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ORIGINAL ARTICLE

Molecular docking studies, biological evaluation and synthesis of novel 3-mercapto-1,2,4-triazole derivatives

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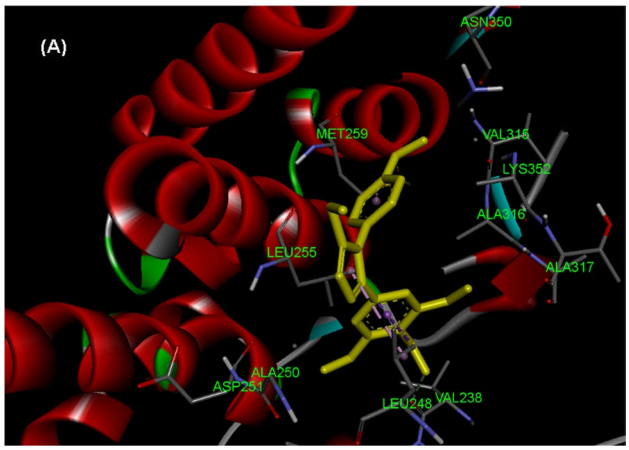
Abstract

Synthesis of bioactive heterocyclic compounds having effective biological activity is an essential research area for wide-ranging applications. In this study, a conventional methodology has been developed for the synthesis of a series of new 3-mercapto-1,2,4-triazole derivatives **4a–f.** The purity and structure of the synthesized molecules were confirmed by ^{1}H NMR, ^{13}C NMR and elemental analysis. In addition, the prepared compounds were screened for their anti-proliferative activity against three human cancer cell lines including A549 (lung cancer), MCF7 (breast cancer) and SKOV3 (ovarian cancer) using MTT reduction assay. All the tested compounds demonstrated remarkable cytotoxic activity with IC_{50} values ranging from 3.02 to 15.37 μM. The heterocyclic compound bearing 3,4,5-trimethoxy moiety was found to be the most effective among the series displaying an IC_{50} of 3.02 μM specifically against the ovarian carcinoma cancer cell line (SKOV3). Moreover, Annexin V-FITC/propidium iodide staining assay indicated that this compound can induce apoptosis in SKOV3 cells. Furthermore, cell cycle assay showed a significant cell cycle arrest at the G2/M phase in a dose-dependent manner for this compound. The molecular docking results was showed binding modes of potent compound **4d** perfectly corroborated the suggestion of binding to the colchicine site. The entire results conclude that 3-mercapto-1,2,4-triazole derivatives can be synthesized by a green method for biological and pharmacological applications.

Graphic abstract

New analogs of 3-mercapto-1,2,4-triazole potential derivatives for anti-proliferative activity were synthesized. Cytotoxic activity of all synthesized compounds was evaluated against tree human cancer cell lines: lung (A549), breast (MCF7) and ovarian (SKOV3).



Keywords Mercapto-triazole derivatives · Molecular docking studies · Anti-proliferative activity · Cell cycle arrest

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Introduction

The existence, number and position of nitrogen in the heterocyclic compounds play important roles in many chemical properties and application effectiveness. In general, the heterocyclic compounds are part of synthetic chemicals, natural products and bioactive pharmaceuticals that improve the quality of life [1–3]. 1,2,4-Triazoles and N-bridged heterocyclic derivatives show various biological activities [4, 5] and have been utilized in medicinal chemistry with incorporation into a wide-ranging diversity of medicinally interesting drug candidates [6–8]. Although many triazoles compounds are commercially available, the 1,2,4-triazole nucleus and *N*-bridged heterocyclic derivatives having novel advantages can be developed by the advance modern heterocyclic chemistry methods. Today, a variety of biological activities have been reported possessing various types of biological activity such as anti-inflammatories, antifungal, antiviral, antimicrobial agents, anti-leishmanial, antihypertensive and anti-migraine activities [9–15]. In the market, there are many drugs containing the 1,2,4-triazole moieties, e.g., triazolam, alprazolam, etizolam, furacilin, ribavirin, hexaconazole, triadimefon, myclobutanil, rizatriptan, propiconazole, flutrimazole, etc. [16–19]. Importantly, sulfur-containing heterocyclic compounds are very essential for practical medical applications. In addition, a recent literature survey reveals that the presence of the mercapto- and thione-substituted 1,2,4-triazole moieties is an important structural feature of a wide range of synthetic medicines [20–22]. Furthermore, many synthesized 1,2,4-triazole ring moieties containing sulfur have been pragmatically applied for their anticancer, antifungal, anti-mycobacterial, anti-tubercular, diuretic and hypoglycemic properties [23–25] through a large number of their derivatives. Owing to their wide variety of biological activities and important chemotherapeutics attributes, substituted 1,2,4-triazole moieties such as letrozole, vorozole and anastrozole (Fig. 1) have been used for the treatment of breast cancer [26]. In the continuation of our research on the synthesis of new heterocyclic compounds exposing active biological properties on human cancerous cell lines [27–31], the triazole nucleus has gained much attention in recent years, due to its metabolic stability, good solubility and capability for hydrogen bonding [32] like para-methyl derivative of trimethoxyphenyl-based 5-amino-1,2,4-triazoles as potent antimitotic agents with IC50 values of 0.21 and 3.2 nM against CEM and HeLa cells, respectively [33]; we have synthesized compounds **4a–f** with 68–86% yields (Scheme 1). We have also investigated the anti-proliferative effects of new analog 3-mercapto-1,2,4-triazoles on three human cancer cell lines: A549 (lung cancer), MCF7 (breast cancer) and SKOV3 (ovarian cancer) with different concentrations of compounds **4a–f.** The effect of these synthesized compounds was evaluated on cell cycles for 24 h using MTT reduction assay. Molecular docking studies and Annexin V-FITC/propidium iodide staining assay were also investigated.

