4g /2 mL $H_2O$) was added drop wise with stirring for 30 minutes. After complete addition, the mixture was left under stirring for another 30 minutes. The formed solid was collected by filtration, dried and crystallized from ethanol. $C_{10}H_9N_5S$: Pale buff crystals (ethanol); m.p. (129$^oC$); yield (70 %); IR (KBr ν $cm^{-1}$) 3099, 3068, 2937, 1605; $^1H$ NMR ($CDCl_3$) $\delta$ ppm: 2.0 (m, 4H, $2CH_{2cyclohexane}$), 3.0 (m, 2H, $CH_{2cyclohexane}$), 3.2 (m, 2H, $CH_{2cyclohexane}$), 9.5 (s, $1H_{pyrimidine}$); $^{13}C$ NMR ($CDCl_3$): 22.4, 22.5, 25.4, 26.2, 125.4, 128.4, 134.0, 142.2,, 152.6, 165.0; Anal. Calcd: C, 51.93; H, 3.92; N, 30.28; Anal. Found: C, 51.73; H, 3.88; N, 30.22.

**Formation of 5-phenyl-2-(5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-2,4-dihydro-3H-pyrazol-3-one (11):**

A mixture of compound **1** (1.1 g; 0.005 mole) and ethyl benzoyl acetate (1 mL; 0.005 mole) was fused at 180$^oC$ for 5 h. When the reaction was completed, the formed solid was triturated by petroleum ether (60-80), filtered and crystallize from the suitable solvent. $C_{19}H_{16}N_4OS$: Brown crystals (ethanol); m.p. (209$^oC$); yield (50%); IR (KBr ν $cm^{-1}$) 3231, 2917, 2848, 1665; $^1H$ NMR

(DMSO-$d_6$) $\delta$ ppm: 1.7-1.9 (m ,4H, $2CH_{2cyclohexane}$), 2.7 (m ,2H, $CH_{2cyclohexane}$), 2.9 (m, 2H, $CH_{2cyclohexane}$), 7.3-7.8 (m, $5H_{aromatic}$), 7.9 (s, $1H_{pyrazolone}$), 8.4 (s, $1H_{pyrimidine}$), 10.4 (s, 1H, OH, $D_2O$ exchangeable); $^{13}C$ NMR ($CDCl_3$): 22.1, 23.2, 25.3, 25.5, 86.8, 120.6, 127.6(2), 128.5, 128.7(2), 129.3, 134.4, 138.4, 144.3, 148.6, 154.4, 159.8, 166,1; MS *m/z* 347 (M-1, 7.58); Anal. Calcd: C, 65.50; H, 4.63; N, 16.08; Anal. Found: C, 65.45; H, 4.53; N, 15.98.

**Formation of 4-(2,2-dibenzylhydrazinyl)-5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidine (12)**

A mixture of compound **1** (1.1 g; 0.005 mole) and benzyl chloride (1mL; 0.007 mole) in tetrahydrofuran was refluxed 4h. After being completed, the precipitated solid was filtered off and crystallized from the suitable solvent. $C_{24}H_{24}N_4S$: Brown crystals (ethanol); m.p. (290$^o$C); yield (65%); IR (KBr cm $^1$), 3322, 3138, 2929, 2857; $^1H$ NMR (DMSO–$d_6$) $\delta$ ppm: 1.7-1.8 (m ,4H ,$2CH_{2cyclohexane}$), 2.1 (d ,2H, $CH_2Bz$), 2.2 (s, 2H, $CH_2Bz$), 2.8 (m, 2H, $CH_{2cyclohexane}$), 3.0 (m, 2H, $CH_{2cyclohexane}$), 7.4-7.9 (m, $10H_{aromatic,}$), 8.4 (s, $1H_{pyrimidine}$), 9.6 (broad, 1H, NH, $D_2O$ exchangeable); $^{13}C$ NMR (DMSO): 22.2, 23.0, 25.3, 25.6, 62.2(2), 117.4, 126.7(2), 127.2, 128.6(4), 129.2(4), 135.9, 140.6(2), 146.5, 154.0, 166.3; Anal. Calcd: C, 71.97; H, 6.04; N, 13.99; Anal. Found: C, 71.93; H, 6.00; N, 13.89.

