Accepted Manuscript

New 1,2,4-triazole-Chalcone hybrids induce Caspase-3 dependent apoptosis in A549 human lung adenocarcinoma cells

Fatma F. Ahmed, Amer Ali Abd El-Hafeez, Samar H. Abbas, Dalia Abdelhamid, Mohamed Abdel-Aziz

PII: S0223-5234(18)30316-7

DOI: 10.1016/j.ejmech.2018.03.073

Reference: EJMECH 10338

To appear in: *European Journal of Medicinal Chemistry*

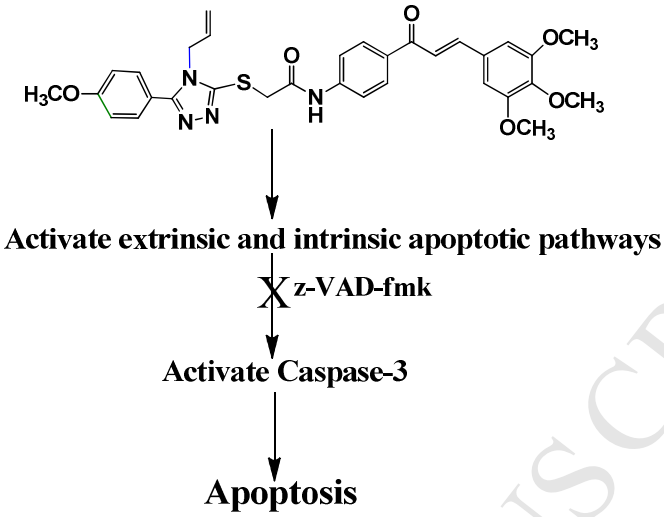
Received Date: 13 September 2017

Revised Date: 23 March 2018

Accepted Date: 24 March 2018

Please cite this article as: F.F. Ahmed, A.A. Abd El-Hafeez, S.H. Abbas, D. Abdelhamid, M. Abdel-Aziz, New 1,2,4-triazole-Chalcone hybrids induce Caspase-3 dependent apoptosis in A549 human lung adenocarcinoma cells, *European Journal of Medicinal Chemistry* (2018), doi: 10.1016/j.ejmech.2018.03.073.

Graphical Abstract



New 1,2,4-Triazole-Chalcone Hybrids Induce Caspase-3 Dependent Apoptosis in A549 Human Lung Adenocarcinoma Cells

Fatma F. Ahmed[a], Amer Ali Abd El-Hafeez[b,c,**], Samar H. Abbas[a*], Dalia Abdelhamid[a], Mohamed Abdel-Aziz[a]

[a]Medicinal Chemistry Department, Faculty of Pharmacy, Minia University, Minia 61519, Egypt.

[b]Pharmacology and Experimental Oncology Unit, Cancer Biology Department, National Cancer Institute, Cairo University, Cairo, 11796, Egypt.

[c]Pharmacotherapy Department, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan.

***** Corresponding Author

Samar H. Abbas; Phone: (+20) 100-542-4005. Fax: (+20) 086-236-9075. E-mail: drsamar77@hotmail.com, samar_hafez@mu.edu.eg.

****** Corresponding Author

Amer Ali Abd El-Hafeez; Phone: (+81)-80-4556-7496, Fax: (+81)-82-424-7000,

E-mail: amer.ali@nci.cu.edu.eg

Abstract

A series of novel 1, 2, 4-triazole/chalcone hybrids was prepared and identified with different spectroscopic techniques. The prepared compounds showed remarkable cytotoxic activity against different cancer cell lines. Compounds **24**, **25**, **27**, **41** and **47** had shown the highest cytotoxicity among the tested compounds against human lung adenocarcinoma A549 cells with IC_{50} ranging from 4.4 to 16.04 µM compared to cisplatin with IC_{50} of 15.3 µM. Flow cytometric analysis of the tested compounds showed an increase in the number of apoptotic cells in a dose-dependent manner. The further mechanistic study demonstrated that 1, 2, 4-triazole-chalcone hybrids induced apoptosis via increased level of proapoptotic protein Bax, release of cytochrome c from mitochondria and activation of caspase-3/8/9 proteins. However, general caspase inhibition by the pan-caspase inhibitor, z-VAD-fmk, significantly decreased the apoptosis induced by the tested hybrids, suggesting dependency of apoptosis on activation of the caspase-3 pathway.

Key words

1, 2, 4-triazole; chalcone; caspase-3; apoptosis; A549 human lung; NCI.

1. Introduction

Nowadays, cancer is the third leading cause of the death globally [1]. Development of drugs for cancer treatment based on the ability to block cell proliferation and/or to induce apoptosis has received great efforts in the last years [2]. Apoptosis is a form of physiological cell death that is essential for normal tissue development and homeostasis [3,4]. This process can clearly be identified by characteristic changes in the cells (i.e., caspase activation, DNA fragmentation and cell fragmentation through the formation of apoptotic bodies) [5]. Chalcones are important class of flavonoids due to their broad spectrum of biological activities including anticancer [6-9], antioxidant [10,11], antimicrobial [12,13], anticonvulsant [14,15], anti-inflammatory [16,17], antiviral [18,19], and antimalarial [20,21] activities. Chalcones exhibited remarkable anticancer activity against cancer cells and this activity may be attributed to induction of apoptosis [22], blocking cell cycle progression in the G2/M phase [23], anti-estrogenic activity [24], inhibition of tubulin polymerization [25] and inhibition of angiogenesis [22]. Chalcone derivative **I** (**Fig. 1**) exhibited promising anticancer activity with IC_{50} ranging from 5.25 to14.49 µg/mL against different cancer cell lines and it induced apoptosis through the intrinsic and extrinsic pathways in MCF-7 cells [8]. Chalcones **IIa-b** (**Fig. 1**) showed cytotoxic activity against leukemia cell lines through induction of apoptosis by activating the intrinsic pathway through reduction of the mitochondrial membrane potential, reduction in Bcl-2 expression, increase in Bax expression and increase in the active caspase-3 [26]. Moreover, amino chalcone derivative **III** (**Fig. 1**) exhibited remarkable anticancer activity through induction of apoptosis via increasing the death receptors expression TNF-related apoptosis-inducing ligand (TRAIL-R1 and TRAIL-R2) and also activation of p21, Bad, Bim, Bid, Bax, Smac, caspase-3, and caspase-8 and reducing the antiapoptotic markers livin, XIAP, and HSP27 [27]. Additionally, naphthyl chalcones derivatives **IV** (**Fig. 1**) showed anticancer activity through the apoptotic pathway by activation of caspase-8, caspase-9, and caspase-12 and increase CHOP expression [28].