Experimental section

Chemicals and instrumentation

All starting materials and reagents were obtained from Sigma-Aldrich, and Merck Company. commercial solvents were used without any pretreatment or purification. The ^{1}H NMR and ^{13}C NMR spectra were recorded using tetramethylsilane (TMS) as the internal standard on a Bruker BioSpin spectrometer at 400 and 100 MHz, respectively. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane, and coupling constants were measured in hertz. The melting points were determined in a capillary tube using a Stuart Scientific apparatus and are uncorrected. The IR spectra were recorded by employing an FT-IR PerkinElmer spectrometer using KBr disks. The high-resolution mass spectra were obtained using an HP 5937 mass-selective detector (Agilent technologies). The analytical results for C, H, N and chlorine were within 0.4% of the theoretical values.

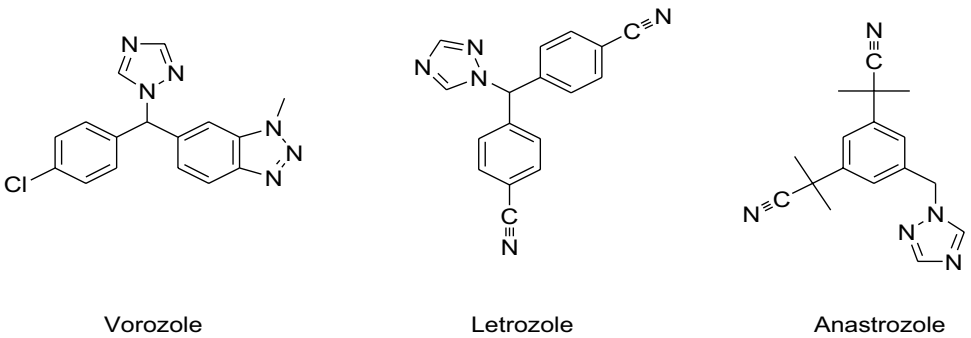
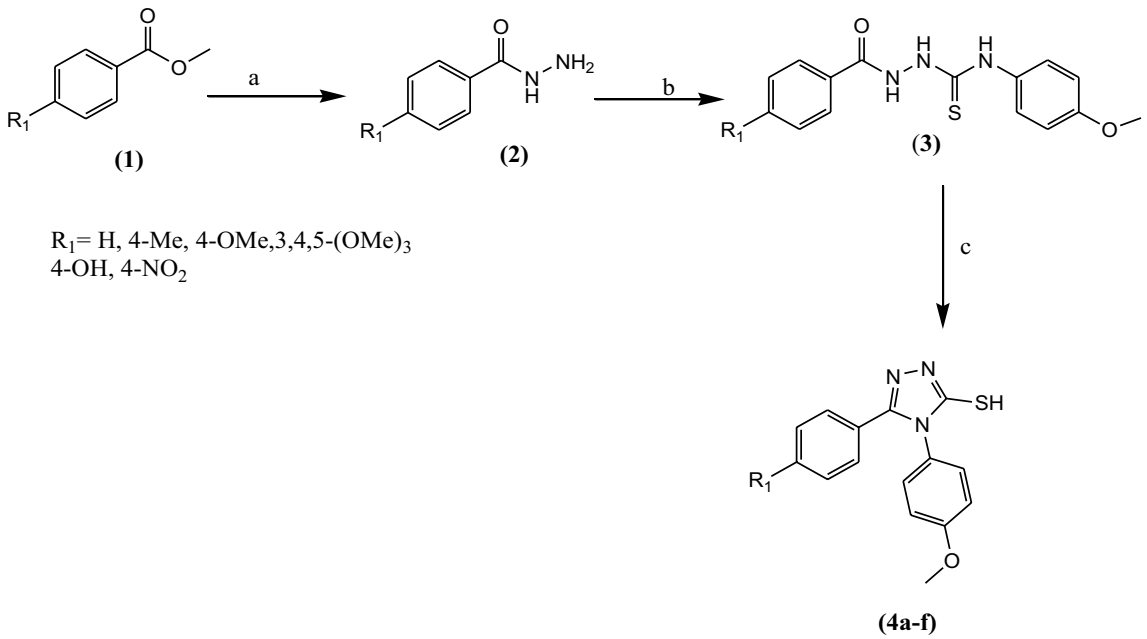


Fig. 1 Chemical structures of some breast cancer drugs



Scheme 1 General procedure for synthesis of target compounds **4a–f**. Reagent and conditions: (a) $NH_2NH_2{\cdot}H_2O$ (80%), ethanol, reflux; (b) 4-methoxy phenyl isothiocyanate, ethanol, reflux; (c) 1 M NaOH, reflux, and then 1 M HCl

