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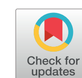
# One-pot synthesis of thiazolo[3,2-*a*]pyrimidine derivatives, their cytotoxic evaluation and molecular docking studies



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ABSTRACT

An economical, simple and efficient one-pot method has been developed for the synthesis of thiazolo[3,2-*a*]pyrimidine hydrobromide derivatives. 2,4-diaryl-6,7,8,9-tetrahydro-4*H*-benzo[4,5]thiazolo[3,2-*a*]pyrimidine hydrobromides were synthesized by the α-bromination of cyclohexanone with N-Bromosuccinamide (NBS) and followed by cyclization with 3,4-dihydropyrimidine-2(1*H*)-thiones, respectively, in the presence of p-toluenesulfonic acid (PTSA) in acetonitrile. However when cyclohexanone was replaced by acetyl acetone and alpha-tetralone gave the corresponding 1-(3-methyl-5,7-diaryl-5*H*-thiazolo[3,2-*a*]pyrimidin-2-yl)ethan-1-one hydrobromide and 9,11-diaryl-6,11-dihydro-5*H*-naphtho[1′,2′:4,5]thiazolo[3,2-*a*]pyrimidine hydrobromide derivatives, respectively. The significant features of this method are novel, simple, inexpensive experimental procedure, short reaction time, and good yield. The some of the synthesized compounds were evaluated for cytotoxic activity against human lung adenocarcinoma cell line (A549), human breast carcinoma cell line (MCF-7), human cervical cancer cell line (HeLa) and human neuronal carcinoma cell lines (SKNSH). Tested compounds **5(b-e)** showed the excellent anticancer activity against various cell lines. Particularly compound **5c** with $IC_{50}$ value of 2.2 ± 0.6 μM against A549 and compound **5e** with $IC_{50}$ value of 5.6 ± 0.4 μM against HeLa showed best cytotoxic effects. Furthermore, Molecular docking study was performed for some of the synthesized compounds **5(b-e)** against topoisomerase-II by using Auto dock method. Docking results of the compounds **5c**, **5d**, and **5e** exhibited higher cytotoxic activity than the standard doxorubicin.

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## 1. Introduction

Cancer is the most dangerous disease in which abnormal cells divide uncontrollably. This can result in tumors, damage to immune system, and destroy the body tissues [1]. Lots of people lost their lives due to cancer [2]. Present treatments for cancer handling are not up to mark due to comparatively major side effects [3]. Despite of various anticancer drugs available, the development of new anticancer agents for the treatment of cancer without side effects is a significant goal for scientists [4]. Heterocyclic compounds have attracted the attention of scientist over the years due to their interesting medicinal properties [5]. As several enzyme binding pockets are subjected to interacting with heterocycles. Heterocycles are a best choice when designing molecules that will interact with targets and disrupt the biological pathways associated with cancer evaluation [6]. Pathways related to cell growth and development often targeted by such anti-cancer treatments. Moreover, the which relative ease by which heterocyclic rings can be changed with additional functional group substituents allows them to cover a broad area of chemical space, further qualifying them as best initial points for anticancer drug developments [7]. The presence of heterocycle ring in all kind of organic compounds of interest in biology, optics, electronics, pharmacology and material science and so on is very well known [8]. Heterocyclic molecules show exciting medicinal properties including anticancer [9]. Sulfur and nitrogen containing heterocyclic compounds have continued the interest of scientists over the years of historical development and these heterocycles proven to be important in the process of drug discovery [10].

Thiazolopyrimidines are an interesting group of heterocyclic compounds. The compounds having thiazolo[3,2-*a*]pyrimidine moiety shows interesting a variety of medicinal properties such as anticancer [11], antimicrobial [12], anti-inflammatory [13], antioxidant [14], anti-human cytomegalovirus activity [15], antiparkinsonian [16], antitumor and antiviral [17,18]. Hence the synthesis of various thiazolo[3,2-*a*]pyrimidines derivatives is of great importance. Based on the reported literature around the importance of thiazolopyrimidines in medicinal

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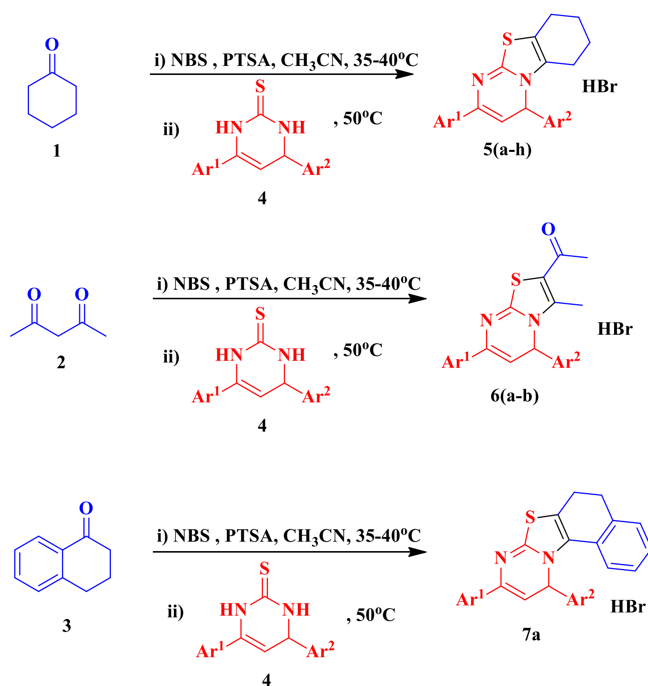


**Scheme 1.** Synthesis of thiazolo[3,2-*a*]pyrimidine derivatives.

chemistry, we reported here in synthesis of some thiazolopyrimidines and tested their anticancer activity against human lung adenocarcinoma cell line (A549), human breast carcinoma cell line (MCF-7), human cervical cancer cell line (HeLa) and human neuronal carcinoma cell lines (SKNSH).

Environmental viability, safety, economical sustainable and efficiency are significant issues in organic chemistry [19]. All need to be addressed carefully when making sensitive and valuable compounds over multi steps. In recent days, developed one-pot synthesis for produce elaborate biologically active molecules has become a significant area of medicinal research in organic and combinatorial chemistry [20]. One-pot synthesis is in which two or more steps into one-pot operation to form desired product without isolation of any intermediate. The applications of one-pot synthesis are minimize effluent, environmentally safe, economically viable, operational simplicity and short reaction time [21]. In fact; one-pot synthesis has been used extensively in synthetic organic chemistry for a long time. Robinson's one-pot synthesis of tropinone was reported 100 years ago, it is a landmark achievement in organic chemistry [22].