**Formation of 3-(2-(5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)hydrazinyl)propanenitrile (13):**

A mixture of compound **1** (1.1 gm., 0.005 mole) and acrylonitrile (1 mL; 0.005 mole) was boiled in dioxane (10 mL) under reflux for 8 h. After the reaction being completed the reaction mixture was concentrated and left to cool down. The precipitated solid was then filtered off and crystalized from the suitable solvent. $C_{13}H_{15}N_5S$, Faint creamy crystals (methanol); m.p. (115$^o$C);

yield (55 %); IR (KBr ν $cm^{-1}$) 3382, 3289, 2918, 2852, 2252, 1557; $^1$H NMR ($CDCl_3$) $\delta$ ppm: 1.7 (s, 1H, NH), 1.9 (m, 4H, $2CH_{2cyclohexane}$), 2.7 (m, 4H, $CH_{2cyclohexane}$ and 1H, NH), 3.0 (m, 2H, N-$C\underline{H}_{2aliphatic.}$), 3.3 (m ,2H, $CH_2CN$), 9.1 (s ,$1H_{pyrimidine}$); $^{13}$C NMR ($CDCl_3$): 14.7, 22.1, 23.0, 25.4, 25.5, 51.4, 120.1, 129.0, 135.1, 138.8, 149.3, 153.6, 165.0; Anal. Calcd: C, 57.12; H, 5.53; N, 25.62; Anal. Found: C, 57.02; H, 5.33; N, 25.54.

**Formation 3-(3-amino-2-(5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)-2,5-dihydro-1H-pyrazol-1-yl)propanenitrile (14):**

A mixture of compound **1** (1.1 g; 0.005 mole) and acrylonitrile (0.265 mL, 0.005 mole) was fused at $180^0$C in an oil bath for 4 h. The reaction mixture was then cooled, triturated with methanol to give white solid that was crystallized from ethanol. $C_{16}H_{18}N_6S$: White crystals (ethanol); m.p. (140$^o$C); yield (10 %); IR (KBr ν $cm^{-1}$) 3378, 3029, 2944, 2246, 1553; $^1$H NMR ($CDCl_3$) $\delta$ ppm: 1.9 (m, 4H, $2CH_{2cyclohexane}$), 2.6 (m, 4H, $2CH_{2cyclohexane}$), 2.7 (m, 2H, $CH_{2pyrazol}$), 2.8 (m, 2H, N-$C\underline{H}_{2aliphatic}$), 2.9 (m, 2H, $CH_2CN$), 3.4-3.6 (bro, 3H, 1H =CH, 2H $NH_2$, $D_2O$ exchangeable), 8.4 (s,$1H_{pyrimidine}$); $^{13}$C NMR ($CDCl_3$): 17.1, 22.4, 22.8, 25.2, 25.5, 51.8 ,77.4, 115.6, 119.3, 125.1, 131.4, 134.8, 141.3, 152.0, 157.1, 166.1; Anal. Calcd.: C, 58.87; H, 5.56; N, 25.75; Anal. Found: C, 58.56; H, 5.48; N, 25.66.

**Formation of 3-(2-hydroxyphenyl)-2-((5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d] pyrimidin-4-yl)amino)isothiazolidin-4-one (15):**

A mixture of compound **1** (1.1g; 0.005 mole), salicylaldehyde (0.53g; 0.005 mole), thioglycolic acid (0.46 gm, 0.005 mole) in acetic acid was refluxed for 3 h. After the reaction completion, the mixture was poured over ice- water mixture and the solid product was collected by filtration, dried, and crystallized from ethanol. $C_{19}H_{18}N_4O_2S_2$: buff crystals (ethanol), m.p.