General synthetic procedure for preparation of compounds 4a–f

A mixture of different methyl benzoate (1 mmol) and hydrazine hydrate (0.75 g, 15 mmol, 80%) in 7 mL of ethanol was heated under reflux for 6 h. Excess ethanol was distilled and the contents were allowed to cool. The solid obtained product was filtered, washed with water and dried to afford the corresponding benzohydrazide, which was used for the next reaction. Then, a solution of different benzohydrazides (1 mmol) and 4-methoxy phenyl isothiocyanate (1 mmol, 0.165 g) in ethanol (10 mL) was heated at reflux for 5 h and cooled down to room temperature gradually. The suspension was filtered, and the solid was washed with ethanol and dried to give the intermediate hydrazine carbothioamide as a white solid product. 1 M NaOH (10 mL) was added to this solid, and the mixture was heated at reflux for 1 h. The resulting solution was cooled at room temperature and acidified to pH 5–6 with 1 M HCl. The precipitate was filtered, washed with water and dried to obtain the title compounds as white solid.

4-(4-Methoxyphenyl)-5-phenyl-4H-1,2,4-triazole-3-thiol (4a) Yield: 86%; m.p.; 110–112 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.79 (S, 3H, OMe), 7.00 (dd, 2H, J = 4.8 and 2.0 Hz, H-3′ and H-5′), 7.23 (dd, 2H, J = 4.8 and 2.0 Hz, H-2′ and H-6′), 7.30–7.35 (m, 3H, H-3, H-4 and H-5), 7.35–7.42 (m, 2H, H-2 and H-6). ^{13}C NMR (100 MHz, DMSO-d_6): δ 56.37, 107.77, 119.48, 120.12, 129.55, 132.37, 134.10, 153.10, 166.60, 167.37. Anal. Calcd. for $C_{15}H_{13}N_3OS$:C, 63.58; H, 4.62; N, 14.83. Found: C, 63.83; H, 4.60; N, 14.88.

4-(4-Methoxyphenyl)-5-p-tolyl-4H-1,2,4-triazole-3-thiol (4b) Yield: 82%; m.p.; 122–124 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.05 (S, 3H, Me), 3.79 (S, 3H, OMe), 6.73 (d, 2H, J = 8.4 Hz, H-3′ and H-5′), 6.99 (d, 2H, J = 8.8 Hz, H-3 and H-5), 7.19 (d, 2H, J = 6.4 Hz, H-2′ and H-6′), 7.40 (d, 2H, J = 7.6 Hz, H-2 and H-6). ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.76, 55.67, 114.76, 122.66, 128.90, 129.47, 134.40, 138.08, 148.39, 157.31. Anal. Calcd. for $C_{16}H_{15}N_3OS$:C, 64.62; H, 5.08; N, 14.13. Found: C, 64.36; H, 5.100; N, 14.07.

4,5-Bis(4-methoxyphenyl)-4H-1,2,4-triazole-3-thiol (4c) Yield: 68%; m.p.; 128–130 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.70 (S, 3H, OMe), 3.78 (S, 3H, OMe), 6.80 (d, 2H, J = 8.8 Hz, H-3′ and H-5′), 6.93 (dd, 2H, J = 4.4 and 2.0 Hz, H-2′ and H-6′), 7.02 (d, 2H, J = 6.8 Hz, H-3 and H-5), 7.13 (d, 2H, J = 8.8 Hz, H-2 and H-6). ^{13}C NMR (100 MHz, DMSO-d_6): δ 55.52, 55.70, 114.05, 114.17, 128.78, 130.16, 150.36, 158.59, 159.30, 169.13. Anal. Calcd. for $C_{16}H_{15}N_3O_2S$:C, 61.32; H, 4.82; N, 13.41. Found: C, 61.07; H, 4.83; N, 134.46.

5-(3,4,5-Trimethoxyphenyl)-4-(4-methoxyphenyl)-4H-1,2,4-triazole-3-thiol (4d) Yield: 76%; m.p; 134–136 °C; ^{1}H NMR (400 MHz, DMSO-d_6): 3.73 (S, 3H, OMe), 3.83 (S, 6H, OMe), 7.23 (S, 2H, H-2 and H-6). 7.27 (d, 2H,

$J = 8.0$ Hz, H-3 and H-5), 7.35 (d, 2H, $J = 6.00$ Hz, H-2 and H-6). ^{13}C NMR (100 MHz, DMSO-d_6): δ 55.67, 56.37, 60.56, 106.97, 112.92, 118.38, 124.34, 126.35, 141.80, 153.10, 167.37. Anal. Calcd. for $C1_8H_{19}N_3O_4S$: C, 57.89; H, 5.13; N, 11.25. Found: C, 58.12; H, 5.15; N, 11.20.

4-(5-Mercapto-4-(4-methoxyphenyl)-4H-1,2,4-triazole-3-yl) phenol (4e) Yield: 73%; m.p.; 121–123 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.78 (S, 3H, OMe), 6.73 (d, 2H, $J = 8.4$ Hz, H-3 and H-5), 6.93 (dd, 2H, $J = 4.4$ and 2.4 Hz, H-3′ and H-5′), 7.02 (d, 2H, $J = 6.8$ Hz, H-2′ and H-6′), 7.19 (d, 2H, $J = 6.8$ Hz, H-2 and H-6). ^{13}C NMR (100 MHz, DMSO-d_6): δ 56.37, 107.77,119.48, 102.12, 129.55, 132.37, 134.10, 153.10, 166.60, 167.37. Anal. Calcd. for $C_{15}H_{13}N_3O_2S$: C, 60.18; H, 4.38; N, 14.04; O, 10.69. Found: C, 59.93; H, 4.39; N, 13.98.