There are several methods are reported for the preparation of thiazolo[3,2-*a*]pyrimidines [23–28]. In this paper, as part of our current research on developing efficient and simple synthesis of biologically potential products, herein we reported a commercially viable and convenient novel one-pot protocol for the synthesis of thiazolo[3,2-*a*]pyrimidine hydrobromide derivatives.2,4-diaryl-6,7,8,9-tetrahydro-4*H*-benzo[4,5]thiazolo[3,2-*a*]pyrimidine hydrobromides **5(a-h)** were synthesized by the α-bromination of cyclohexanone with N-Bromosuccinamide (NBS) and followed by cyclization with 3,4-dihydropyrimidine-2(1*H*)-thiones (thiopyrimidines) **4**, respectively, in the presence of PTSA in acetonitrile. However when cyclohexanone was replaced by acetyl acetone and alpha-tetralone gave the corresponding 1-(3-methyl-5,7-diaryl-5*H*-thiazolo[3,2-*a*]pyrimidin-2-yl) ethan-1-one hydrobromide **6(a-b)** and 9,11-diaryl-6,11-dihydro-5*H*-naphtho[1′,2′:4,5]thiazolo[3,2-*a*]pyrimidine hydrobromide **7a** derivatives, respectively (Scheme 1). The significant features of this method are simple, inexpensive experimental procedure, short reaction time, and good yield. More number of naphthalene substituted thiazolopyrimidine derivatives has been prepared with reason of naphthalene substituted heterocyclic molecules are reported with variety of medicinal properties [29–31].

## 2. Results & discussion

Cyclohexanone **1** was allowed to react with N-bromosuccinamide (NBS) in acetonitrile in the presence of PTSA at 35-40 °C to afford alpha-bromocyclohexanone, then in one-pot way without isolation of this intermediate (alpha-bromocyclohexanone) undergo cyclization with thiopyrimidines **4** at 50 °C to afford thiazolopyrimidine hydrobromides **5** with good yields. However when cyclohexanone was replaced by acetyl acetone **2** and alpha-tetralone **3** gave the corresponding thiazolopyrimidine hydrobromide **6** and **7** derivatives, respectively.

In order to determine the most appropriate reaction conditions, comparative studies has been made with different type of halogenating agents, solvents and temperatures. Initially we focused our efforts on optimizing the suitable reaction condition by conducting a model reaction using the cyclohexanone **1** (10 mmol), thiopyrimidine **4b** (10 mmol), NBS as halogenating agent in the presence of catalytic amount of PTSA in DMF as solvent (Table 1).The bromination of cyclohexanone **1** was carried out with 15 mmol of NBS in DMF in the presence of PTSA (5 mmol). It is followed by cyclization with thiopyrimidine **4b** at 50 °C, resulted the desired product **5b** with 34% yield. To increase the product yield, the reaction was carried out with 10 mmol of NBS, the product **5b** was increased to 51%. Thereafter, different brominating reagents like $Br_2$, $CuBr_2$ were investigated. However, it was observed that the reaction carried out with these reagents under similar conditions did not give much satisfactory results and the best yields were obtained with NBS as the brominating agent. Later the reaction was also observed with different solvents such as acetonitrile,

**Table 1**
Optimization of the reaction conditions for the synthesis of thiazolo[3,2-*a*]pyrimidines **5(a-h), 6(a-b), 7a.**

| Entry | Reagent (mmol) | PTSA(mmol) | Solvent | Temp °C | Time (h) | Yield[a] (%) |
|---|---|---|---|---|---|---|
| 1 | NBS (15) | PTSA (5) | DMF | 50 | 2 | 34 |
| 2 | NBS (10) | PTSA (5) | DMF | 50 | 2 | 51 |
| 3 | $Br_2$(10) | PTSA (5) | DMF | 50 | 2 | 32 |
| 4 | $CuBr_2$(10) | PTSA (5) | DMF | 50 | 2 | Trace |
| 5 | NBS (10) | PTSA (5) | DCM | 50 | 2 | 34 |
| 6 | NBS (10) | PTSA (5) | EtOH | 50 | 2 | 65 |
| 7 | NBS (10) | PTSA (5) | THF | 50 | 2 | 27 |
| 8 | NBS (10) | PTSA (5) | Acetonitrile | 50 | 2 | 82 |
| 9 | NBS (10) | PTSA (5) | Acetonitrile | 35 | 2 | 45 |
| 10 | NBS (15) | PTSA (5) | Acetonitrile | 50 | 2 | 61 |
| 11 | NBS (10) | PTSA (2.5) | Acetonitrile | 50 | 2 | 69 |

Optimal condition: Ketone (Cyclohexanone **1**) (10 mmol), PTSA (5 mmol), N-Bromosuccinamide (10 mmol) in 30 ml Acetonitrile at 35-40 °C for 30 min, then Thiopyrimidine **4b** (10 mmol) at 50 °C for 2 h.

[a] Isolated yields.

**Table 2**
Synthesis of thiazolo[3,2-*a*]pyrimidines derivatives.[a]

| Entry | Ketone | Ar[1] | Ar[2] | Product | Yield[b] |
|---|---|---|---|---|---|
| 1 | Cyclohexanone | 2-Naphthyl | Phenyl | 5a | 84 |
| 2 | Cyclohexanone | Phenyl | Phenyl | 5b | 82 |
| 3 | Cyclohexanone | 2-Naphthyl | 3,4,5-Trimethoxyphenyl | 5c | 87 |
| 4 | Cyclohexanone | 2-Naphthyl | 2,6-Dichlorophenyl | 5d | 92 |
| 5 | Cyclohexanone | 2-Naphthyl | 3-Bromophenyl | 5e | 87 |
| 6 | Cyclohexanone | 2-Naphthyl | 3,4,-Dimethoxyphenyl | 5f | 84 |
| 7 | Cyclohexanone | 2-Naphthyl | 1-Naphthyl | 5g | 86 |
| 8 | Cyclohexanone | 2-Naphthyl | 4-Fluorophenyl | 5h | 82 |
| 9 | Acetyl acetone | Phenyl | Phenyl | 6a | 81 |
| 10 | Acetyl acetone | 2-Naphthyl | Phenyl | 6b | 84 |
| 11 | Alpha-tetralone | Phenyl | Phenyl | 7a | 77 |

[a] Reaction conditions (Optimal condition): Ketone (Cyclohexanone **1**/Acetyl acetone **2**/alpha-Tetralone **3**) (10 mmol), PTSA (5 mmol), N-Bromosuccinamide (10 mmol) in 30 ml Acetonitrile at 35-40 °C for 30 min, then Thiopyrimidines **4** (10 mmol) at 50 °C for 2 h.
[b] Isolated yields after purification.

dichloromethane, tetrahydrofuran and ethanol (Table 1) on the model reaction. Thus we observed that the product 5(b) (entry 8, Table 1) yield was 82% with acetonitrile compared with others solvents such as 65% with EtOH, 34% with dichloromethane, 27% with tetrahydrofuran (Table 1).

After the optimization of reaction conditions, we subsequently applied various substituted thiopyrimidines to the model reaction. It was observed that halogen substituted aryl groups and electron releasing groups of 3,4-dihydro-4,6-diarylpyrimidine-2(1*H*)-thiones **(Entry 3,** Table 2) under optimized reaction conditions gave the corresponding products in excellent yields. A high yield was reported with 4-(2,6-dichlorophenyl) of 3,4-dihydro-4,6-diarylpyrimidine-2 (1*H*)-thione **(Entry 4,** Table 2). However, similar yields were obtained with acetyl acetone under the same optimized reaction conditions. (Table 2).