(234$^{o}$C); yield (80%); IR (KBr ν cm$^{-1}$) 3184 ,3067, 2939, 1650; $^{1}$H NMR (DMSO-$d_6$) $\delta$ ppm: 1.8 (m, 4H, 2$CH_{2cyclohexane}$), 2.6 (m, 2H, $CH_{2cyclohexane}$), 2.9 (m, 2H, $CH_{2cyclohexane}$), 3.6 (m, 2H, $CH_2S$), 5.2 (s, 1H, -CO-CH-N), 6.8-7.3 (m, 4$H_{aromatic}$),, 9.6 (s, 1$H_{pyrimidine}$), 10.4 (s, 1H, NH, $D_2O$ exchangeable), 11.6 (s ,1H, OH, $D_2O$ exchangeable); $^{13}$C NMR (DMSO): 22.1, 23.0, 25.4, 25.6, 54.9, 87.5, 110.6, 111.8, 116.5, 120.1, , 127.5, 128.7, 130.2, 136.8, 144.9, 149.6, 159.7, 162.4, 206.5; MS *m/z*: 400.07 (M+2 8.77 %), 398.97 (M+ 4.93 %), 350.15 (65.85 %), 312.08 (100 %); Anal. Calcd: C, 57.27; H, 4.55; N, 14.06; Anal. Found: C, 57.16; H, 4.43; N, 13.96.

**Formation of 8,9,10,11-tetrahydrobenzo[4,5]thieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine-3-thiol (16):**

Ether excess carbon disulphide was added to a solution of compound **1** (1.1 g; 0.005 mole) in pyridine or 0.005 mole of phenyl or α-naphthylisothiocyanate was added to a solution of **1** (1.1 g; 0.005 mole) ethanol 20 mL and boiled under reflux on water bath for 2 h, where a precipitated solid was formed. After the reaction being completed, the formed solid was collected by filtration and crystalized from ethanol. $C_{11}H_{10}N_4S_2$, yellow crystals (Ethanol); m.p. (246 $^{0}$C); yield (60 %); IR (KBr ν cm$^{-1}$) 3080, 3060, 2932, 2860; $^{1}$H NMR ($CDCl_3$) $\delta$ ppm, 1.8 (m, 4 H, 2$CH_{2cyclohexane}$), 2.8 (m,2H, $CH_{2cyclohexane}$), 3.1 (m, 2 H, $CH_{2cyclohexane}$), 8.8 (s, 1$H_{Pyrimidine}$), 12.8 (s ,1H, SH); $^{13}$C NMR (DMSO-$d_6$), 21.9, 22.1, 25.0, 25.1, 124.6, 129.8, 131.8, 137.5, 148.7, 154.9; Anal. Calcd: C, 50.36; H, 3.84; N, 21.36; Anal. Found: C, 50.20; H, 3.66; N, 21.19.

**N-phenyl-2-(5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidin-4-yl)hydrazine-1-carboxamide (17):**

To a solution compound **1** (1.1 g; 0.005 mole) in 30 mL ethanol was added (0.005 mole) of phenyl isocyanate and the reaction mixture was refluxed for 4 h. When the reaction was

completed, the solid product was filtered off and crystallized from DMF. $C_{17}H_{17}N_5OS$: White crystals (DMF); m.p. (218$^{o}$C); yield (60 %); IR ( KBr ν $cm^{-1}$) 3332, 3272 (NH), 2929, 1653; $^{1}$H NMR (DMSO-$d_6$) $\delta$ ppm: 1.9 (m, 4H, $2CH_{2cyclohexane}$), 2.7 (m, 2H, $CH_{2cyclohexane}$), 3.0 (m, 2H, $CH_{2cyclohexane}$), 6.5 - 7.5 (m, $5H_{aromatic}$), 8.0 (s, 1H, NH, $D_2O$ exchangeable), 8.5 (s ,$1H_{pyrimidine}$), 8.9 (s, 1H, NH, $D_2O$ exchangeable), 12.4 (s, 1H ,NH, $D_2O$ exchangeable); $^{13}$C NMR (DMSO-$d_6$), 22.1, 22.3, 24.4, 24.5, 113.8, 121.7, 128.7, 128.8, 135.0, 138.2, 139.6, 148.5, 150.1, 152.3, 156.0, 157.7, 165.0. Anal. Calcd.: C, 60.16; H, 5.05; N, 20.63; Anal. Found: C, 60.06; H, 4.95; N, 20.43.