4-(4-Methoxyphenyl)-5-(4-nitrophenyl)-4H-1,2,4-triazole-3-thiol (4f) Yield: 82%; m.p.; 142–144 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.75 (S, 3H, OMe), 6.90 (d, 2H, $J = 8.8$ Hz, H-3′ and H-5′), 7.27 (d, 2H, $J = 8.0$ Hz, H-2 and H-6), 8.17 (d, 2H, $J = 8.8$ Hz, H-2′ and H-6′), 8.35 (dd, 2H, $J = 5.2$ and 2.0 Hz, H-3 and H-5). ^{13}C NMR (100 MHz, DMSO-d_6): δ 55.67, 109.77, 113.74, 120.06, 123.86, 128.90, 129.87, 132.40, 139.06, 141.19, 149.79, 157.30. Anal. Calcd. for $C_{15}H_{12}N_4O_3S$: C, 54.87; H, 3.68; N, 17.06; O, 14.62. Found: C, 54.65; H, 3.69; N, 16.99.

Biology

In vitro MTT assay

The cytotoxic effects of all newly synthesized compounds **4a–f** were evaluated by using MTT assay on three human cancer cell lines (MCF-7, A549 and SKOV3). 1×10^4 cells per well were seeded in 96-well culture plates in culture medium supplemented with 10% FBS and incubated for 24 h at 37 °C in a CO_2 incubator. After incubation, cells were treated with 100 µL of test compounds and were diluted to the desired concentrations in culture. After 48 h of incubation, 10 µL MTT (5 mg/mL) was added to each well and the plates were further incubated until a purple precipitate was visible (4 h). Then, the medium was carefully removed, the precipitates were dissolved in 200 µL of DMSO, and the absorbance of the solution at 490 nm was measured. IC_{50} values, at which cell growth was inhibited by 50% were calculated with GraphPad Prism Software version 7.0 [34].

Annexin V-FITC for apoptosis

The apoptotic effect of compound **4d** was further evaluated by Annexin V-FITC/PI assay. SKOV3 cells were cultured in 6-well plates (1×10^6 cells/well) and incubated for 24 h. Then, the medium was replaced with complete medium containing compounds **4d** at 25, 50 and 100 µM concentrations. After 48 h of drug treatment, cells from the supernatant were harvested by trypsinization, washed with PBS at 3000 rpm and stained with 5 mL of Annexin V-FITC (Sigma Chemical Co) in PBS at room temperature for 15 min and then PI solution (Keygen Biotech, China) for another additional 10 min. Flow cytometry was performed using a FACScan (Becton–Dickinson) equipped with a single 488 nm argon laser. The percentage of apoptotic cells was calculated using the EXPO32 ADC analysis software.

Cell cycle analysis

SKOV3 cells were seeded in 6-well plates (3×10^5 cells/well), incubated in the presence or absence of compound **4d** at the 25, 50 and 100 µM concentrations for 24 h, harvested by centrifugation and then fixed in ice-cold 70% ethanol overnight. After that, ethanol was removed and the cells were re-suspended in ice-cold PBS, treated with RNAse A (Keygen Biotech, China) at 37 °C for 30 min and then incubated with the DNA staining solution propidium iodide (PI, Keygen Biotech, China) at 4 °C for 30 min. Approximately, 10,000 events were detected by flow cytometry (Beckman Coulter, Epics XL) at 488 nm. The data regarding the number of cells in different phases of the cell cycle were analyzed using EXPO32 ADC analysis software.

Molecular docking studies

In order to determine the basic structure of the biological results discussed above, the binding mode of promising compounds **4d** to the colchicine-binding site of tubulin was investigated using AutoDock software (version 4.2). The 3D structures for the compounds were built and optimized by ChemBioDraw Ultra 16.0 and ChemBio3D Ultra 16.0 software. These optimized 3D structures are utilized for docking. The β-subunit of the target (chain D) was taken into account, and the rest was deleted. The AutoDock protocol [35] was followed to prepare the final receptor pdbqt file. A grid map with 40, 40 and 40 grid points (X, Y, Z dimensions, resp.) and centroid: $X = 17.0192$, $Y = 65.9939$, $Z = 43.3901$ was selected. The best scoring pose of each complex as judged by the AutoDock was chosen and visually analyzed for interactions. The 3D, 2D poses of most active compound **4d** taken using discovery studio 2016.

Results and discussion

Chemistry and cytotoxicity assay

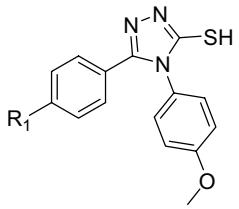
Pursuant to a survey of the literature, the substituted 1,2,4-triazole ring is found to be an interesting group of heterocyclic compounds that are highly regarded by contemporary medicinal chemists owing to their appreciable anticancer effects on different types of cancer cell lines [36, 37]. Furthermore, the introduction of proper heterocyclic compounds would be a good strategy to overcome some going problems such as metabolic instability, low oral bioavailability, poor aqueous solubility and undesired side effects [38]. The structure–activity relationship studies indicated that the presence of a 3,4,5-trimethoxyphenyl ring-A is one of the essentials for potent anticancer activity [39]. The steric and electronic effects of different electron-donating alkoxy groups on the benzene moiety were chosen to improve the cytotoxicity and physicochemical properties of these compounds.