The structure of the products **5(a-h), 6(a, b) 7a** were confirmed by IR, $^1$H NMR, $^{13}$C NMR and HRMS. IR spectra of **5a** showed strong absorption band at 1634 $cm^{-1}$, indicating the presence of C═N group and 1541 $cm^{-1}$ indicating the presence of C═C group. The $^1$H NMR (400 MHz in $CDCl_3$) spectrum of **5a** exhibited multiplet at 1.39–2.30 ppm for $CH_2$ protons, two singlet's at 5.47, 5.91 ppm for C—H thiazolopyrimidine protons and singlet at 12.67 ppm for N.HBr. The $^{13}$C spectra of **5a** showed characteristic peaks of C-SP$^3$ at 20.94, 21.64, 22.51, 59.79 and 160.12 ppm (C—S). IR spectra of **6a** showed prominent sharp C═O stretching in region 1620 $cm^{-1}$ and 1598 $cm^{-1}$ for C═N group, 1384 and 1120 $cm^{-1}$ for C═C, C—O groups. The $^1$H NMR (400 MHz in $CDCl_3$) spectrum of **6a** exhibited two singlet's for two $CH_3$ at 2.35 and 2.41 ppm, two singlet's at 5.49, 6.43 ppm for C—H thiazolopyrimidine protons. A multiplet at 7.22–7.67 ppm was assigned to aromatic protons. The $^{13}$C spectra of **6a** showed characteristic peaks of C-SP$^3$ at 13.90, 30.27, 60.65 ppm, 161.57 ppm (C—S) and 188.64 ppm (C=O). IR spectra of **7a** showed strong absorption band at 1628 $cm^{-1}$, indicating the presence of C═N group. The $^1$H NMR (400 MHz in $CDCl_3$) spectrum of **7a** exhibited two singlet's at 5.63, 6.82 ppm for C—H thiazolopyrimidine protons and singlet at 13.01 ppm for N.HBr.

### 2.1. Cytotoxic evaluation

The results shown in Table 3 and Fig. 1 revealed that among the synthesized thiazolopyrimidines, some of the tested compounds **5(b-e)** showed the very good anticancer activity against various cancer cell lines. Particularly compounds **5c** showed excellent activity on A549 (**2**.2 ± 0.6) and **5e** showed on HeLa (5.6 ± 0.4). The present research results may be considered for designing novel anticancer compounds. Compound **5b** thiazolopyrimidine with only phenyl substitutions showed very less activity on cell lines. Compound **5c** thiazolopyrimidine with naphthyl and trimethoxy phenyl substitutions showed very good activity on cell lines. The above results revealed that compounds with naphthyl and methoxypheny groups as substituents on thiazolopyrimidine showed potent anticancer activity. So, the results will be more significant in developing effective and safe anticancer drugs.

Exponentially growing cells were treated with different of **5b, 5c, 5d, 5e** derivatives (S. No. 1 to 4) for 24 h and cell growth inhibition was estimated by performing MTT assay.

$IC_{50}$ is defined as the concentration, which causes 50% decrease in the cell number when compared with that of the control cultures in the absence of an inhibitor.

$IC_{50}$ values were calculated from the plotted absorbance data for the dose-response curves.

The values (in μM) represent the mean ± SD of three independent experiments. Doxorubicin was used as positive control.

### 2.2. Molecular docking

Topoisomerases are enzymes that catalyze topological alterations of DNA. Human topoisomerases II are occurred in humans two types of isoforms such as (i) topoisomerase IIα and (ii) topoisomerase IIβ. Topoisomerase IIα has two different types of DNA-independent binding pockets, which are present in the catalytic domain and N-terminal ATP-binding domain [32]. TopIIα is involved in the DNA replication during the cell cycle and it regulates chromosome segregation, chromosome

**Table 3**
$IC_{50}$ of the synthesized compounds **5(b-e)** against cancer cell lines.

| S·No | Sample code | $IC_{50}$ in μM | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | A549 | | MCF-7 | | HeLa | | SKNSH | |
| | | 24 h | 48 h | 24 h | 48 h | 24 h | 48 h | 24 h | 48 h |
| 1 | 5b | 9.6 ± 0.4 | 8.5 ± 0.2 | 18.3 ± 0.6 | 15.2 ± 0.3 | 17.3 ± 0.5 | 16.6 ± 0.6 | 15.8 ± 0.4 | 9.6 ± 0.3 |
| 2 | 5c | 5.1 ± 0.3 | 2.2 ± 0.6 | 15.6 ± 0.4 | 10.2 ± 0.3 | 8.4 ± 0.5 | 5.2 ± 0.4 | 10.8 ± 0.2 | 6.6 ± 0.3 |
| 3 | 5d | 14.3 ± 0.3 | 11.1 ± 0.4 | 17.6 ± 0.6 | 13.6 ± 0.4 | 21.4 ± 0.3 | 15.3 ± 0.7 | 14.6 ± 0.4 | 9.7 ± 0.4 |
| 4 | 5e | 16.8 ± 0.6 | 9.8 ± 0.2 | 21.6 ± 0.3 | 15.8 ± 0.6 | 18.8 ± 0.5 | 5.6 ± 0.4 | 11.3 ± 0.6 | 8.5 ± 0.3 |
| 5 | Doxorubicin | 0.8 ± 0.04 | 0.5 ± 0.03 | 1.2 ± 0.3 | 0.7 ± 0.1 | 0.4 ± 0.06 | 0.3 ± 0.01 | 1.4 ± 0.4 | 0.8 ± 0.2 |

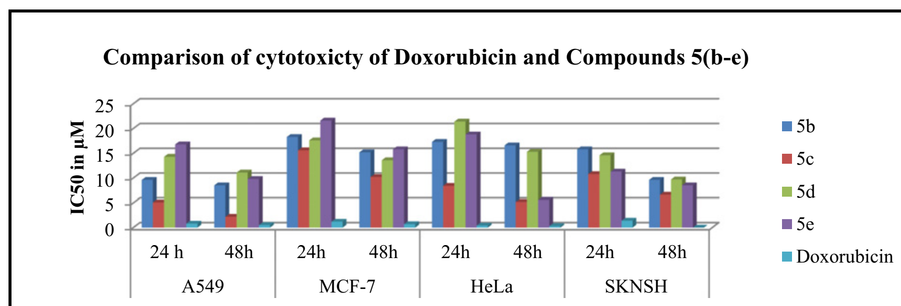


**Fig. 1.** Comparison of cytotoxicity of Doxorubicin and compounds **5(b-e)** against A549, MCF-7, HeLa, SKNSH cancer cell lines.

condensation, arrest in meiosis I and recombination suppression [33]. Topo II concentrations are dramatically up-regulated in cancer cells, it could be effects of cell proliferation [34]. Therefore, numbers of anti-cancer agents are designed with topo II as a potential target [35].