### 3.2. Biology

#### 3.2.1 Antiproliferative (cytotoxic) activity (MTT assay) of Thienopyrimidine Derivatives

The cytotoxic activity of thienopyrimidine derivatives against the growth of human cancer epithelial cell lines; liver (HepG2) and breast (MCF7), in addition to normal fibroblasts (WI-38) was estimated using the MTT assay as described elsewhere [43]. This assay is based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. Cell lines were cultured in RPMI-1640 medium with 10% fetal bovine serum. Penicillin (100 units/ml) and streptomycin (100µg/ml) were added and cells were incubated at 37 °C in a 5% $Co_2$ incubator. The cells were seeded in a 96-well plate at a density of $10^4$ cells/well at 37 °C for 48 h under 5% $Co_2$. After incubation the cells were treated with different concentration of compounds (1-100 µM) in DMSO and incubated for 24 h. After 24 h of treatment, 20 µl of MTT solution (5mg/ml) was added and incubated for 4 h. The formazan formed in every well was dissolved in DMSO (100 µL) and the optical density was recorded at 570 nm using a plate reader (ELX 800, USA). Control untreated cells in addition to doxorubicin treated cells were performed for comparison. The data are expressed as mean ± SEM for two independent experiments

### 3.2.2 Assessment of the expression of p53, cdk1, caspase-3, and topo II by qRT-PCR

The liver HepG2 cells were treated with the new derivatives at the $IC_{50}$ concentrations reported in Table **1** for 8 hrs. The derivatives were dissolved in DMSO. Total RNA was isolated from liver cells using TRIzol reagent from Sigma (St. Louis, MO, USA). Specific primers for p53, cdk1, caspase-3, topo II and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a house-keeping gene (Table **4**) were used in reverse transcription (RT) and q-PCR. RT and q-PCR were executed using Maxima SYBR Green qPCR Master Mix (Bioline, London, UK) as described before [44]. Every sample (n=4) was analyzed in triplicate. The $^{\Delta\Delta}$Ct (cycle threshold, Ct) method was used to determine the differences in gene expression between groups [11]. The changes were normalized against GAPDH of the same sample and expressed as fold change compared with the control untreated cells.

**Table 4: Sequences of primers used for the Real-time PCR analysis**

| Gene | Forward primer | Reverse primer |
|---|---|---|
| p53 | CTGCCCTCAACAAGATGTTTTG | CTATCTGAGCAGCGCTCATGG |
| Cdk1 | CCGAAATCTGCCAGTTTGAT | CTGGCCAGTTCATGGATTCT |
| Caspase-3 | TTAATAAAGGTATCCATGGAGAACACT | TAGTGATAAAAATAGAGTTCTTTTGTGAG |
| Topo II | TGCCTGTTTAGTCGCTTTC | TGAGGTGGTCTTAAGAAT |
| GAPDH | TCAAGAAGGTGGTGAAGCAG | AGGTGGAAGAATGGGAGTTG |

### 3.2.3 Molecular modeling

The crystal structure of DNA-Topo II was retrieved from Protein Data Bank [http://www.rscb.org./pdb; code: 3QX3, resolution 2.16 Å] and considered as target for docking simulation. The docking analysis was performed using MOE software to evaluate the free energies and binding mode of the designed molecules against topoisomerase II. At first, water molecules were deleted from the complex, the protein structures were protonated, and the hydrogen atoms were hidden. Then, the energy was minimized, and the binding pockets of the protein were defined. The 2D structures of the synthesized compounds and doxorubicin were sketched using ChemBioDraw Ultra 16.0 and saved as MOL format. Then, the saved files were opened using MOE and 3D structures were protonated. Next, energy minimization was applied. Before docking the synthesized compounds, validation of the docking protocol was carried out by running the simulation only using the co-crystallized ligands and low RMSD between docked and crystal conformations. The molecular docking of the synthesized compounds and the co-crystallized ligand was performed using a default protocol. In each case, 50 docked structures were generated using genetic algorithm searches. Finally, the most ideal pose was selected according to the minimum free energy of the DNA-Topo II ligand interactions.

### 3.2.4 Inhibitory activity of novel derivatives on topoisomerase II (Topo II)

The inhibitory effect of novel thienopyrimidine derivatives on Topo II was determined using Mybiosource (San Diego, CA, USA) based on sandwich ELISA method. The optical density was recorded at 450 and 570 nm.