In this study, the heterocyclic compounds were synthesized by employing the sequence outlined following the procedure depicted in Scheme 1: firstly, the methyl ester derivatives (**1**) converted to the corresponding acid hydrazides (**2**) by treating hydrazine hydrate 80% in almost quantitative yield. After cooling the mixture to room temperature, the solid hydrazide product was washed with water and then dried. The reaction of different acid hydrazides (**2**) with 4-methoxy phenyl isothiocyanate in the presence of sodium hydroxide as a base in refluxing water followed by acidification with HCl 1 M to pH = 5–6 resulted in carbothioamide salts (**3**) which then underwent intramolecular ring closure to generate the corresponding substituted 3-mercapto-1,2,4-triazole derivatives **4a–f**.

The in vitro anti-proliferative activity of synthesized compounds against three human cancer cell lines including adeno carcinomic human alveolar basal epithelial cells (A549), breast cancer cells (MCF7) and human ovarian carcinoma cells (SKOV3) was evaluated by using standard MTT assays according to the literature method, and etoposide was chosen as a standard drug [40]. Cytotoxicity values against different cancer cell lines expressed as the compound concentration (μM) that causes 50% inhibition of cell growth (IC_{50}) are shown in Table 1 and Fig. 2.

All the tested compounds presented considerable cytotoxic activity against cell lines, in particular, compounds **4d** and **4e** with IC_{50} values of 3.02–5.87 μM. The best IC_{50} values were obtained against SKOV3 cell line. In addition, most compounds showed superior growth inhibitory activity against all tested cell lines to that of reference drug etoposide. A survey on the structures and activities in Table 1 revealed that the IC_{50} values of compounds with

Table 1 Cytotoxic activities of compounds **4a–f** against cancer cells (A549, MCF-7 and SKOV3)



Compound		*IC_{50} ± SD values against cancer cell lines (μM)		
	R_1	A-549	MCF-7	SKOV3
4a	H	9.18 ± 0.13	12.45 ± 0.21	10.48 ± 37
4b	4-Me	12.26 ± 0.28	11.87 ± 0.26	9.13 ± 0.29
4c	4-OMe	8.90 ± 0.17	9.25 ± 0.42	6.28 ± 0.31
4d	$3,4,5\text{-}(OMe)_3$	4.32 ± 0.12	4.65 ± 0.32	3.02 ± 027
4e	4-OH	6.16 ± 0.18	7.61 ± 0.12	5.87 ± 0.12
4f	$4\text{-}NO_2$	12.87 ± 0.45	13.46 ± 0.17	15.37 ± 0.28
Etoposide		2.99 ± 0.16	3.89 ± 0.1	4.74 ± 0.14

*IC_{50} values are indicated as the mean of three independent experiments

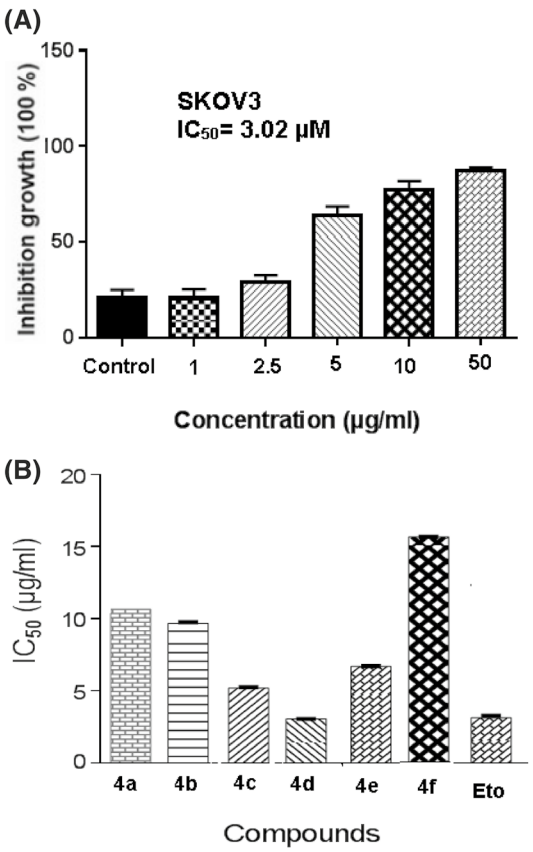


Fig. 2 **a** Inhibition growth of **4d** on SKOV3 cells. **b** Cytotoxicity activities of compounds **4a–f** and etoposide against SKOV3 cell