Molecular docking is a significant computer-assisted drug design method for predicting the main binding modes of a ligand with a protein of known three-dimensional structure [36]. In order to know the possible binding studies of the thiazolopyrimidines **5(b-e)**, molecular interaction studies of the active anti-cancer compounds were performed against topoiosmerase-II α enzyme, which are targets of cancer treatments. DNA topoisomerase-II has been attracted much attention among the many anticancer targets for cancer treatment and inhibitors include doxorubicin, etoposide and mitoxantrone [37]. Often the binding of the drug is reversible, but if a replication fork runs into the blocked topoisomerase, then a piece of the gapped DNA strand not bound by the topoisomerase could be released, making a permanent breakage in the DNA that leads to cell damage and induce apoptosis or other types of cell death. More number of anti-cancer drugs are being increased due to eukaryotic topoisomerases II is the target for these drugs that act to inhibit these enzymes by blocking the reaction that reseals the breaks in the DNA.

## 3. Methodology

The derivative compounds were drawn using Chemsketch 12.0 allotted with 3D orientation and analyzed for bond order error connection using the structures. Energy minimization of each compound was done by Dundee PRODRG2 server and used as input files for Autodock 4.2 to study the docking simulations [38]. Structure of Topoisomerase II alpha (1ZXM) was collected using PDB database. Heteroatoms were removed and made complex receptor free from all ligands before docking. To run and analyze docking simulations Autodock tools graphical user interface program was used. Protein was prepared by adding solvation parameters, polar hydrogen's and Kollman united atom charges in docking simulation. To the derivative compounds, Gasteiger charge was given and then non-polar hydrogen's were merged. In Autodock for each atom type in ligand requires pre-calculated grid maps to store potential energy coming from the interactions with protein and this must cover the active site region of the protein. In this study, the active site residues was selected based on the involvement in binding with phosphoaminophosphonic acid-adenylate ester and magnesium ion of Topoisomerase II alpha, obtained from PDB with ID 1ZXM which would be the best accurate active site as it is identified by experimental crystallographic data. The grid was generated surrounding active site including amino acid residues as in Fig. 2. The grid box size was set at 80, 65, and 64 Å for *x*, y and z respectively, and the grid center was set to 32.43, 16.432 and −4.21 for *x*, *y* and *z* respectively, which covered all the amino acid residues in the considered active pocket. To produce grid maps AutoDock 4.2 docking software program supplied with AutoGrid 4.0 and AutoDock 4.0 was used. The spacing between grid points was 0.375 Å. Best conformers were chosen by the Lamarckian Genetic Algorithm (LGA). During the docking studies, for each compound a maximum of 10 conformers was considered. All the AutoDock docking runs were performed in Intel Centrino Core2Duo CPU @ 2.20 GHz of IBM system origin, with 2 GB DDR2 RAM. AutoDock 4.0 was compiled and run under Microsoft Windows XP operating system.