### 3.2.5 Measurement of the active cleaved caspase 3 activity

The active cleaved caspase-3 activity was determined in HepG2 cells after an incubation with the $IC_{50}$ concentration of the investigated compounds for 12h according to the method of Hasegawa *et al*., [45] using a colorimetric assay kit provided from Abcam (UK).

## Conflict of interest

The authors declare no conflict of interest.

## References


1. D. J. Brown, Pyrimidines; Their Benzo Derivatives, in Comprehensive Heterocyclic Chemistry (Eds. A. R. Katritzky and C. W. Rees), Pergamon Press, Oxford 1984, Vol. 3, p. 443.
2. B. Roth, C. Cheng; Progress in Medicinal chemistry (Eds. G. P. Ellis and G. B. West), Elsevier Biomedical Press, New York 1982, Vol. 19, p. 267.
3. C. R. Petrie, H. B. Cottam, P. A. Mckernan, R. K. Robins, G. R. Revankar; Synthesis and biological activity of 6-azacadeguomycin and certain 2,4,6-trisubstituted pyrazolo3,4-dpyrimidine ribonucleosides, J. Med. Chem. 28 (1985) 1010–1016.
4. M. S. A. E.-A. El-Gaby, S. G. Abdel-Hamide, M. M. Ghorab, S. M. El-Sayed; Synthesis and anticancer activity in vitro of some new pyrimidines, Acta Pharm. 49 (1999) 149–158.
5. M. N. Nasr, M. M. Gineinah; Pyrido2,3-dpyrimidines and pyrimido5',4':5,6pyrido2,3-dpyrimidines as new antiviral agents: Synthesis and biological activity, Arch. Pharm. 335 (2002) 289–295.
6. P. G. Baraldi, M. G. Pavani, M. Nunez, P. Brigidi, B. Vitali, R. Gambari and R. Romagnoli; Antimicrobial and antitumor activity of N-heteroimine-1,2,3-dithiazoles and their

transformation in triazolo-, imidazo- and pyrazolopyrimidines, Bioorg. Med. Chem. 10 (2002) 449–456.

7. S. M. Sondhi, M. Johar, S. Rajvanshi, S. G. Dastidar, R. Shukla, R. Raghubir and J. W. Lown; Anticancer, anti-inflammatory and analgesic activity evaluation of heterocyclic compounds synthesized by the reaction of 4-isothiocyanato-4-methylpentan-2-one with substituted o-phenylenediamines, o-diaminopyridine and (un)substituted o-diaminopyrimidines, Australian J. Chem. 54 (2001) 69–74.
8. A. Z. M. S. Chowdhury, M. M. Matin, M. N. Anwar; Synthesis and antimicrobial activities of fused pyrimidines: Benzothieno2,3-dimidazo1,2-cpyrimidine, Chittagong Univ. Stud. Part II: Sci. 21 (1997) 79–83; ref. Chem. Abstr. 130 (1999) 237530p.
9. G. Mangalagiu, M. Ungureanu, G. Grosu, I. Mangalagiu, M. Petrovanu; New pyrrolo-pyrimidine derivatives with antifungal or antibacterial properties, Ann. Pharm. Fr. 59 (2001) 139– 140.
10. G. P. Ellis; Synthesis of Fused Heterocycles, The Chemistry of Heterocyclic Compounds (Ed. E. C. Taylor), Wiley, New York 1987, Vol. 47, p. 226.
11. S. El-Metwally, A. Khalil, A. M. El-Naggar, W. El-Sayed; Novel Tetrahydrobenzo [b] Thiophene Compounds Exhibit Anticancer Activity through Enhancing Apoptosis and Inhibiting Tyrosine Kinase. Anti-Cancer Agents in Medicinal Chemistry, 18(12), (2018), 1761-1769.
12. S. Bugge, A. F. Buene, N. Jurisch-Yaksi, I. U. Moen, E. M. Skjønsfjell, B. H. Hoff; Extended structure–activity study of thienopyrimidine-based EGFR inhibitors with evaluation of druglike properties. Eur. J. Med. Chem., 107, (2016), 255-274.