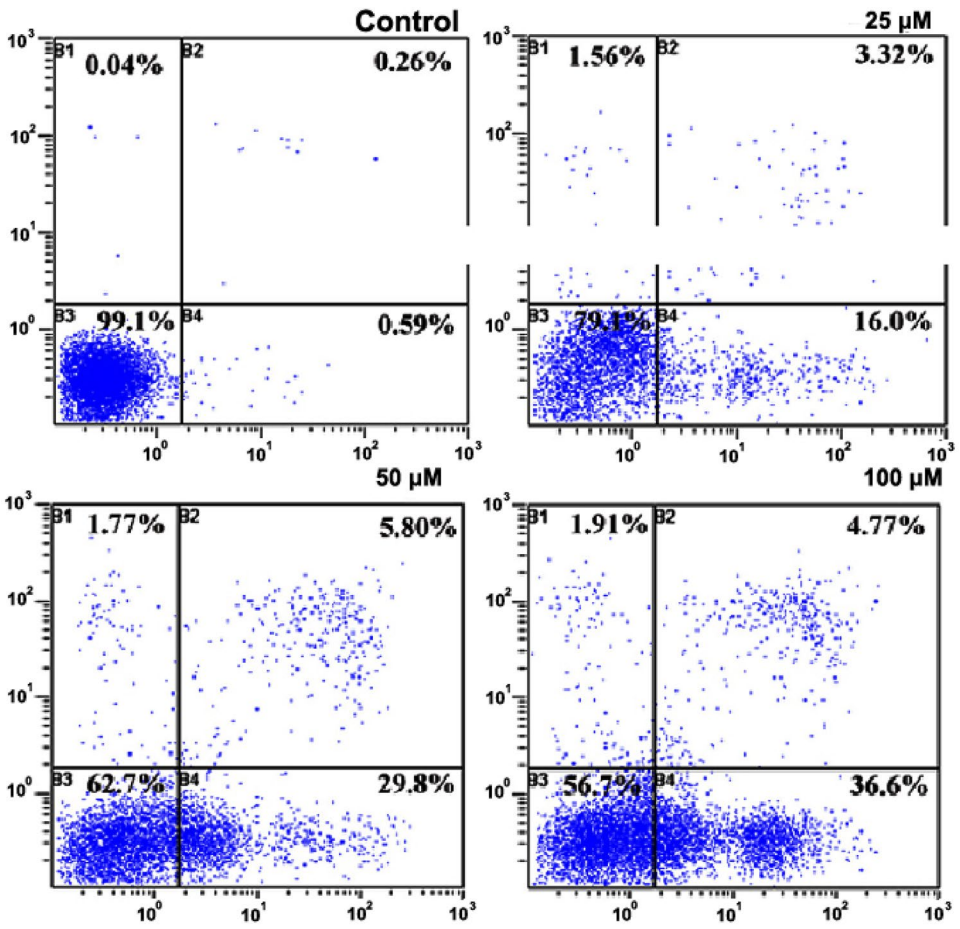


Fig. 3 Compound **4d** induced the apoptosis of SKOV3 cells. The percentages of cells in each stage of apoptosis were quantitated by flow cytometry. (B1, upper left quadrant), necrotic cells; (B2, upper right quadrant) late apoptotic cells; (B3, bottom left quadrant) live cells; and (B4, bottom right quadrant) early apoptotic cells

different substituents are related to their substituent and also the effect of the substituent on the cytotoxic activity depends on the type of tested cell line. For example, the comparison of un-substituted compound **4a** and 4-methyl derivative 4b reveals that the introduction of 4-methyl group improves the activity against MCF7 and SKOV3 cells while diminishes the activity toward A549 cells. Interestingly, the highest inhibition was obtained with 3,4,5-trimethoxy derivative **4d** against all tested cell lines, displaying IC_{50} values in the range of 3.02–4.65 μM. Its activity was better than that of etoposide against SKOV3 cell line. Comparison of the anti-proliferative activities of **4d** with other derivatives obviously indicates that the existence of 3,4,5-trimethoxyphenyl moiety plays a vital role in presenting improved anti-proliferative activity. A simple structure–activity relationship also indicated that the anti-proliferative activity was related to the substituted groups R_1. The strong inhibitory potential for **4d** and **4e** derivatives could be attributed to the electron-donating 3,4,5-trimethoxy and hydroxyl group present at para position of the aryloxy ring. A slight decrease in activity was observed when these groups was replaced by a methyl or electron-withdrawing group 4-NO_2 in compounds **4a** and **4f**. For example, regarding the IC_{50} value on SKOV3 cell compound **4d** (3.02 μM) with the electron-donating group, 3,4,5-trimethoxy **4e** (5.87 μM) and the hydroxyl group at R_1 compared to un-substituted derivative **4a** (10.48 μM), 4-methyl derivative **4b** (9.13 μM) and 4-NO2 derivative **4f** (15.37 μM) having electron-withdrawing group can donate an electron at the para position of phenyl ring strongly improving cytotoxicity of these compounds. In contrast, withdrawing the group led to a reduction in the potency of these compounds to inhibit cancer cells.