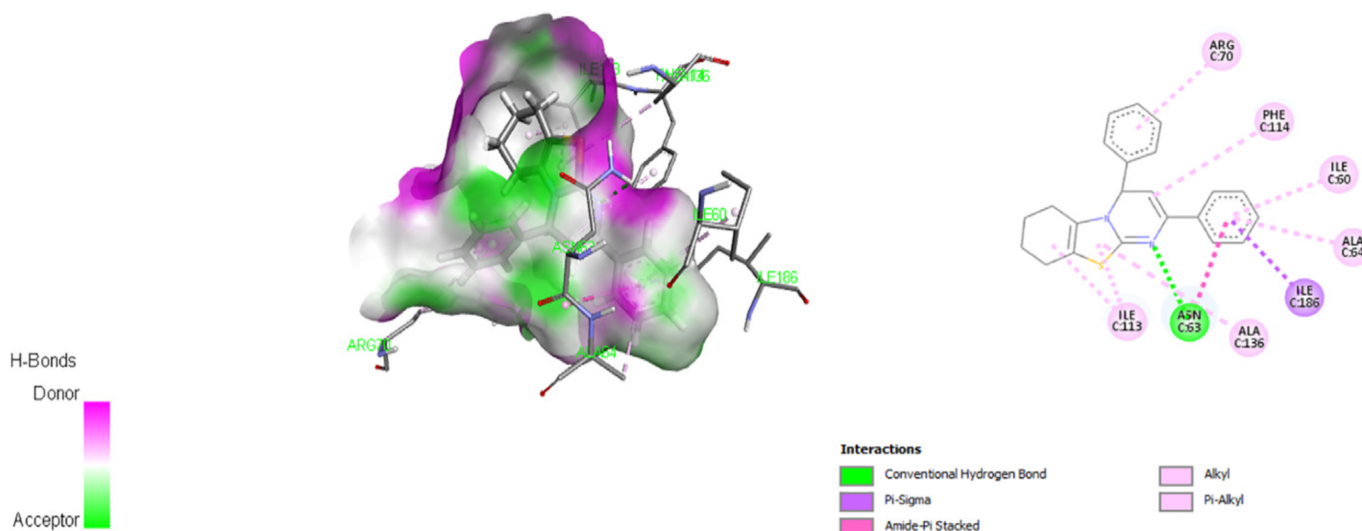


**Fig. 2.** Molecular docking of 5**b** with topoisomerase-II.

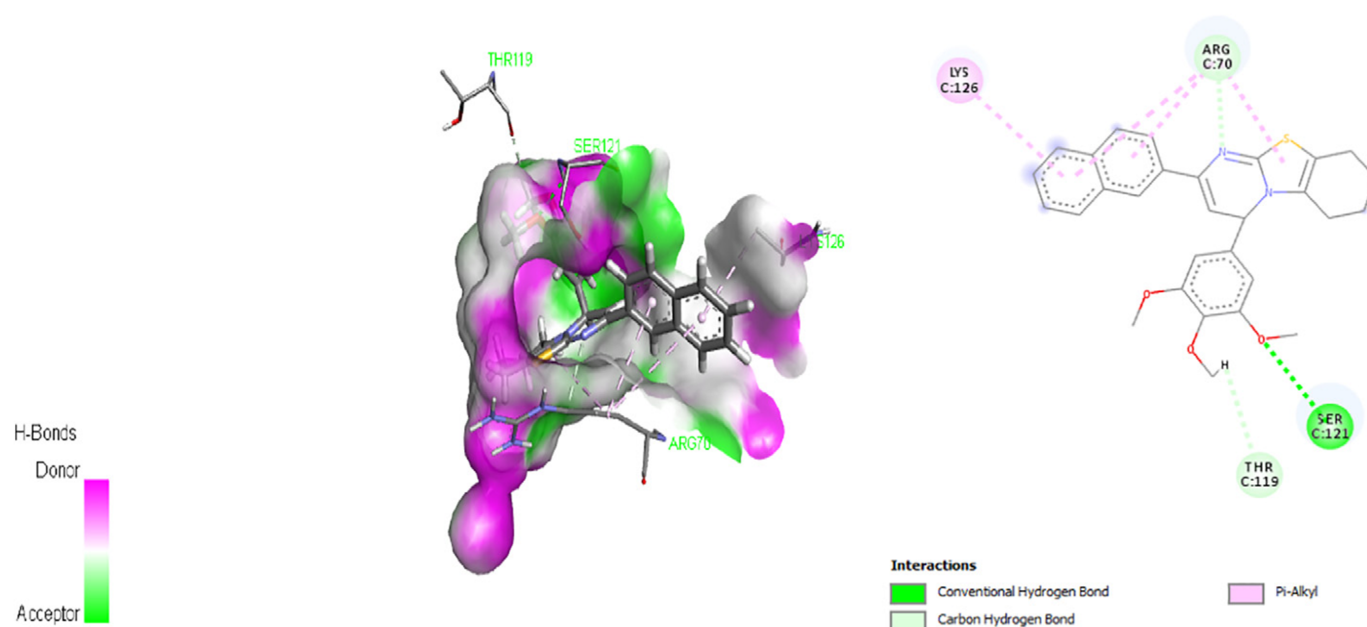


**Fig. 3.** Molecular docking of 5**c** with topoisomerase-II.

## 4. Results

Considering the well obtained *in vitro* results, it was thought worthy to perform molecular docking studies, hence screening the compounds, inculcating both *in silico* and *in vitro* results. Considering Topoisomerase II alpha as the target receptor, comparative and automated docking studies with newly synthesized candidate lead compounds was performed to determine the best *in silico* conformation. The Lamarckian genetic algorithm, inculcated in the docking program AutoDock 4.2, was employed to satisfy the purpose. Fig. 3 shows the native crystal structure of Topoisomerase II alpha (A chain) in complex was obtained from Protein Data Bank (http://www.pdb.org/pdb/home/home.do) with the PDB ID 1ZXM which was resolved at 1.8 Å using X-ray diffraction.

The docking of Topoisomerase II alpha with newly synthesized compounds showed well established hydrogen bonds with more than one amino acid in the active site. The active pocket was considered to be the region where Topoisomerase II alpha formed complex with compounds. The active pocket consisted of amino acid residues as Asn63, Arg70, Phe114, Ile60, Ala64, Ile186, Ala136 and Ile113 as shown in Fig. 2**.** The synthesized compounds having 2D structure were then converted to energy minimized 3D structures and were further used for protein–ligand docking study. All the synthesized molecules were docked to the protein. Fig. 3 shows the docked images of selected compounds including the considered standard drug *i.e.* Doxorubicin. Table 4 shows the Binding Energy and Inhibition Constant of compounds including the standard. *In silico* studies revealed all the synthesized molecules showed good binding energy toward the target protein ranging from −9.4 to −10.8 kJ $mol^{-1}$.

The docking studies from Table 4 reveals that the docking score correlates with the all docked ligands, which were found to have some interaction between atoms of the ligands and atoms of topoisomerase-II enzyme. Among the series, compound **5e** possessed best dock score of −10.8 comparable to the standard doxorubicin. It was observed that the presence of bromo group at meta position of phenyl ring may become more selective toward the topoisomerase-II activity. Moreover, these docked conformations also formed H-bonding interaction within the active site of enzyme. Among the tested compounds, nitrogen of thiazolopyrimidine **5b** formed H-bonds with amino-acid residues *i.e.* Asn C:63, with docking score −9.4 nearer to standard doxorubicin. Compound **5c** containing –$OCH_3$ on phenyl ring forms H-bond only with Ser C: 121 and its docking score −10.5 higher than standard. The compound **5d** (Fig. 4) with Sulfur in thiazolopyrimidine forms H-bond with Arg C:70 having a docking score −10.7 higher than standard. The nitrogen of thiazolopyrimidine in compound **5e** (Fig. 5)forms H-bond with Asn C:122 with docking score −10.8 higher than standard. The docking studies reveals that the thiazolopyrimidines having naphthyl, –$OCH_3$, Cl and Br moieties as substituents on phenyl ring in the compounds **5c**, **5d**, and **5e** showed good anticancer activity and their interaction with the enzyme topoisomerase-II is much higher than the standard.

## 5. Experimental section

### 5.1. General consideration

All chemicals were obtained from commercial suppliers and were used without purification. Infrared spectra were recorded on a Bruker WM-4× spectrophotometer with KBr pellets. All the reactions were monitored by TLC performed on precoated silica-gel 60 $F_{254}$ (Merk), compounds were visualized with UV light at 254 nm and $I_2$. $^1$H NMR (400 MHz, 300 MHz) and $^{13}$C NMR (100 MHz, 75 MHz) spectra were recorded on a JEOL Resonance 400 MHz and Bruker 300 MHz spectrometer in $CDCl_3$ or DMSO-$d_6$ with Tetramethylsilane (TMS) as internal standard. High resolution mass spectra (HRMS) were recorded using a Bruker spectrometer.

**Table 4**
Molecular docking studies of synthesized thiazolo[3,2-*a*]pyrimidine derivatives by using Autodockvina.

| Compound | Binding energy (KJ/mol) | Active site residues | No. of H-bonds |
|---|---|---|---|
| 5b | −9.4 | Asn C: 63 | 1 |
| 5c | −10.5 | Ser C: 121 | 1 |
| 5d | −10.7 | Arg C: 70 | 1 |
| 5e | −10.8 | Asn C: 122 | 1 |
| Doxorubicin | −10.4 | Asn C: 63, Ile C: 60, Tyr D: 6 | 3 |

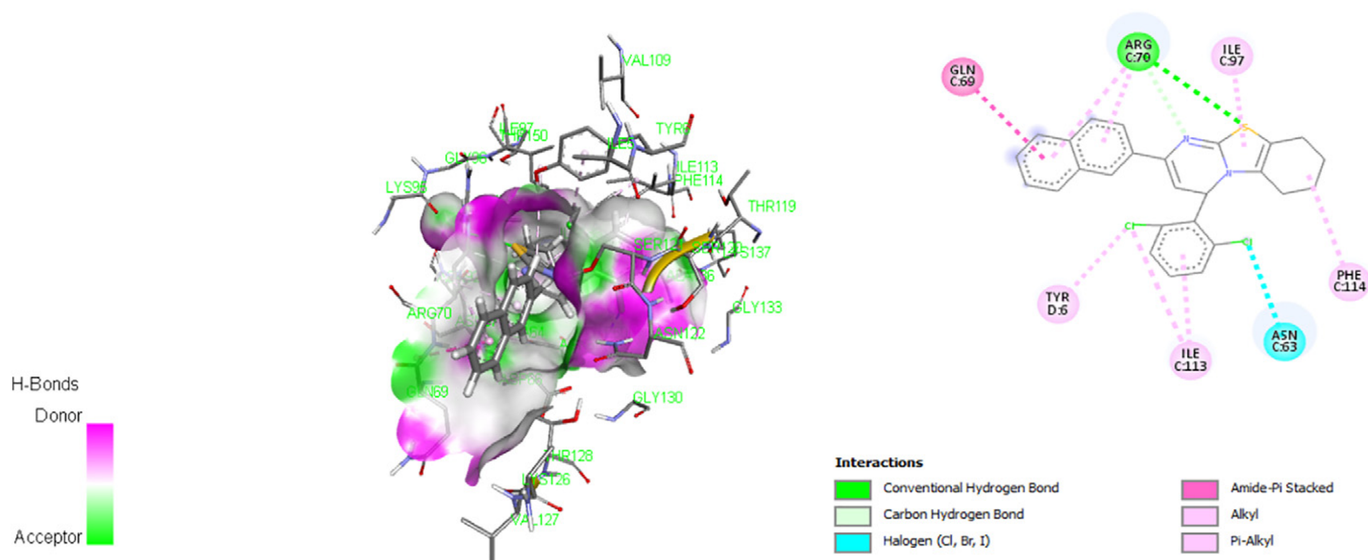


**Fig. 4.** Molecular docking of 5**d** with topoisomerase-II.

*5.2. General procedure for the synthesis of thiazolo[3,2-a]pyrimidines 5(a-h), 6(a-b), 7a*