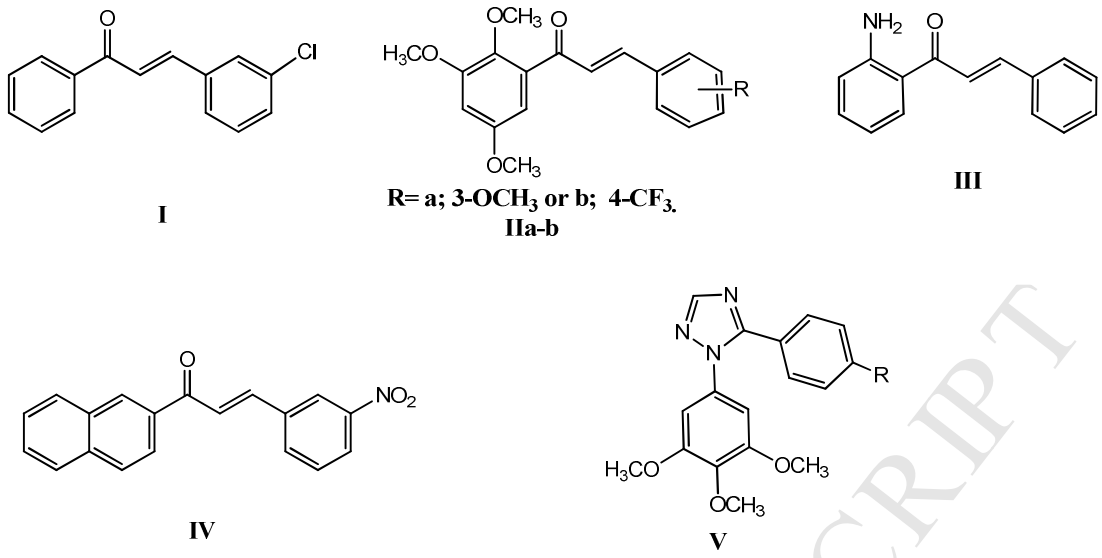
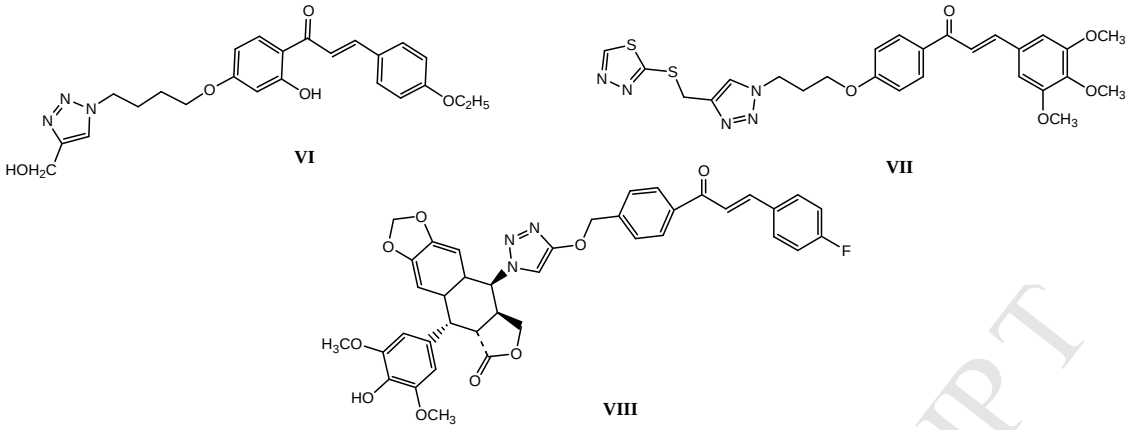


Fig. 1. Structures of compounds **I-V**.

On the other hand, triazoles are important class of heterocyclic compounds which have been studied extensively due to their numerous biological activities such as antibacterial [29], antifungal [30], antimicrobial [31], antioxidant [32], analgesic [33], .anticancer [34-36], .anticonvulsant [37],.antiviral [38] and.anti-inflammatory [39,40] activities. Triazoles are readily able to bind to variable receptors and enzymes in biological systems by diverse non-covalent interaction [41,42]. The anti-proliferative activity of 1,2,4-triazole derivative **V** (**Fig. 1**) was attributed to cell cycle arrest in the G2/M phase, tubulin polymerization inhibition, mitochondrial depolarization, and induction of apoptosis through activation of caspase-3 [43]. Moreover, efforts have been made in the last decades to improve the anticancer activity of the drugs, to solve the drug resistance, lack of selectivity and side effects of other drugs. One of these efforts was the hybridization between different chalcone derivatives and heterocyclic derivatives such as piperazine [44], quinolone [45], quinolone [46], coumarin [47,48], quinazolinone [49] and 1,2,3-triazoles [50-52] derivatives aiming to obtain synergistic anticancer activity. For example, chalcone/1,2,3-triazole hybrid **VI** (**Fig. 2**) showed cytotoxic activity with an IC_{50} value of 13.03 µM against HeLa cells relative to cisplatin as a reference drug [51]. Also, chalcone-1,2,3-triazole hybrid **VII** (**Fig. 2**) showed remarkable anticancer activity with IC_{50} of 1.52 µM against SK-N-SH cell line through induction of apoptosis [52]. Additionally, epipodophyllotoxin/chalcone hybrid **VIII** (**Fig. 2**) exhibited remarkable anticancer activity with IC_{50} ranging from 0.35 to 12.45µM, with high selectivity against SW-620 and SKN-SH cell lines [53].



Fig, 2. Structures of compounds **VI-VIII**.

However, 1,2,3- triazole-chalcone hybrids showed synergistic anticancer activities with apoptotic capacity, these hybrids lack its ability to activate the caspase-3 apoptotic pathway in low micromolar concentrations. A series of 1,2,3- triazole-chalcone was synthesized that can activate caspase-3 at 50 µM [54]. Recently, the research has been partially shifted toward 1,2,4-triazole nucleus that has been found to exhibit anticancer activity due to their interaction at the active site of a receptor as hydrogen bond acceptor and as a donor [55,56]. However, the apoptotic capacity of the 1,2,4-triazole nucleus through caspase-mediated pathway as well as its hybridization with chalcones to augment this apoptotic potency are completely unknown. Herein, we aimed to clarify and augment the apoptotic induction capacity of the 1,2,4-triazole nucleus through the caspase-3-mediated pathway via synthesis a novel series of 1,2,4-triazole-chalcone hybrids through S-alkylation of 1,2,4-triazoles with different acetylated chalcones as shown in **Fig. 3**. The chemical structures were designed so that these analogues will possess different electron donating or electron withdrawing substituents. The prepared compounds were evaluated for their cytotoxic activity against different cell lines. Moreover, the mechanistic studies of the anticancer activity of the tested compounds against A549 human lung adenocarcinoma cells were also evaluated. To the best of our knowledge, this is the first report that explains the apoptotic potency of 1,2,4-triazole-chalcone hybrids, at low micromolar concentrations, via a caspase-3-mediated pathway.

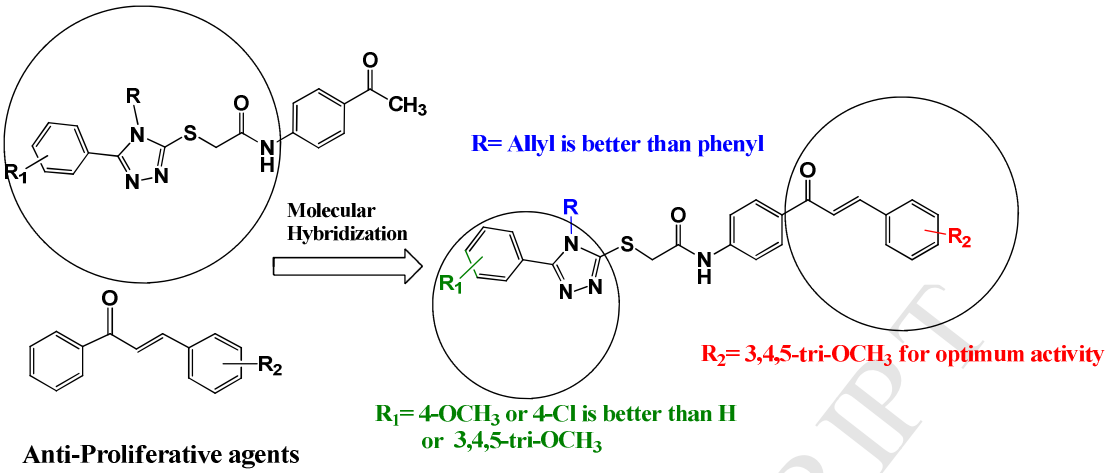


Fig. 3. Pharmacophore of the target compounds.