13. A. Gryshchenko, V. Bdzhola, A. Balanda, N. Briukhovetska, I. Kotey, A. Golub, T. Ruban, L. Lukash, S. Yarmoluk; Design, synthesis and biological evaluation of N-phenylthieno [2, 3-d] pyrimidin-4-amines as inhibitors of FGFR1. Bioor. Med. Chem., 2015, 23(9), 2287-2293.

14. S. Pédeboscq, D. Gravier, F. Casadebaig, G. Hou, A. Gissot, C. Rey, F. Ichas, F. De Giorgi, L. Lartigue, J.P. Pometan; Synthesis and evaluation of apoptosis induction of thienopyrimidine compounds on KRAS and BRAF mutated colorectal cancer cell lines. Bioor. Med. Chem., 2012, 20(22), 6724-6731.

15. C.H. Park, C. Lee, J.S. Yang, B.Y. Joe, K .Chun, H. Kim, H.Y. Kim, J.S. Kang, J.I. Lee, M.H. Kim; Discovery of thienopyrimidine-based FLT3 inhibitors from the structural modification of known IKKβ inhibitors. Bioor. Med. Chem. Lett., 2014, 24(12), 2655-2660.

16. D. P. Sutherlin, D. Sampath, M. Berry, G. Castanedo, Z. Chang, I. Chuckowree, J. Dotson, A. Folkes, L. Friedman, R. Goldsmith; Discovery of (thienopyrimidin-2-yl) aminopyrimidines as potent, selective, and orally available pan-PI3-kinase and dual pan-PI3-kinase/mTOR inhibitors for the treatment of cancer. J. Med. Chem., 2010, 53(3), 1086-1097.

17. [A] M. D. Bhuiyan, K. M. Rahman, M. D. Hossain, A. Rahim, M. Hossain, M. Abu Naser; Synthesis and antimicrobial evaluation of some new thienopyrimidine derivatives; Acta Pharm. 56 (2006) 441–450.

18. Y. Pommier, E. Leo, H. Zhang, C. Marchand; DNA topoisomerases and their poisoning by anticancer and antibacterial drugs. Chem. Bio., 2010, 17, 421-433.

19. Liang X, Wu Q, Luan S, Yin Z, He C, Yin L, Zou Y, Yuan Z, Li L, Song X, He M, Lv C, Zhang W. A comprehensive review of topoisomerase inhibitors as anticancer agents in the past decade. Eur J Med Chem. 2019;171:129-168.
20. Hevener K, Verstak TA, Lutat KE, Riggsbee DL, Mooney JW. Recent developments in topoisomerase-targeted cancer chemotherapy. Acta Pharm Sin B. 2018;8(6):844-861.
21. Sordet O, Khan QA, Kohn KW, Pommier Y. Apoptosis induced by topoisomerase inhibitors. Curr Med Chem Anticancer Agents. 2003;3(4):271-90.
22. El-Naggar AM, Khalil AK, Zeidan HM, El-Sayed WM. Eco-friendly synthesis of pyrido[2,3-d]pyrimidine analogs and their anticancer and tyrosine kinase inhibition activities. Anti-Cancer Agents Med Chem, 17(12):1644-1651, 2017.
23. Ismail MA, Youssef MM, Arafa RK, Al-Shihry SS, El-Sayed WM. Synthesis and antiproliferative activity of monocationic arylthiophene derivatives. Eur J Med Chem, 126, 789-798, 2017.
24. Ismail MA, Negm A, Arafa RK, Abdel-Latif E, El-Sayed WM. Anticancer activity, dual prooxidant/antioxidant effect and apoptosis induction profile of new bichalcophene-5-carboxamidines. European Journal of Medicinal Chemistry, 169:76-88, 2019.
25. Elmetwally SA, Saied KF, Eissa IH, Elkaeed EB. Design, synthesis and anticancer evaluation of thieno[2,3-d]pyrimidine derivatives as dual EGFR/HER2 inhibitors and apoptosis inducers. Bioorg Chem. 2019;88:102944.
26. J. Herrero-Ruiz, Mar Mora-Santos, S. Giráldez, C. Sáez, M. Á. Japón, M. Tortolero, F. Romero. βTrCP controls the lysosome-mediated degradation of CDK1, whose accumulation correlates with tumor malignancy, Oncotarget., 5 (2014) 7563-7574.