To a solution of ketone **(1/2/3)** (10 mmol) in 30 ml acetonitrile, PTSA (5 mmol) and NBS (10 mmol) were added and mixture was allowed to stirred at 35-40 °C for 30 min. Subsequently Thiopyrimidine **4** (10 mmol) was added to reaction mixture and stirred for 2 h at 50 °C. The reaction was monitored by TLC. After completion of the reaction, acetonitrile was evaporated under vacuum and the residue was washed with water. The residue mass was recrystallized from isopropyl alcohol and diethyl ether to get pure thiazolopyrimidine **5/6/7**.

*5.3. Spectral characterization Data for 5(a-h), 6(a-b) and 7a*

**5a:2-(naphthalen-2-yl)-4-phenyl-6,7,8,9-tetrahydro-4*H*-benzo[4,5]thiazolo[3,2-*a*]pyrimidine hydrobromide**: Pale yellow solid; Yield: 84%; IR (KBr) $cm^{-1}$: 2923 (C—H), 1634 (C=N), 1541 (C=C); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 12.67 (1H, s, N.HBr), 8.68 (1H, s, Ar—H), 8.04 (1H, s, Ar—H), 7.64–7.75 (3H, m, Ar—H), 7.46 (2H, s, Ar—H), 7.29 (3H, t, Ar—H), 7.12 ($J$ = 6.4 Hz, 2H, d, Ar—H), 5.91 (1H, s, CH), 5.47 (1H, s, CH), 2.15–2.30 (3H, m, –CH-$CH_2$), 1.39–1.58 (5H, m, CH-$CH_2$-$CH_2$); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ ppm 160.12, 140.52, 133.72, 133.54, 133.06, 131.68, 129.73, 129.34, 129.26, 128.53, 127.90, 127.42, 127.29, 126.86, 126.20, 125.90, 122.80, 120.60, 101.19, 59.79, 22.51, 21.64, 20.95; MS ($m/z$) = 395.15 $[M + H]^+$ (—Br).

**5b: 2,4-diphenyl-6,7,8,9-tetrahydro-4*H*-benzo[4,5]thiazolo[3,2-*a*]pyrimidine hydrobromide:** Half white solid; Yield: 82%; IR (KBr) $cm^{-1}$: 2924 (C—H), 1630 (C=N), 1543 (C=C); $^1$H NMR (300 MHz, $CDCl_3$ + DMSO-$d_6$) $\delta$ ppm 12.53 (1H, s, N.HBr), 7.64–7.67 (5H, m, Ar—H), 7.27–7.38 (5H, m, Ar—H), 6.07 (1H, s, CH), 5.34 (1H, s, CH), 2.20–2.32 (2H, s, $CH_2$), 1–50 -1.71 (6H, m, $CH_2$-$CH_2$-$CH_2$); $^{13}$C NMR (75 MHz, $CDCl_3$ + DMSO-$d_6$) $\delta$ ppm 160.86, 142.50, 140.47, 139.62, 133.96, 132.63, 131.31, 129.64, 129.29, 128.78, 128.54, 126.10, 125.92, 125.74, 120.89, 120.62, 100.38, 59.68, 22.73, 21.75, 21.25, 21.04; HRMS: ($m/z$) Calcd for $[M + H]^+$, 345.142; Found, 345.142 (—Br).

**5c:2-(naphthalen-2-yl)-4-(3,4,5-trimethoxyphenyl)-6,7,8,9-tetrahydro-4*H*-benzo[4,5]thiazolo[3,2-*a*]pyrimidine hydrobromide:** Yellow solid; Yield: 87%; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ ppm 12.50 (1H, s, N.HBr), 8.73 (1H, s, Ar—H), 8.08 ($J$ = 6.1, dd, 3.4 Hz, 1H, Ar—H), 7.72–7.76 (3H, m, Ar—H), 7.46–7.48 (2H, m, Ar—H), 6.30 (1H, s, Ar—H), 5.85 (1H, s, Ar—H), 5.50 ($J$ = 4.0 Hz, 1H, d, CH), 5.51 (1H, m,

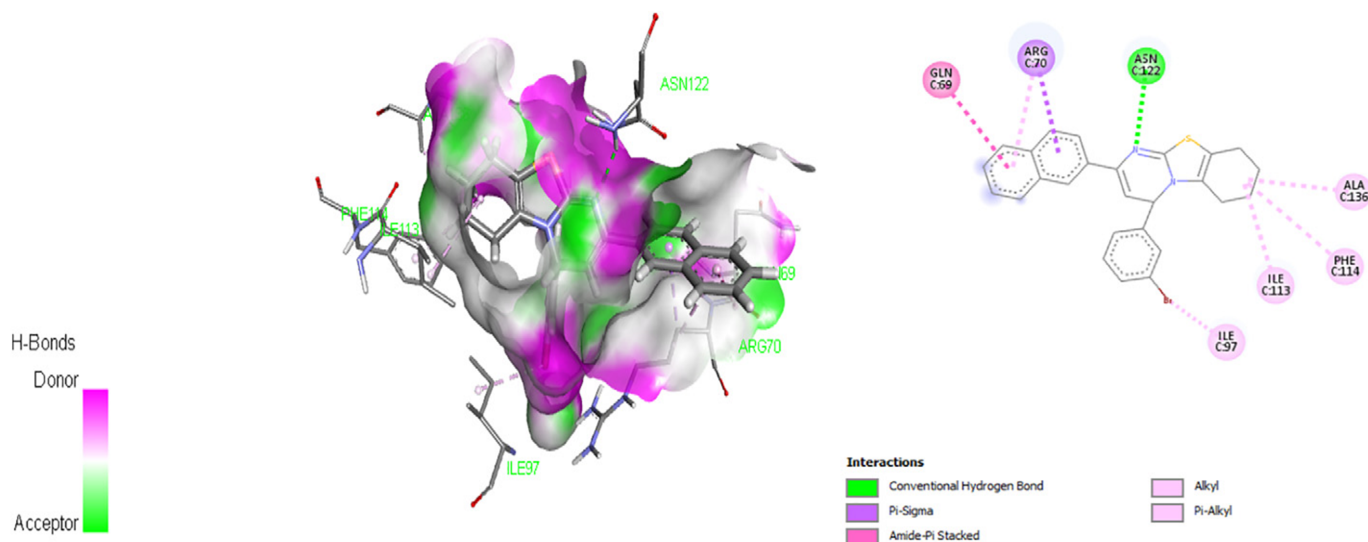


**Fig. 5.** Molecular docking of 5**e** with topoisomerase-II.

CH), 3.74–3.76 (9H, s, 3 × $OCH_3$), 2.24–2.30 (1H, m, CH), 2.03–2.03 (2H, m, $CH_2$), 1.52–1.54 (m, 3H, CH-$CH_2$), 1.33–1.35 (m, 2H, $CH_2$); ESI-MS: 485.18 $[M + H]^+$ (—Br).