Hany et al. have reported a series of novel compounds carrying 1,2,4-triazole a scaffold as potent anti-tumor agents. 4-Me and 4-$(OMe)_3$ derivatives with IC_{50} values of 1.5 and 1.2 μM showed potent cytotoxic activity against PaCa-2 and HT-29 cell lines [41]. In the other study, the cytotoxicity activity of a series of novel S-substituted 1H-3-R-5-mercapto-1,2,4-triazoles was tested on the HT-29 colorectal cancer cell line and results revealed that 4-methyl derivative (R = 4-Me) with IC_{50} values of 87 μM had the highest activity against tested cell line [42]. A series of 3-[3-[4-(substituted)-1-cyclicamine]propyl]thio-5-substituted[1,2,4]triazoles derivatives were designed and synthesized by Murty and coworkers. The cytotoxicity

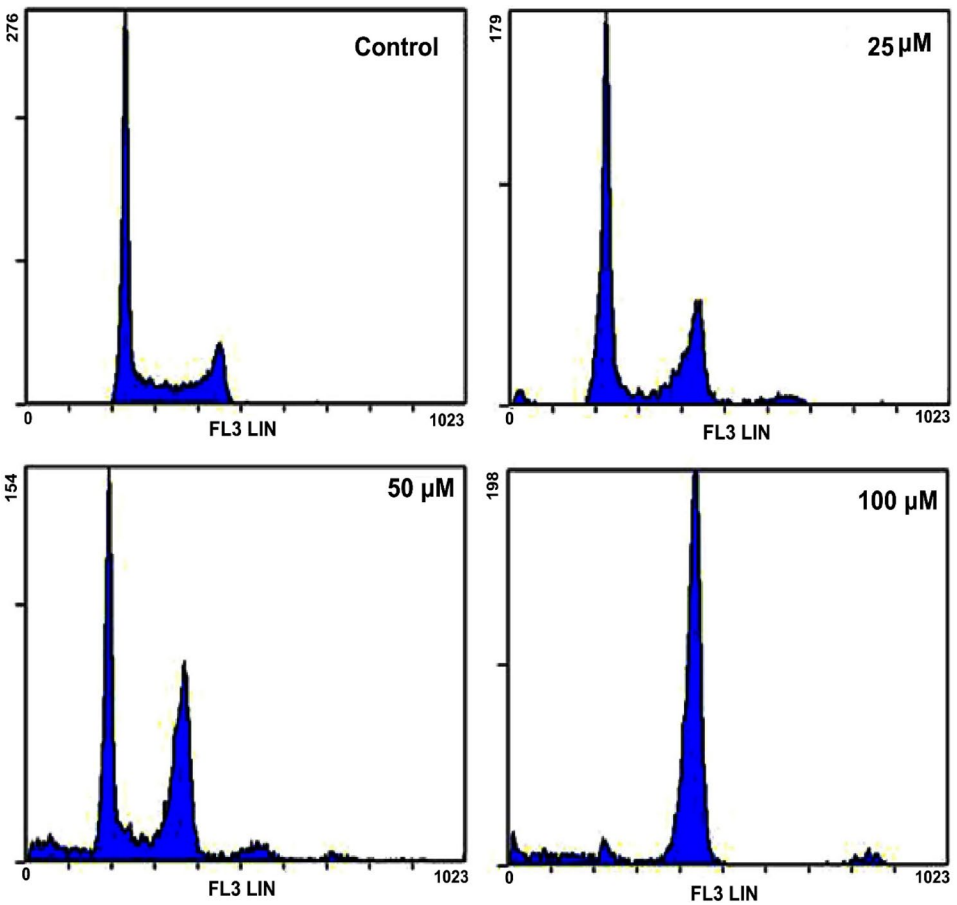


Fig. 4 Cell cycle arrest effect of **4d**. SKOV3 cells were treated with **4d** at the indicated concentrations for 24 h

assay revealed that 3-Me and 3-Cl derivatives were more potent than other synthesized compounds against HL-60 cells [43]. As shown by our results (Table 1), derivatives with 4-OMe, 4-OH and 4-$(OMe)_3$ substitutions also exhibited the highest level of cytotoxicity with an IC_{50} value of 3.02–9.25 µM against all tested cell lines.

Annexin V-FITC flow cytometric analysis of apoptosis

To clarify the possibility of phosphatidylserine externalization and investigate the consequence of apoptosis or non-specific necrosis, we evaluated the apoptotic effect on **4d** compound without any additive and with DMSO addition (0.01%) separately, using control cells, by Annexin V-FITC/PI dual staining assay [44]. SKOV3 cells were treated with the aforementioned compounds at 25, 50 and 100 µM for 48 h. As shown in Fig. 3, dose-dependent apoptosis occurred in SKOV3 cells treated with compound **4d** with different concentrations. The results demonstrated increasing percentages of apoptotic cells with increasing doses. Compound **4d** exhibited 19.32, 35.6 and 41.37% apoptosis at 25, 50 and 100 µM concentrations for 48 h, respectively.

Analysis of cell cycle arrest

To evaluate the cell cycle arrest effect of compound **4d**, SKOV3 cells were treated with DMSO (0.01%) at various concentrations (25, 50 and 100 µM) for 24 h. According to the results shown in Fig. 4, compound **4d** resulted in significant cell cycle arrest at the G2/M phase in a dose-dependent manner. As shown in Fig. 4, in the compound **4d**-treated group, 30% of the cells were at the G2/M phase 24 h after the treatment, whereas for the DMSO-treated group, 19% of the cells were in this phase at the same time after treatment. The percentage of cells at the G2/M phase correspondingly increased to 43% and 72%, respectively, after increasing the concentration of **4d** to 50 and 100 µM for 24 h.