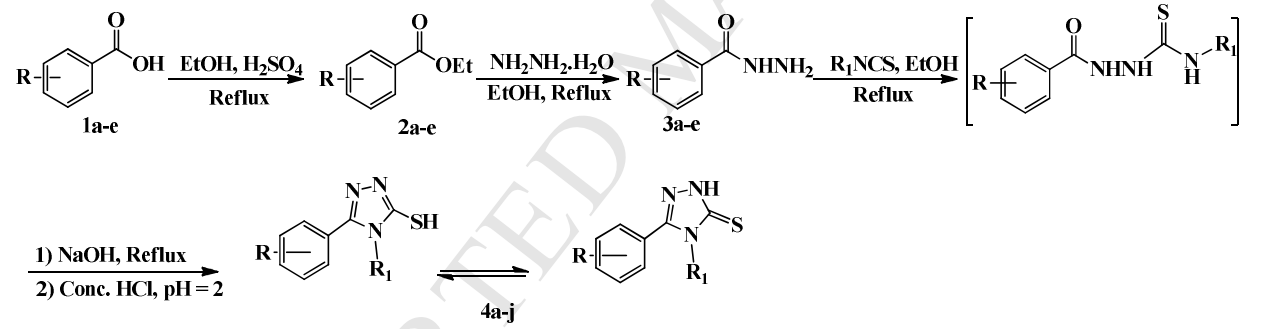
2. Results and discussion

2.1.Chemistry

The target compounds and intermediates were prepared as outlined in **Schemes 1**. The benzoic acid derivatives **1a-e** were converted to ethyl esters **2a-e** *via* Fisher esterification [57] using absolute ethanol and *conc.* H_2SO_4. The obtained esters were then converted to the corresponding hydrazides **3a-e** using hydrazine hydrate [58, 59], **Scheme 1**. Heating at reflux of equimolar amounts of the hydrazides **3a-e** and allyl/phenyl isothiocyanate in ethanol affords the corresponding 1,4-disubstituted thiosemicarbazides [58,60] which were used as crude products for the next step. The 1,2,4-triazole-3-thiol derivatives **4a-j** were prepared by intramolecular cyclization of the 1,4-disubstituted thiosemicarbazides in presence of aqueous 2N KOH followed by the acidification with concentrated hydrochloric acid [59].

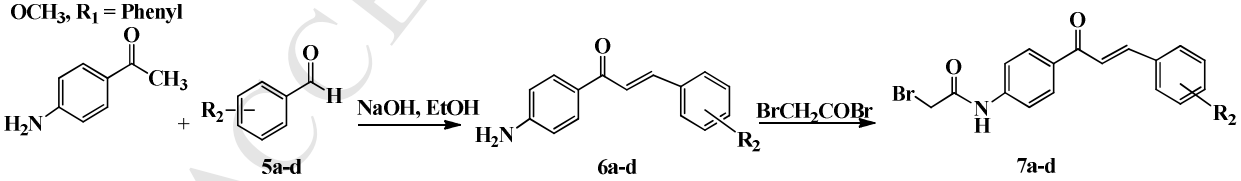
Synthesis of the acetylated chalcones starts with a reaction of various aromatic aldehydes **5a-d** with *p*-aminoacetophenone through usual Clasien Schmidt condensation to form the corresponding chalcones **6a-d**. The acetylated chalcone intermediates **7a-d** were prepared as reported [7, 46] by reaction of the appropriate chalcone with bromoacetyl bromide using potassium carbonate as a base in dichloromethane. Alkylation of triazole derivatives **4a-j** with acetylated chalcone derivatives **7a–d** was achieved in acetonitrile in the presence of TEA to afford the target compounds **8-47** in a good yield [46]. ^{1}H NMR and ^{13}C NMR confirmed the formation of target compounds. ^{1}H NMR spectra of compounds **8-47** showed a singlet signal at δ 3.96-4.32 ppm related to (S-C$\underline{H_2}$-CO) of the linker. Furthermore, The two

chalcone protons appear at the aromatic region as doublet signals at δ 7.35-7.64 ppm and 7.60-7.82 ppm with coupling constant *J* = 15.00-15.84 Hz, The amide proton NH appears as singlet signals at δ 10.83-11.22 ppm. Moreover, compounds **8-27** showed characteristic allyl signals as a doublet at δ 4.53-4.62 ppm related to (N-C<u>H</u>$_2$-CH=CH2) with coupling constant *J* = 4.00-4.86 Hz, doublet of doublet at δ 5.03-5.13 ppm and 5.32-5.45 ppm for (N-CH$_2$-CH=C<u>H</u>$_2$), with coupling constant *J* = 17.10-17.22 Hz and 9.72-11.04 Hz, respectively. Due to restricted rotation around the double bond; Multiplet signal appears at δ 5.87-6.02 ppm related to (N-CH$_2$-C<u>H</u>=CH$_2$). On the other hand; allyl signals are disappeared in ^{1}H NMR spectra for the compounds **28-47** but the aromatic protons are increased by five protons of the phenyl ring. The ^{13}C NMR spectra of compounds **8-47** revealed the presence of two carbonyl groups appeared δ 188.43-189.25 ppm and δ 166.15-168.15 ppm related to C=O of chalcone and N-C=O, a characteristic signal of SCH$_2$ appears at 35.81-37.54 ppm. Moreover, NCH$_2$ signal appears at δ 45.90-47.60 ppm in compounds **8-27**.

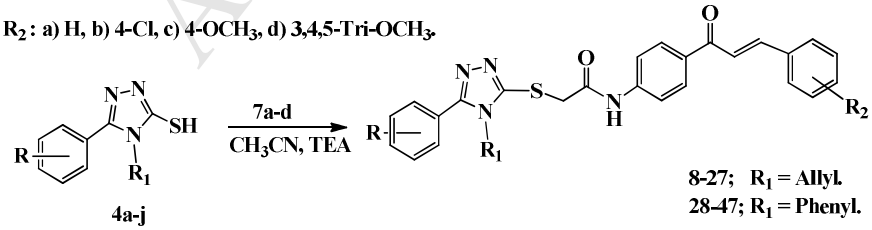


4a; R = H, R_1 = Allyl; 4b; R = 4-Cl, R_1 = Allyl; 4c; R = 4-OCH_3, R_1 = Allyl; 4d; R = 3,4-DiOCH_3, R_1 = Allyl; 4e; R = 3,4,5-Tri-OCH_3, R_1 = Allyl

4f; R = H, R_1 = Phenyl; 4g; R = 4-Cl, R_1 = Phenyl; 4h; R = 4-OCH_3, R_1 = Phenyl; 4i; R = 3,4-DiOCH_3, R_1 = Phenyl; 4j; R = 3,4,5-Tri-OCH_3, R_1 = Phenyl



R_2 : a) H, b) 4-Cl, c) 4-OCH_3, d) 3,4,5-Tri-OCH_3.



8-27; R_1 = Allyl.
28-47; R_1 = Phenyl.

Compd.	R_1	R_2	Compd.	R_1	R_2
8, 28	H	H	**18, 38**	H	4-OCH_3
9, 29	4-Cl	H	**19, 39**	4-Cl	4-OCH_3
10, 30	4-OCH_3	H	**20, 40**	4-OCH_3	4-OCH_3
11, 31	3,4-di-OCH_3	H	**21, 41**	3,4-di-OCH_3	4-OCH_3
12, 32	3,4,5-tri-OCH_3	H	**22, 42**	3,4,5-tri-OCH_3	4-OCH_3
13, 33	H	4-Cl	**23, 43**	H	3,4,5-tri-OCH_3
14, 34	4-Cl	4-Cl	**24, 44**	4-Cl	3,4,5-tri-OCH_3
15, 35	4-OCH_3	4-Cl	**25, 45**	4-OCH_3	3,4,5-tri-OCH_3
16, 36	3,4-di-OCH_3	4-Cl	**26, 46**	3,4-di-OCH_3	3,4,5-tri-OCH_3
17, 37	3,4,5-tri-OCH_3	4-Cl	**27, 47**	3,4,5-tri-OCH_3	3,4,5-tri-OCH_3

Scheme1:-Synthesis of the target compounds **8-47**.