**5d:4-(2,6-dichlorophenyl)-2-(naphthalen-2-yl)-6,7,8,9-tetrahydro-4*H*-benzo[4,5]thiazolo[3,2-*a*]pyrimidine hydrobromide:** Pale yellow solid; Yield: 92%; $^1$H NMR (400 MHz, $CDCl_3$) δ ppm 12.84 (1H, s, N.HBr), 8.72 (1H, s, Ar—H), 8.06 (*J* = 2.9 Hz, 1H, d, Ar—H), 7.71–7.80 (3H, m, Ar—H), 7.49–7.72 (5H, m, Ar—H), 7.05 (1H, s, CH), 5.23 (1H, s, CH), 2.33–2.49 (2H, m, $CH_2$), 1.42–1.82 (6H, m, $CH_2$-$CH_2$-$CH_2$); $^{13}$C NMR (100 MHz, $CDCl_3$) δ ppm 158.65, 141.61, 137.97, 137.96, 136.83, 135.09, 134.51, 134.02, 130.79, 129.70, 129.52, 128.62, 128.36, 128.29, 128.16, 128.02, 127.01, 126.50, 124.78, 120.38, 112.56, 46.91, 25.76, 24.57, 23.91, 23.69; HRMS: (*m*/*z*) Calcd for $[M + H]^+$, 463.0797; Found, 463.080 (—Br).

**5e: 4-(3-bromophenyl)-2-(naphthalen-2-yl)-6,7,8,9-tetrahydro-4*H*-benzo[4,5]thiazolo[3,2-*a*]pyrimidine hydrobromide:** Yellow solid; Yield: 87%; $^1$H NMR (400 MHz, $CDCl_3$) δ ppm 12.21 (1H, s, N.HBr), 8.15 (1H, s, Ar—H), 7.92–7.98 (3H, m, Ar—H), 7.43–7.68 (7H, m, Ar—H), 6.47 (*J* = 4.6 Hz, 1H, d, CH), 5.90 (*J* = 4.56 Hz, 1H, d, CH), 2.23–2.58 (2H, m, $CH_2$), 1.68–1.74 (2H, m, $CH_2$), 1.50–1.54 (4H, m, $CH_2$-$CH_2$); HRMS: (*m*/*z*) Calcd for $[M + H]^+$, 473.0682; Found, 473.0722 (—Br).

**5f:4-(3,4-dimethoxyphenyl)-2-(naphthalen-2-yl)-6,7,8,9-tetrahydro-4H-benzo[4,5]thiazolo[3,2-*a*]pyrimidine hydrobromide:** Yellow solid; Yield: 84%; $^1$H NMR (400 MHz, $CDCl_3$) δ ppm 12.45 (s, 1H, N.HBr), 8.71 (s, 1H, Ar—H), 8.18 (dd, *J* = 6.1, 3.4 Hz, 1H, Ar—H), 7.71–7.79 (m, 4H, Ar—H), 7.42–7.58 (m, 2H, Ar—H), 6.39 (s, 2H, Ar—H), 5.82 (d, *J* = 4.0 Hz, 1H, CH), 5.52 (t, 1H, CH), 3.73 (d, 6H, 2 × $OCH_3$), 2.24–2.31 (m, 1H, CH), 2.04–2.05 (m, 2H, $CH_2$), 1.55–1.57 (m, 3H, CH-$CH_2$), 1.33–1.35 (m, 2H, $CH_2$); ESI-MS: 455.15 [M + H]] + (—Br).

**5g: 4-(naphthalen-1-yl)-2-(naphthalen-2-yl)-6,7,8,9-tetrahydro-4*H*-benzo[4,5]thiazolo[3,2-*a*]pyrimidine:** Yellow solid; Yield: 86%; $^1$H NMR (400 MHz, $CDCl_3$) δ ppm 8.45 (s, 1H, Ar—H), 8.08 (d, *J* = 8.4 Hz, 1H, Ar—H), 7.89–7.91 (m, 2H, Ar—H), 7.78–7.81 (m, 1H, Ar—H), 7.68–7.74 (m, 2H, Ar—H), 7.60–7.68 (m, 2H, Ar—H), 7.52–7.56 (m, 2H, Ar—H), 7.40–7.54 (m, 2H, Ar—H), 7.19 (d, 1H, Ar—H), 6.75 (d, *J* = 4.0 Hz, 1H, CH), 5.59 (d, *J* = 4.3 Hz, 1H, CH), 2.22–2.36 (m, 3H, CH-$CH_2$), 1.51–1.68 (m, 5H, CH-$CH_2$-$CH_2$); ESI-MS: 445.17 $[M + H]^+$.

**5h: 4-(4-fluorophenyl)-2-(naphthalen-2-yl)-6,7,8,9-tetrahydro-4*H*-benzo[4,5]thiazolo[3,2-*a*]pyrimidine hydrobromide:** Yellow solid; Yield: 82%; $^1$H NMR (300 MHz, $CDCl_3$) δ ppm 12.49 (s, 1H, N.HBr), 8.43 (s, 1H, Ar—H), 7.19–7.92 (m, 10H, Ar—H), 5.99 (s, 1H, CH), 5.53 (s, 1H, CH), 2.28 (s, 2H, $CH_2$), 1.25–1.63 (m, 6H, $CH_2$-$CH_2$-$CH_2$); $^{13}$C NMR (75 MHz, $CDCl_3$ and DMSO-$d_6$) δ ppm 165.80, 144.31, 141.72, 139.71, 138.69, 138.01, 137.22, 133.89, 133.82, 133.77, 133.63, 132.74, 132.43, 132.07, 131.06, 130.29, 128.06, 125.55, 121.98, 121.69, 106.84, 63.88, 27.99, 26.94, 26.40, 26.15; ESI-MS: 413.14 $[M + H]^+$ (—Br).

**6a: 1-(3-methyl-5,7-diphenyl-5*H*-thiazolo[3,2-*a*]pyrimidin-2-yl) ethan-1-one:** Half white solid; Yield: 81%; FT-IR (KBr) $cm^{-1}$: 2923 (C—H),1620 (C=O), 1598 (C=N), 1384 (C=C), 1120 (C—O) 696 (C—S); $^1$H NMR (400 MHz, $CDCl_3$) δ ppm 7.66 (d, *J* = 4.9 Hz, 3H, Ar—H), 7.22–7.37 (m, 7H, Ar—H), 6.43 (s, 1H, CH), 5.49 (s, 1H, CH), 2.41 (s, 3H,$CH_3$), 2.35 (s, 3H, $CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) δ ppm 188.64, 161.57, 143.56139.79, 131.77, 130.52, 130.08, 129.78, 129.04, 128.89, 126.11, 125.93, 122.77, 102.36, 60.64, 30.27, 13.90; HRMS: (*m*/*z*) Calcd for $[M + H]^+$, 347.1213; Found, 347.1215.