Molecular docking studies

Previous studies have shown that trimethoxyphenyl moiety acts as a pharmacophoric group for the anticancer effect of some natural anticancer compounds such as colchicines, combretastatin, podophyllotoxin and polymethoxychalcone [45, 46]. Mechanistically, these compounds were found to be tubulin inhibitors that bind to the

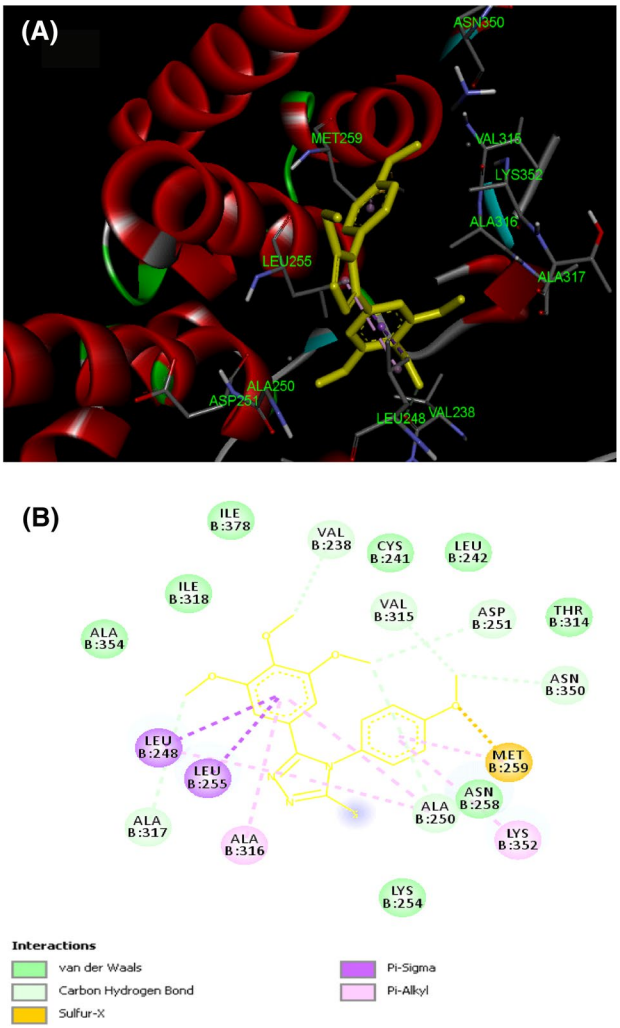


Fig. 5 3D and 2D representation of binding mode of compound **4d** (**a** and **b**) with tubulin (PDBID: 4O2B)

colchicine-binding site, leading to microtubule depolymerization. Microtubules have critical roles in several cellular processes, particularly in mitosis. Therefore, microtubules have been considered as an attractive target for anticancer drug discovery [47]. Molecular modeling was performed to investigate promising binding modes for the potential ligands toward tubulin polymerization into the colchicine-binding site of tubulin by using the AutoDock software (version 4.2). The visualization was carried out using Discovery Studio 2016 software. The most active compounds **4d** were docked into the colchicine-binding site of tubulin using the high-resolution crystal structure of the tubulin–colchicine complex screening from protein data bank (PDB entry: 4O2B) [48]. Colchicine was first docked into the pocket of the tubulin as the representative and susceptible compound in this study. Accordingly, the presence of 3,4,5-trimethoxy phenyl and nitrogen groups in the ring of the synthesized compounds may intensify the attachment of these compounds to the relevant receptor and provide a proper orientation toward the hydrogen bond formation [49, 50]. As shown in Fig. 5a, trimethoxyphenyl moiety of **4d** is surrounded by several amino acid residues such as βAla354, βIle378, βIle318, βVal238, βCys241, βLeu242, βVal315, βAsp25, βThr314, βLeu248, βLeu255, βAla317 and βAla316, located in a hydrophobic pocket. 1,2,4-Triazole moiety is buried in the amino acid residues including βAla316, βLys254, βAla317 and βLeu255. Molecular docking studies indicate the formation of two Pi-Sigma between the trimethoxyphenyl ring and Leu255 and βLeu248. Moreover, as shown in 2D representation of compound **4d** (Fig. 5b), the formation of Pi-alkyl and alkyl bonds between the p-methoxyphenyl ring of compound **4d,** and amino acid residues βLys352 between the trimethoxyphenyl ring, and Lys352 and Ala316 could be detected. A sulfur-X interaction between p-methoxyphenyl ring and βMet259 is also clarified.

Conclusion

In this study, we designed and synthesized new analogs of 3-mercapto-1,2,4-triazole potential derivatives for anti-proliferative activity. Cytotoxic activity of the synthesized compounds was evaluated against three human cancer cell lines: lung (A549), breast (MCF7) and ovarian (SKOV3). Anti-proliferative results showed that all tested compounds potentially could inhibit the growth of cancerous cells, in particular, in case of compounds **4d** and **4e**. Furthermore, Annexin V-FITC assay suggested that compound **4d** significantly induces apoptosis in SKOV3 in a concentration-dependent manner and arrests cell cycle progression at the G2/M phase due to microtubule depolymerization and cytoskeletal disruption. Molecular docking study get an obvious insight into the binding site of compound **4d** with tubulin indicating that the binding of this compound to the colchicine binding site of beta-tubulin that causing improved anti-proliferative activity. The synthesized 3-mercapto-1,2,4-triazoles may find potential practical applications as a new class of cytotoxic agents as the anti-proliferative activity of these compounds could be also useful.

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