2.2. Biological evaluation

2.2.1. Cytotoxic assays

2.2.1.1. *In vitro* one dose assay

Compounds **9-11, 13-19, 21-27, 29-35, 37-41, 43-45** and **47** were selected by the National Cancer Institute (NCI), USA for *in vitro* anticancer screening. Compounds were screened at 10 μM dose using Sulforhodamine B colorimetric assay, against full NCI 60 cell lines derived from nine tumor subpanels, including leukemia, lung, colon, melanoma, renal, prostate, CNS, ovarian, and breast cancer cell lines.

Results showed that the tested compounds had a promising anticancer activity (**Table 1, 2, supporting information**) against human cancer cells especially compounds **24**, **25**, **27**, **41** and **47** (mean growth inhibition percentages were 82.79, 108.20, 65.81, 61.60 and 92.19%, respectively (**Fig. 80, supporting information**)).

Furthermore, eighteen compounds; **9, 11, 13-15, 17, 18, 22-25, 27, 31, 41, 43-45** and **47** exhibited remarkable anticancer activity against RPMI-8226 cell line with cell growth inhibition% ranging from 68.90 to 133.10%. Additionally, compounds; **9, 11, 13, 14, 17, 18, 22-25, 27, 31, 32, 34, 40, 41, 43, 44** and **47** showed remarkable anticancer activity against HCT-116 cell line with cell growth inhibition% ranging from71.98–191.71%.

On The other hand; only the compounds; **11,13, 18, 22-25, 27, 41, 44** and **47**

exhibited remarkable anticancer activity against MCF7 cell line with cell growth inhibition% ranging from 68.59 – 108.34%.

2.2.1.2 *In vitro* five-dose assay

Compounds **26, 27, 29, 43** and **49** were selected for advanced five-dose testing against the full panel of 60 human tumor cell lines. All the 60 cell lines representing nine tumor subpanels were incubated at five different concentrations (0.01, 0.1, 1, 10 and 100 µM). This revealed their great potency against almost cell lines. Data of five dose assay results showed that the five selected compounds exhibited a remarkable and broad-spectrum antitumor activity against all cell lines used with GI_{50} ranging from 0.15 to 23.30 µM (**Table 1**) with no selectivity (**Tables 3-7, supporting information**).

Table (1): GI_{50} of compounds 24, 25, 27, 41 and 47 against 60 cell lines of 9 different cancer panels tested using NCI's in vitro five dose anticancer assay.

Panel/Cell Line	GI_{50} (μM) Compound					Panel/Cell Line	GI_{50} (μM) Compound				
	24	**25**	**27**	**41**	**47**		**24**	**25**	**27**	**41**	**47**
Leukemia						**Melanoma**					
CCRF-CEM	2.38	0.34	3.33	1.85	nd	**LOX IMVI**	1.66	0.21	2.29	1.46	nd
HL-60(TB)	2.65	0.32	2.88	2.57	nd	**MALME-3M**	6.69	1.28	11.10	1.75	nd
K-562	1.70	0.40	3.44	3.02	nd	**M14**	2.43	0.32	2.96	1.80	nd
MOLT-4	2.66	0.33	4.22	2.26	nd	**MDA-MB-435**	1.58	0.27	2.72	1.91	nd
RPMI-8226	0.25	0.15	1.51	1.72	0.27	**SK-MEL-2**	4.52	1.78	14.80	5.03	nd
SR	1.52	0.23	2.34	0.72	0.32	**SK-MEL-28**	4.23	0.71	3.29	5.78	nd
Non-Small Cell Lung Cancer						**SK-MEL-5**	2.53	0.42	4.27	1.81	nd
A549/ATCC	3.61	0.46	6.46	14.90	nd	**UACC-257**	6.38	nd	7.45	12.0	nd
EKVX	2.92	1.33	4.03	3.05	nd	**UACC-62**	2.94	0.85	3.31	2.26	nd
HOP-62	3.76	0.28	5.16	4.36	1.84	**Ovarian Cancer**					
HOP-92	2.47	6.58	12.50	1.56	2.06	**IGROV1**	2.76	0.33	3.42	1.85	nd
NCI-H226	1.94	0.22	1.98	3.92	nd	**OVCAR-3**	2.41	0.25	2.98	1.72	nd
NCI-H23	2.70	0.43	3.41	1.80	nd	**OVCAR-4**	3.10	0.56	5.67	1.96	1.80
NCI-H322M	4.89	0.60	6.85	13.60	nd	**OVCAR-5**	4.75	1.37	3.97	14.00	nd
NCI-H460	3.41	0.34	3.76	5.05	nd	**OVCAR-8**	2.79	0.46	5.94	3.09	nd
NCI-H522	3.25	0.39	6.90	1.48	2 .05	**NCI/ADR-RES**	4.74	1.01	15.30	2.66	nd
Colon Cancer						**SK-OV-3**	6.01	0.64	5.08	13.10	nd
COLO 205	4.92	0.40	4.94	2.10	nd	**Renal Cancer**					
HCC-2998	2.36	0.18	2.05	2.97	nd	**786-0**	2.16	0.31	1.85	1.78	1.05
HCT-116	0.47	0.15	2.09	1.17	nd	**A498**	2.47	4.11	7.40	14.70	1.35
HCT-15	2.68	0.22	1.86	1.80	nd	**ACHN**	3.67	3.43	4.16	2.38	1.61
HT29	2.33	0.24	2.66	1.86	nd	**RXF 393**	1.47	0.20	2.16	1.66	1.33
KM12	1.97	0.30	2.82	2.24	nd	**SN12C**	3.43	0.46	4.74	2.25	nd
SW-620	3.00	0.36	3.37	1.96	nd	**TK-10**	7.87	1.28	23.30	14.60	9.30
CNS cancer						**UO-31**	1.98	0.21	4.18	1.48	nd
SF-268	4.32	0.32	3.99	1.89	nd	**Breast Cancer**					
SF-295	4.49	0.40	5.25	12.20	1.90	**MCF7**	0.38	0.20	1.83	1.41	nd
SF-539	2.96	0.25	2.52	1.80	nd	**MDA-MB231/ ATCC**	0.38	0.42	3.81	1.73	4.09
SNB-19	3.64	0.35	4.30	3.50	2.18	**HS 578T**	5.81	0.74	4.66	7.68	20.40
SNB-75	1.79	0.22	2.71	2.60	0.43	**BT-549**	2.40	0.21	2.14	1.60	nd
U251	nd	0.23	2.15	1.75	0.53	**T-47D**	2.24	0.39	5.44	2.24	nd
Prostate Cancer						**MDA-MB-468**	3.47	0.32	2.45	1.96	nd
PC-3	3.20	0.56	4.29	3.09	nd						
DU-145	3.73	0.44	6.09	3.21	nd						

nd= not detected

2.2.1.3 *In vitro* Anti-proliferative assay on A549 cells

Independently, all the synthesized compounds **8-47** were tested for their inhibitory effect on human non-small cell lung cancer A549 cell growth using BrdU incorporation assay. A549 cells were exposed to different concentrations (0.5, 1, 5, 10, 25, 50 or 100 µM) from chalcone/ 1,2,4-triazole hybrids **8-47** or the reference cisplatin for 24 h using DMSO as a negative control. Data were summarized in **Table2**. Twenty-five compounds out of forty showed significant cell growth inhibition on A549 cells (IC_{50} = 4.4-79.6 µM) in a dose-dependent manner, while fifteen compounds had very weak or no anticancer activity (IC_{50} > 100 µM) in comparison with cisplatin as a positive control (IC_{50} = 15.3 µM). In particular, in agreement with the NCI results, compounds **24**, **25**, **27**, **41** and **47** exhibited the highest activities against A549 cells (IC_{50} = 6.06, 4.4, 7.55, 16.04 and 8.04 µM, respectively).