**6b: 1-(3-methyl-7-(naphthalen-2-yl)-5-phenyl-5*H*-thiazolo[3,2-*a*]pyrimidin-2-yl)ethan-1-one hydrobromide:** Pale yellow solid; Yield: 84%; FT-IR (KBr) $cm^{-1}$: 2927 (C—H),1625 (C=O), 1596 (C=N), 1387 (C=C), 1032 (C—O), 680 (C—S); $^1$H NMR (400 MHz, $CDCl_3$) δ ppm 12.83 (s, 1H, N.HBr), 8.41 (s, 1H, Ar—H), 8.00 (s, 1H, Ar—H), 7.06–7.78 (m, 10H, Ar—H), 6.30 (s, 1H, CH), 5.76 (s, 1H, CH), 2.13 (s, 3H, $CH_3$), 2.02 (s, 3H, $CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) δ ppm 188.12, 161.71, 142.62, 140.10, 139.86, 133.45, 133.02, 131.21, 130.27, 129.89, 129.50, 129.42, 128.88, 128.74, 127.43, 127.00, 126.16, 126.11, 124.80, 122.74, 122.64, 103.48, 60.25, 29.77, 13.52; HRMS: (*m*/*z*) Calcd for $[M + H]^+$, 397.1369; Found, 397.1370 (—Br).

**7a:9,11-diphenyl-5,6-dihydro-11*H*-naphtho[1′,2′:4,5]thiazolo[3,2-*a*]pyrimidine hydrobromide:** Pale yellow solid; Yield: 77%; IR (KBr) $cm^{-1}$: 2923 (C—H), 1628 (C=N), 1384 (C=C), 671 (C—S); $^1$H NMR (400 MHz, $CDCl_3$) δ 13.01 (s, 1H, N.HBr)), 9.38 (d, *J* = 24.9 Hz, 1H), 7.09–7.78 (m, 11H), 6.88 (s, 1H), 6.82 (s,1H, =CH), 5.63 (s, 1H, CH), 2.68–2.73 (m, 2H, $CH_2$), 1.91 (s, 2H, $CH_2$); HRMS: (*m*/*z*) Calcd for $[M + H]^+$, 393.142; Found, 393.142 (—Br).

## 6. Cytotoxic evaluation

### 6.1. Materials and methods

Dulbecco's modified eagle medium (DMEM), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], trypsin-EDTA, antibiotic-antimycotic solution, phosphate buffered saline ($Ca^{2+}$, $Mg^{2+}$ free; PBS), dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA), Foetal bovine serum (FBS) was purchased from Gibco, USA. Cell culture 96 well plates and plastic ware were obtained from Techno Plastic Products (TPP) (CH-8219, Trasadingen, Switzerland). All other chemicals were obtained locally and were of analytical grade. Human lung adenocarcinoma cell line (A549), Human breast carcinoma cell line (MCF-7), Human cervical cancer cell line (HeLa) and Human neuronal carcinoma cell lines (SKNSH) were obtained from National Centre for Cell Science (NCCS) Pune, India.

### 6.2. Cell culture

The cell lines were grown in DMEM culture medium supplemented with 10% FBS, 0.3% sodium bicarbonate, 10 ml/l antibiotic-antimycotic solution (10,000 U/ml penicillin, 10 mg/l streptomycin and 25 μg/ml amphotericin B), culture was maintained in $CO_2$ incubator at 37 °C with a 90% humidified atmosphere and 5s% $CO_2$.

### 6.3. Preparation of samples for MTT assay

Test compounds **5(b-e)** were dissolved in DMSO to prepare a stock solution of 10 μM and various dilutions were made with sterile PBS (1×) to get desired concentrations. All the solutions were filtered with 0.22 μm sterile filter and subjected to 20 min of UV eradication before adding to the 96 well plates containing cells.

### 6.4. Cytotoxicity evaluation (MTT assay)

Cytotoxicity of synthesized compound were assessed using MTT assay to determine the cell viability according to a method described by Hansen et al., 1989 [39]. The assay is based on the reduction of MTT by the mitochondrial dehydrogenase of viable cells into purple formazan crystals which gets dissolved in DMSO. Quantification of purple colour formed spectrophotometrically at 570 nm gives an estimate on the viability of cells. Briefly, $5 \times 10^3$ exponentially growing cells were seeded into each 96 well plate (counted by Trypan blue exclusion dye method) allowed to grow till 60–70% confluence then compounds **5b, 5c, 5d, 5e** were added to the culture medium with the final concentrations ranging from of 0.1, 1, 5 and 10 μM and along with controls (negative (without compound) and positive (Doxorubicin)) incubated for 24 and 48 h in a $CO_2$ incubator at 37 °C with a 90% humidified atmosphere and 5% $CO_2$. Then the media of the wells were replaced with 90 μl of fresh serum free media and 10 μl of MTT (5 mg/mL of PBS), plates were incubated at 37 °C for 2 h, there after the above media was discarded and allowed to dry for 30 min. 100 μl of DMSO was added in each well at 37 °C for 5 min. The purple formazan crystals were dissolved and immediately the absorbance at 570 nm was measured

using Spectra Max plus 384 UV–Visible plate reader (Molecular Devices, Sunnyvale, CA, USA). $IC_{50}$ values were calculated based on the absorbance values relative to the controls (Table 3).

## 7. Conclusion

In, conclusion, an efficient one-pot method has been developed for the synthesis of series of 2,4-diaryl-6,7,8,9-tetrahydro-4*H*-benzo[4,5]thiazolo[3,2-*a*]pyrimidine hydrobromide, 1-(3-methyl-5,7-diaryl-5*H*-thiazolo[3,2-*a*]pyrimidin-2-yl)ethan-1-one hydrobromide and 9,11-diaryl-6,11-dihydro-5*H*-naphtho[1′,2′:4,5]thiazolo[3,2-*a*]pyrimidine hydrobromide derivatives by the α-bromination of ketone (cyclohexanone/acetylacetone/alpha-tetralone) with N-Bromosuccinamide (NBS) and followed by cyclization with 3,4-dihydropyrimidine-2(1*H*)-thiones, respectively, in the presence of p-toluenesulfonic acid (PTSA) in acetonitrile. The significant features of this method are novel, simple, inexpensive experimental procedure, short reaction time, and good yield and moreover some of the synthesized compounds are potent anticancer activity than standard. Particularly compounds **5c** and **5e** substituted with $OCH_3$, Br moieties showed highest cytotoxic activity against cancer cell lines. Molecular docking of synthesized compounds **5(b-e)** against topoisomerase-II reveals the compounds **5c**, **5d**, and **5e** showed best docking score than the standard doxorubicin. The present research results may be considered for designing new class of drugs is potent in anticancer treatment.

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