27. Haider C, Grubinger M, Řezníčková E, Weiss TS, Rotheneder H, Miklos W, Berger W, Jorda R, Zatloukal M, Gucky T, Strnad M, Kryštof V, Mikulits W. Novel inhibitors of cyclin-dependent kinases combat hepatocellular carcinoma without inducing chemoresistance. Mol Cancer Ther. 2013;12(10):1947-57.

28. J. M. Enserink, R. D. Kolodner. An overview of Cdk1-controlled targets and processes, Cell Division., 5 (2010) 1-41

29. T. Ozaki, A. Nakagawara, Role of p53 in Cell Death and Human Cancers, Cancers (Basel)., 3 (2011) 994–1013.

30. E. Senturk, J. J. Manfredi, p53 and cell cycle effects after DNA damage, Methods Mol Biol., 962 (2013) 49–61.

31. Y. Sun, P. Xia, H. Zhang, B. Liu, Y. Shi. P53 is required for Doxorubicin-induced apoptosis via the TGF-beta signaling pathway in osteosarcoma-derived cells. Am J Cancer Res., 6 (2016) 114–125.

32. M. Olsson, B. Zhivotovsky, Caspases and cancer, Cell Death Differ., 18 (2011) 1441-1449.

33. Putt KS, Chen GW, Pearson JM, Sandhorst JS, Hoagland MS, Kwon JT, Hwang SK, Jin H, Churchwell MI, Cho HH, Doerge DR, Helferich WG, Hergenrother PJ. Small-Molecule Activation of Procaspase-3 to Caspase-3 as a Personalized Anticancer Strategy. Nat Chem Biol. 2006; 2:543–550.

34. Soussi T. and Beroud C. Assessing TP53 status in human tumours to evaluate clinical outcome. Nat Rev Cancer 2001;1:233–240.

35. Sharifi S., Barar J., Hejazi M. S., Samadi N.,. Doxorubicin Changes Bax /Bcl-xL Ratio, Caspase-8 and 9 in Breast Cancer Cells. Adv. Pharm. Bull. 2015;5(3):351–359.

36. J. L. Nitiss, DNA topoisomerase II and its growing repertoire of biological functions, Nature Reviews Cancer., 9 (2009) 327–337.

37. E. Willmore, S. de Caux, Sunter N. J. et al, A novel DNA-dependent protein kinase inhibitor, NU7026, potentiates the cytotoxicity of topoisomerase II poisons used in the treatment of leukemia, Blood., 103 (2004) (12) 4659–65.

38. R.P. Hertzberg, M.J. caranfa, S.M. Hecht, On the mechanism of topoisomerase I inhibition by camptothecin: evidence for binding to an enzyme-DNA complex, Biochemistry28 (1989) 4629-4638.

39. M.E. Wall; M.C.Wani; C.E. Cook; K.H.Palmer; A.I.McPhail; G.A.Sim (1966). "Plant antitumor agents. I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from camptotheca acuminate". J. Am. Chem. Soc. 88 (16): 3888–3890.

40. Seiden, MV; Muggia, F; Astrow, A; Matulonis, U; Campos, S; Roche, M; Sivret, J; Rusk, J; Barrett, E (April 2004). "A Phase II Study of Liposomal Lurtotecan (OSI-211) in Patients with Topotecan Resistant Ovarian Cancer". Gynecologic Oncology. 93 (1): 229–32.

41. Lu LY, Kuang H, Korakavi G, Yu X. Topoisomerase II regulates the maintenance of DNA methylation. J Biol Chem. 2015;290:851–60.