In other words, through a simple structure-activity relationship (SAR) analysis, we found that hybrids containing *N*4-allyl triazole were more active than that containing *N*4-phenyl triazole. In addition, substitution in both phenyl rings was essential for anticancer activity. Also, for optimum activity in the allyl triazole hybrids; R_2 must be 3,4,5-trimethoxy groups and the phenyl ring must be substituted with mono substitution either electron donating or electron withdrawing (OCH_3 or Cl) good in *p*-position but not exclusive as in case of unsubstituted ring, the activity decreased slightly, while in case of 3,4-dimethoxy substitution, the activity decreased to great extent but the activity retained in case of trimethoxy substitution. On the other hand; for optimum activity in the phenyl triazole hybrids; R_1 and R_2 must be 3,4,5-trimethoxy groups. The activity decreased slightly if the phenyl ring substituted with mono substitution either electron donating or electron withdrawing (OCH_3 or Cl) in *p*-position. From these results, it is obvious that 1,2,4-triazole/chalcone hybrids especially compounds **24**, **25**, **27**, **41** and **47** had the ability to suppress the cell proliferation in human cancer cells especially A549cells.

Table (2): Cytotoxic activity of triazole-chalcone hybrids, **8-47**, against human lung adenocarcinoma A549 cell line for 24 h.

Compound	IC_{50} (µM)± SD	Compound	IC_{50} (µM)± SD
8	**>100**	**28**	**>100**
9	**>100**	**29**	**19.43±1.7**
10	**>100**	**30**	**24.02±0.9**
11	**23.15±1.3**	**31**	**30.7±1.05**
12	**28.2±0.81**	**32**	**>100**
13	**22.7±0.3**	**33**	**49.82±2.2**
14	**>100**	**34**	**>100**
15	**44.15±2.5**	**35**	**21.9±1.8**
16	**>100**	**36**	**>100**
17	**23.6±1.5**	**37**	**19.58±0.58**
18	**24.6±0.42**	**38**	**>100**
19	**>100**	**39**	**49.25±3.45**
20	**79.6±3.08**	**40**	**46.72±1.85**
21	**>100**	**41**	**16.04±1.32**
22	**16.6±1.6**	**42**	**>100**
23	**21.8±0.9**	**43**	**>100**
24	**6.06±0.5**	**44**	**20.82±0.78**
25	**4.4±0.3**	**45**	**31.5±1.27**
26	**59.7±2.4**	**46**	**>100**
27	**7.55±0.8**	**47**	**8.04±0.59**
Cisplatin	**15.3±1.04**		

2.2.2. Triazole-chalcone hybrids induced apoptosis in A549 human lung adenocarcinoma cells

Apoptosis plays an important role in hemostasis process. Balance between survival and apoptosis is critical for the maintenance of physiologic functions. Imbalance toward the survival in cell results in cancer development and resistance to anticancer therapies such as radiotherapy and chemotherapy. Apoptosis is typically accompanied by the activation of a class of death proteases (caspases) [61]. The activation of the caspase cascade is involved in chemical-and agent-induced apoptosis [62]. Caspase-3 has been shown to be a key component involved in the underlying mechanisms of apoptosis and lies on the action of the initiator caspases including caspase-8 and caspase-9 for its action [63]. Caspase-8 is usually activated through the extrinsic pathway triggered by tumor necrosis factor receptor and Fas/CD95 receptor [64]. In contrast, caspase-9 is activated through the intrinsic pathway, which can be initiated by translocation of Bax into mitochondria [65]. This is followed by an increase in mitochondrial membrane permeability and a release of cytochrome c from mitochondria into the cytosol. In the cytosol, cytochrome c activates caspase-9, which

leads to apoptotic cell death. Both pathways activate caspase-3, eventually causing apoptosis [66]. To study in depth the bioactivities of triazole-chalcone hybrids, compounds **24, 25, 27, 41** and **47,** against A549 cells, the cancer cells were treated with vehicle alone as a control or with one of the five testing compounds at different concentrations (1, 5 and 10 μM). After 24 h, the samples were double-stained with annexin V and PI. The percentages of cell populations at various stages of apoptosis were exhibited in **Fig. 4.** (A and B). The five compounds increased both early and late apoptotic cells percentage in a dose-dependent manner. At 10 μM, the total apoptotic cells percentages (early + late, annexin V positive cells) were higher in compounds **24**, **25** and **27** (78.6, 54.4 and 66.1%, respectively) than in compounds **41** and **47** (24.2 and 42.3 %, respectively) in comparison with control (9.5%). It is obvious from these results that hybrids containing allyl triazole (**24**, **25** and **27**) were more apoptotic inducers than hybrids containing phenyl triazole (**41** and **47**) which confirm our previous cytotoxicity assay results. Evidently, these data pointed out triazole-chalcone hybrids having the ability to induce apoptosis in A549 cells at low micromolar.