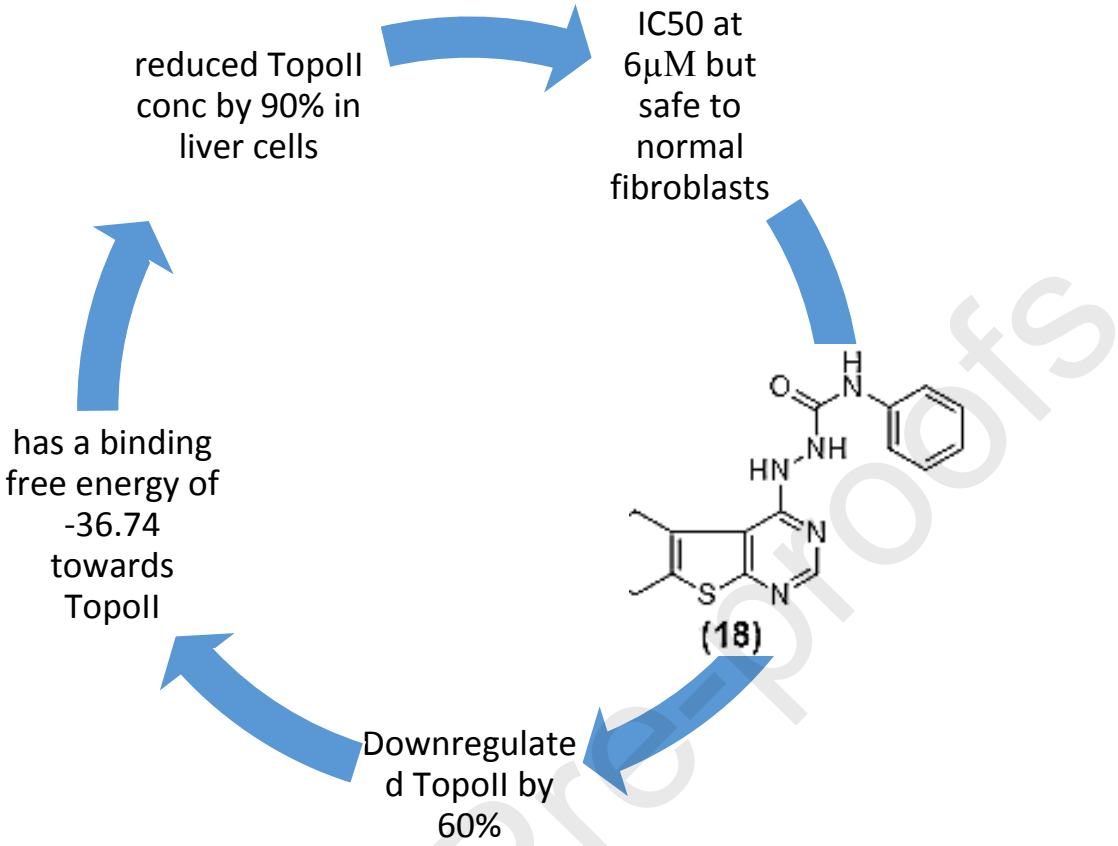
42. Congras A, Caillet N, Torossian N, Quelen C, Daugrois C, Brousset P, Lamant L, Meggetto F, Hoareau-Aveilla C.Doxorubicin-
induced loss of DNA topoisomerase II and DNMT1- dependent suppression of MiR-125b induces chemoresistance in ALK-positive cells.

43. F. Denizot, R. Lang. Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J. Immunol. Methods 89 (1986) 271-277.

44. R.A. Hussein, E.A. El-Husseiny, L.A. Hassanin, W.M. El-Sayed, The Prophylactic and Therapeutic effects of Safranal and Selenite on Liver Damage Induced by Thyrotoxicosis in Adult Male Albino Rats, Int. J. Clin. Pharmacol. Toxicol, 6 (2017) 270-279.

45. S. Kamada, M. Washida, J.I. Hasegawa, H. Kusano, Y. Funahashi, Y.Tsujimoto. Involvement of caspase-4(-like) protease in Fas-mediated apoptotic pathway. Oncogene 15(1997):285–90.

- Thienopyrimidines **5, 15, 17,** and **18** $IC_{50}$ ~ 4-7 μM against liver and breast cells
- They upregulated p53 and caspase 3 (induce apoptosis)
- Derivative **18** reduced the expression of topo II by ~60%
- Derivatives **5** and **18** with high binding energies could bind and inhibit Topo II
- Derivatives **5** and **18** reduced the Topo II conc. by >80%



**Declaration of interests**

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

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

**Soad El-Metwally**

**Ali Khalil**

**Wael El-Saved**