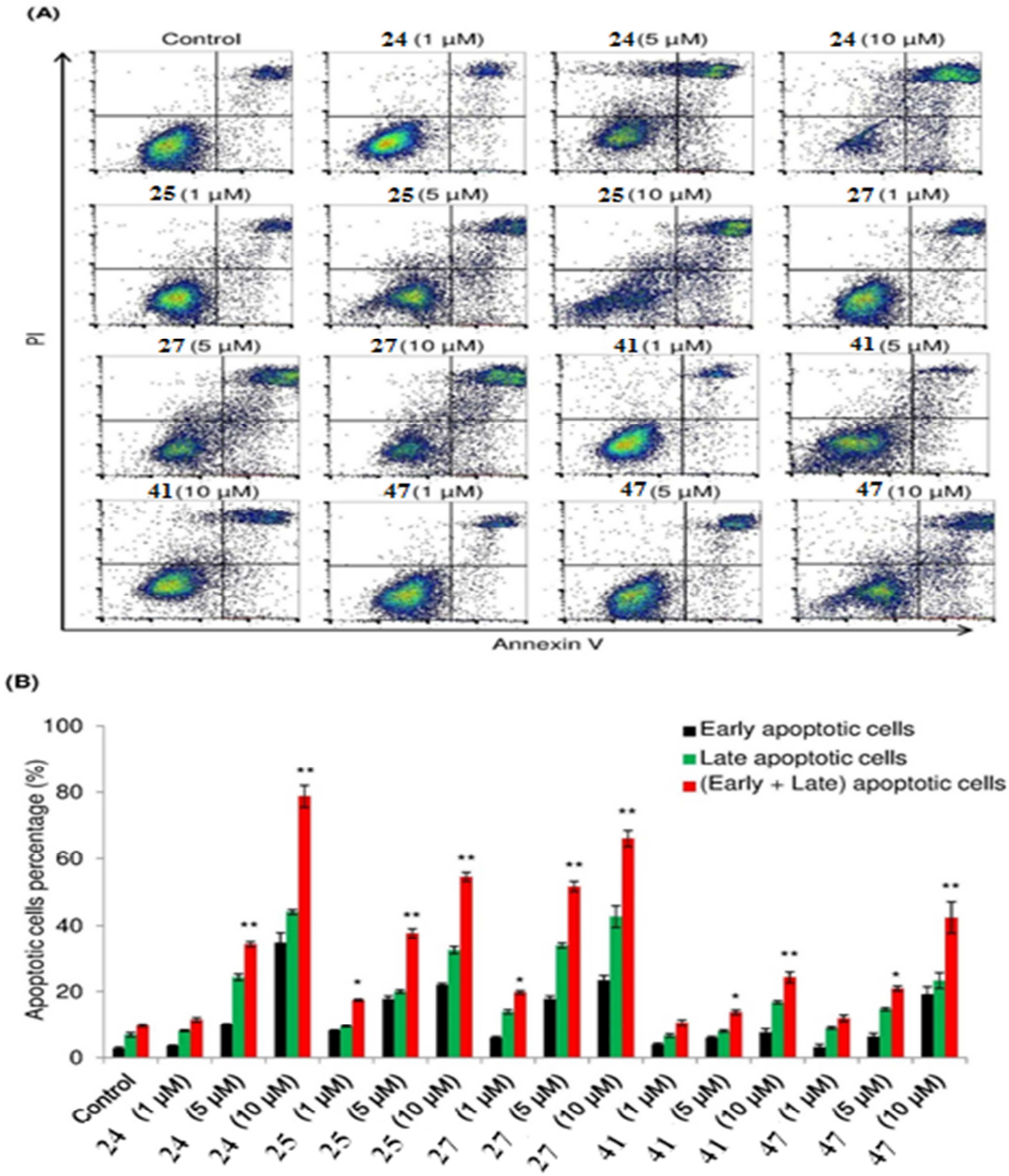


Fig.4. (A) Flow cytometric analysis of A549 cells treated with different concentrations (0, 1, 5, 10 μM) of triazole-chalcone hybrids, (**24, 25, 27, 41** and **47)** for 24 h. **(B)** Apoptotic cell % of early, late and total (early + late) apoptotic cells after incubation of A549 cells with different concentrations (0, 1, 5, 10 μM) of triazole-chalcone hybrids, (**24**, **25**, **27**, **41** and **47**) for 24 h. The values are expressed as the mean ± SD of three different experiments. *, $P < 0.05$ and **, $P < 0.005$ indicate a significant difference compared with vehicle treatment (control).

2.2.3. Triazole-chalcone hybrids induced apoptosis through activation of caspase-3, -8 and -9

The effect of triazole-chalcone hybrids **24, 25, 27, 41** and **47** on the expression of pro-apoptotic markers (Bax, cytochrome c and caspases 3, 8 and 9) was tested to elucidate signal transduction pathway through which these compounds fulfill their apoptotic effect as shown in **Fig.5.** (A-C). All tested compounds upregulated Bax protein in a dose-dependent manner. As a consequence of Bax activation, cytochrome c is also released from the mitochondria and increased in the cytosol, in a dose-dependent manner, after treatment with the mentioned compounds. Furthermore, cleaved caspase-3, -8, and -9 were increased by triazole-chalcone hybrids treatment for 24 h in a dose-dependent manner. From these results, it is observed that the ability of the allyl triazole compounds (**24**, **25** and **27**) to upregulate the tested apoptotic markers was higher than the phenyl triazole compounds (**41** and **47**). Furthermore, it was observed that although compound **47** always had higher activities than **43** but its ability to upregulate cleaved caspase 8 was very weak.

Furthermore, triazole-chalcone hybrids activated both extrinsic and intrinsic apoptotic pathways. The studied compounds activated the extrinsic pathway via activation of caspase-8 while activated the intrinsic pathway through Bax activation, the release of cytochrome *c* from mitochondria and caspase-9 activation. The two apoptotic pathways activated the most effector caspase-3 leading to apoptosis in A549 cells. Triazole alone can induce apoptosis; these results in agreement with the previous reports that showed triazole-chalcone hybrids induce apoptosis through the mitochondria and/or death receptor pathways in several cancer cell lines [67, 54].

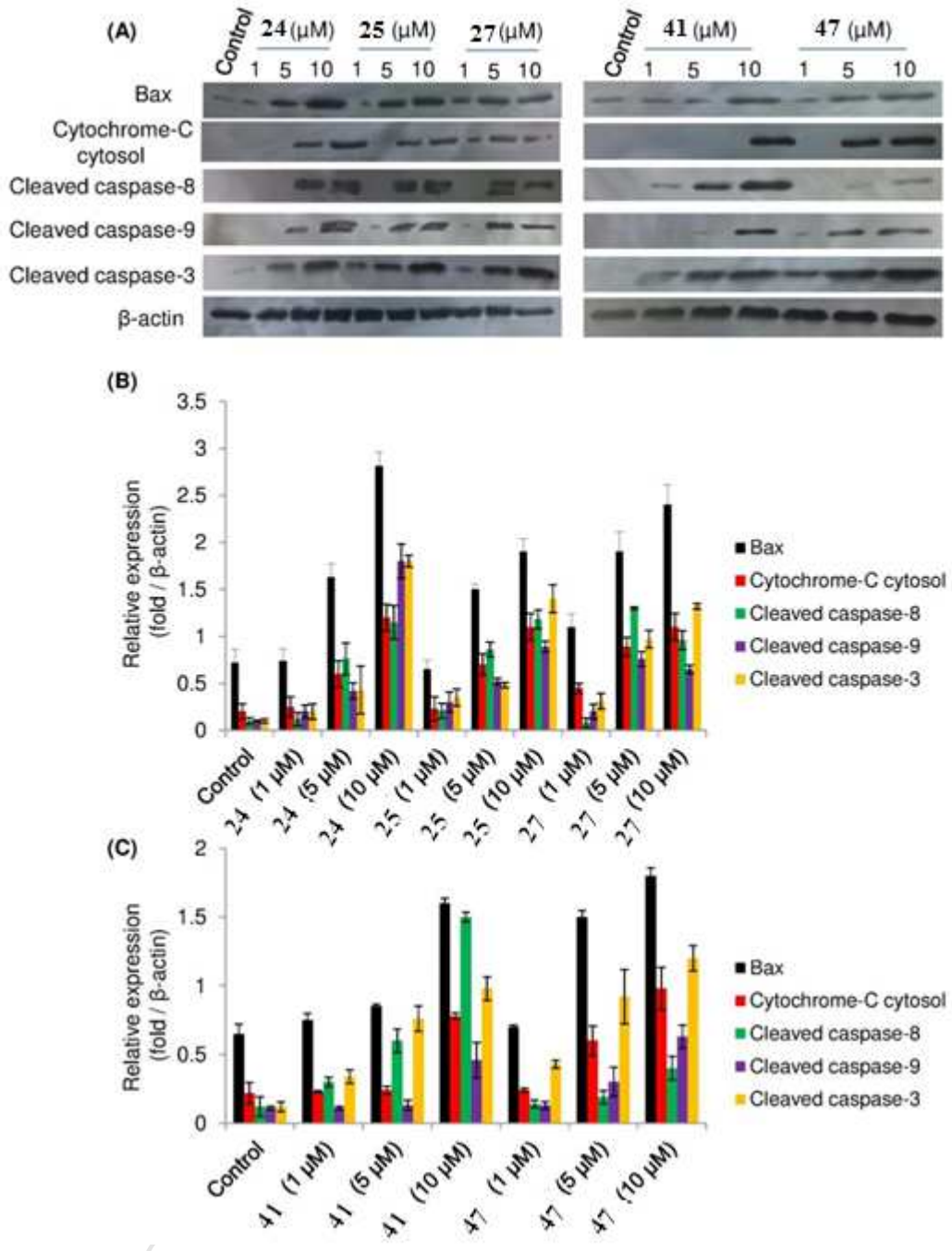


Fig.5. (A) Western blotting analysis of the pro-apoptotic marker proteins, Bax, cytochrome c and cleaved caspase-3/8 and 9 in A549 cells treated with 0, 1, 5, or 10 μM triazole-chalcone hybrids, (**24, 25, 27, 41** and **47**). β-actin served as a loading control. **(B-C)** The relative expression (fold/ β-actin) of Bax, cytochrome c and cleaved caspase-3/8 and 9 compared to β-actin and quantified by Image J. The values are expressed as the mean ± SD of three different experiments.

2.2.4. Pan-caspase inhibitor, z-VAD-fmk, decreased triazole-chalcon hybrids-induced caspase-3 activation in A549 cells

To investigate the relationship between apoptosis induced by triazole-chalcone hybrids in A549 cells and caspase-3, pan-caspase inhibitor z-VAD-fmk was tested. Representative results are shown in **Fig.6A**, and quantitative data from 3 different experiments are summarized in **Fig.6B**. The cleaved Caspase-3 level after triazole-chalcone hybrids treatment in the presence of z-VAD-fmk was significantly decreased at 24 h after treatment, as compared with triazole chalcone hybrids treatment alone.

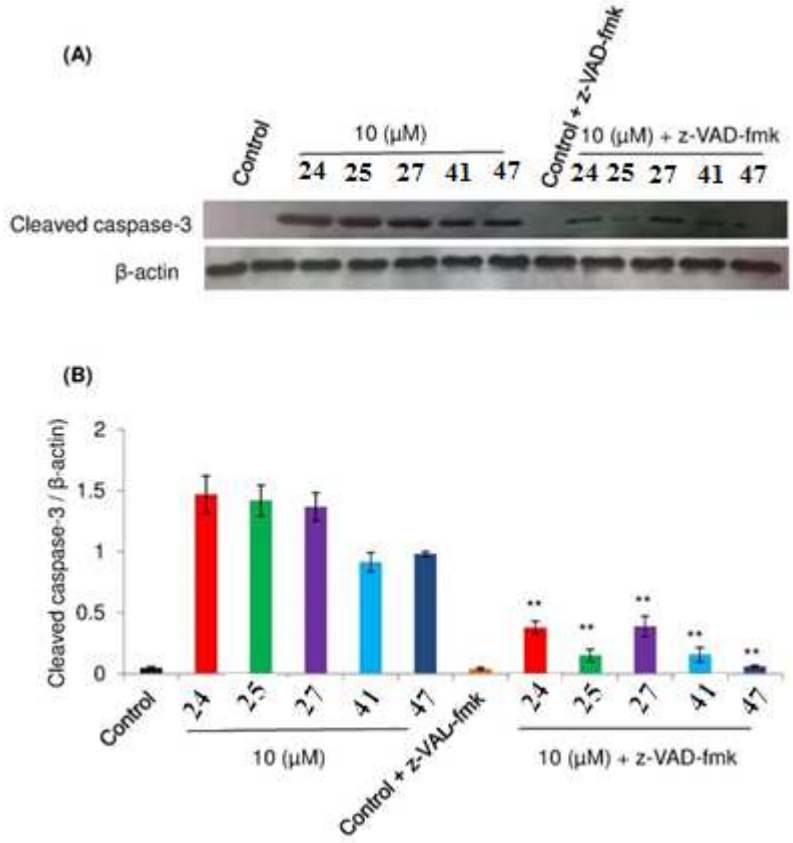


Fig.6. (A) Western blotting analysis of cleaved caspase-3 in A549 cells treated with 0, or 10 μM triazole-chalcone hybrids, (**24, 25, 27, 41** and **47**) in the absence or presence of Pan-caspase inhibitor (z-VAD-fmk) for 24 h. β-actin served as a loading control. **(B)** The relative protein expression levels of cleaved caspase-3 compared to β-actin and quantified by Image J. The values are expressed as the mean ± SD of three different experiments. **, $P < 0.005$ indicates a significant difference vs. triazole-chalcone hybrids-treated cells.

2.2.5. Triazole-chalcone hybrids induce Caspase-3 dependent apoptosis

The effect of the general caspase inhibitor, z-VAD-fmk, on the triazole chalcone hybrids- induced apoptosis was determined by Annexin V assay (**Fig. 7A-B**). Triazole-chalcone hybrids treatment for 24 h in the presence of z-VAD-fmk significantly reduced the total apoptotic cells (early+late) in all tested compounds. The great effect was found in the presence of compound **25**, **27**, **41** and **47** where the total apoptotic cells percentage reduced from 53.8 to 20.09%, 64.4 to 26.46%, 24.04 to 15.1%, and 44.4 to 13.33%, respectively. However, z-VAD-fmk significantly reduced the apoptotic cells percentage, induced by compound **24**, from 78.23 to 50.67 % which still higher than control (11.85 %). These results indicated that triazole-chalcone hybrids induced apoptosis via caspase-3 dependent pathway.

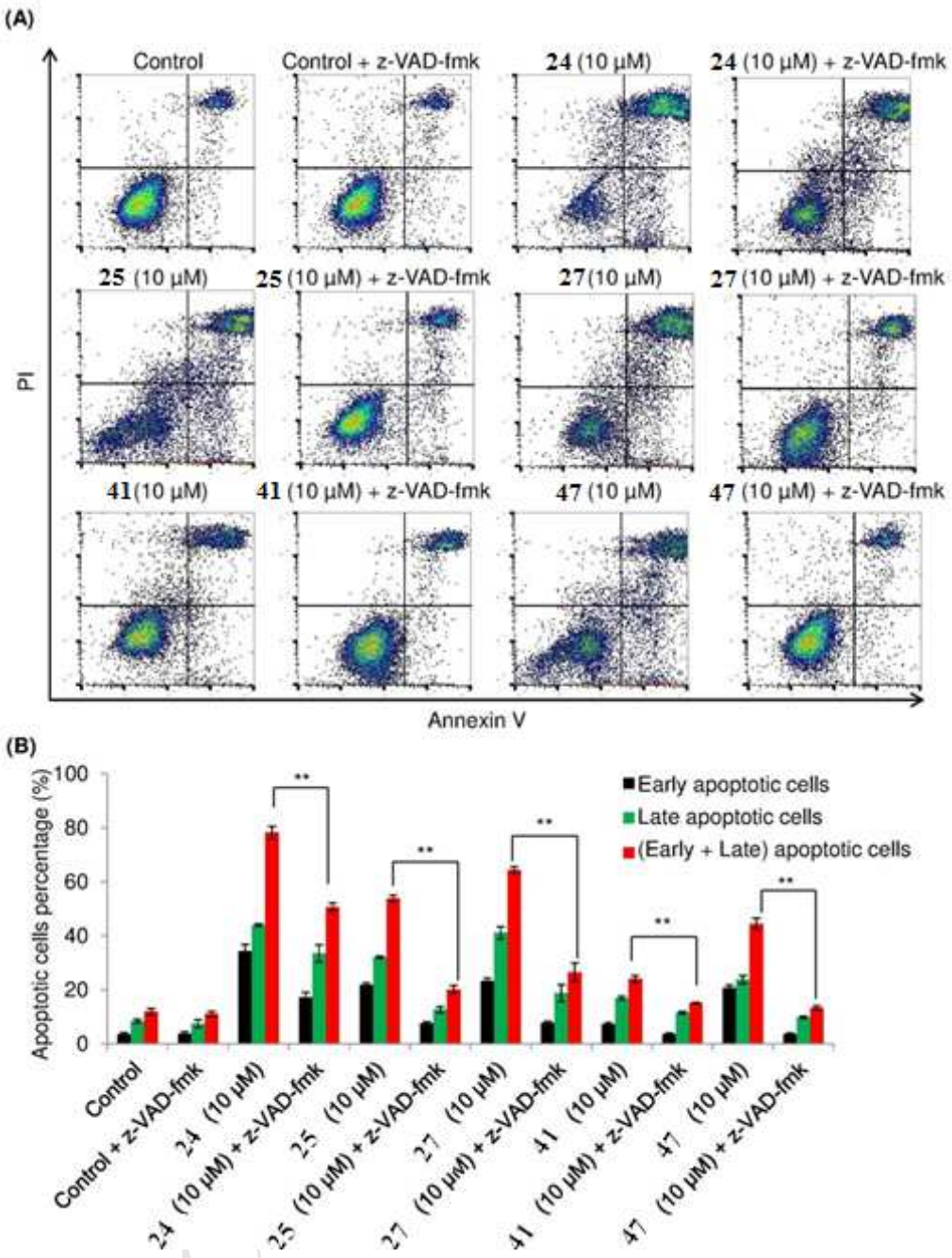


Fig.7. (A) Flow cytometric analysis of A549 cells treated with 0 or 10 μM of triazole-chalcone hybrids, (**24**, **25**, **27**, **41** and **47**) with or without z-VAD-fmk for 24 h. **(B)** Apoptotic cells % of early, late and total (early + late) apoptotic cells after incubation of A549 cells with 0 or 10 μM of triazole-chalcone hybrids, (**24, 25, 27, 41** and **47**) with or without z-VAD-fmk for 24 h. The values are expressed as the mean ± SD of three different experiments. **, $P < 0.005$ indicates a significant difference vs. triazole-chalcone hybrids treated cells.

3. Conclusion

A series of novel 1,2,4-triazole/chalcone hybrids was prepared and identified with different spectroscopic techniques. The prepared compounds showed remarkable cytotoxic activity against different cancer cell lines. Compounds **24**, **25**, **27**, **41** and **47** had shown the highest cytotoxicity among the tested compounds against human lung adenocarcinoma A549 cells with an IC_{50} range from 4.40 to 16.04 µM compared to cisplatin with IC_{50} of 15.3 µM. Primary *in vitro* one dose anticancer and BrdU incorporation assays confirmed that compounds **24, 25, 27, 41** and **47** exhibited the highest activities against human cancer cells. The results indicated that the hybrids containing allyl triazole were more active than that containing phenyl triazole. Also, it was observed that the apoptosis was induced in a dose-dependent manner. Furthermore, triazole-chalcone hybrids activated both extrinsic and intrinsic apoptotic pathways. The studied compounds activated the extrinsic pathway via activation of caspase-8 while activated the intrinsic pathway through Bax activation, the release of cytochrome *c* from mitochondria and caspase-9 activation. Additionally, the apoptotic cells percentages in all tested compounds were significantly reduced after using z-VAD-fmk which indicated the dependency of triazole-chalcone hybrids-induced apoptosis on the caspase-3 pathway. In summary, 1,2,4-triazole/chalcone hybrids induced caspase-3 dependent apoptosis through both extrinsic and intrinsic pathways in A549 cells. The finding that 1,2,4-triazole/chalcone hybrids had an anticancer effect is meaningful for the treatment of lung cancer as an alternative to conventional chemotherapy, which has many side effects. However, further study with *in vivo* models is needed to clarify the efficacy of 1,2,4-triazole/chalcone hybrids as an anticancer agent.

4. EXPERIMENTAL SECTION

4.1.Chemistry section:

- Chemicals and solvents that were used for the analytical grade. The progress of the reactions was monitored by thin layer chromatography pre-coated Merck silica gel 60 F254 aluminum sheets.
- Melting points were determined on Stuart electro-thermal melting point apparatus and were uncorrected.

- ^{1}H spectra were recorded on JEOL JNM-GX-600 spectrometer (600 MHz); Japan and Burker AG, Switzerland, 500 MHz, Faculty of Pharmaceutical Sciences, Umm Al-Qura University, Mecca, Saudi Arabia; chemical shift (δ) in ppm relative to TMS (δ=0 PPM) as internal standard and $CDCl_3$ as a solvent. Chemical shifts (δ) are expressed in parts per million (ppm) and coupling constants (*J*) are expressed in Hertz. The signals are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet.
- ^{13}C spectra were recorded on JEOL JNM-GX-150 MHz; Japan and Burker AG, Switzerland and 125 MHz, faculty of Pharmaceutical Sciences, Umm Al-Qura University, Mecca, Saudi Arabia using TMS as the reference standard and $CDCl_3$ as a solvent. Chemical shifts (δ) are expressed in parts per million (ppm).
- The purity of the compounds was checked by HPLC.
- Elemental analyses were recorded on Shimadzu GC/ MS-QP5050A, The Regional center for Mycology and Biotechnology, Al-Azhar University, Egypt.

4.1.1. General procedure for the synthesis of substituted ethylbenzoate 2a-e [57].

A mixture of the appropriate substituted benzoic acid **1a-e** (10 mmol), absolute ethanol (20 mL) and concentrated sulfuric acid (2 mL) was heated under reflux for 12-18 h. Excess solvent was removed under reduced pressure; The residue extracted with ether (2 X 50 mL) and washed with saturated $NaHCO_3$ (2 X 20 mL). The ether layer was dried over anhydrous magnesium sulphate, and the ether was evaporated under vacuum to give the ethyl ester derivatives **2a-e**.

4.1.2. General procedure for the synthesis of substituted benzohydrazides 3a-e [58, 59].

A solution of the isolated esters **2a–e** (10 mmol) in ethanol (20 mL), hydrazine hydrate (97 %, 3 mL) was added and heated under reflux for 5-8 h. After cooling, the formed precipitate was filtered off, washed with water, dried, and crystallized from ethanol.

Benzohydrazide (3a) [59].

White solid (77.2 % yield); mp 110-112^{o}C (Reported mp = 112-114 ^{o}C).

4-Chloro-benzohydrazide (3b) [68].

White solid (79.3% yield); mp 195-197 ^{o}C (Reported mp = 189-191^{o}C).

4-Methoxybenohydrazide (3c) [59].

White solid (80.2 % yield); mp 134-137 ºC (Reported mp =136-140ºC)

3,4-Dimethoxybenzohydrazide (3d) [59].

White solid (72.7 % yield); mp 145-146 ºC as reported.

3,4,5-Trimethoxybenzohydrazide (3e) [59].

White solid (72.9% yield); mp 158-160^{o}C as reported.

4.1.3. General procedure for the synthesis of 4-allyl/phenyl-5-aryl-4*H*-1,2,4-triazole-3-thiol derivatives (4a-j) [59, 60].

Equimolar quantities of the benzohydrazides **3a-e** (0.1 mol) and allyl/ phenyl isothiocyanate (0.1 mol) in 125 mL of absolute ethanol were heated under reflux for 4 h. The solvent was evaporated under vacuum. The resulting solid was filtered off, dried and used for the following step as a crude product. A mixture of the crude products (10mmol) and 100 mL of 2 N NaOH was heated under reflux for 3 h. The reaction mixture was cooled and acidified to pH 2 with concentrated HCl. The solid that precipitated was filtered off, washed with water, and recrystallized from 95% ethanol [59].

4-Allyl-3-phenyl-4*H*-1,2,4-triazole-3-thiol (4a) [36].

White solid (78.6% yield); mp 120-121^{o}C (Reported mp = 118-119^{o}C).

4-Allyl-3-(4-chlorophenyl)-4*H*-1,2,4-triazole-3-thiol (4b) [69].

White solid (77.9% yield); mp 176-178^{o}C (Reported mp = 180-181^{o}C).

4-Allyl-3-(4-methoxyphenyl)-4*H*-1,2,4-triazole-3-thiol (4c) [36].

White solid (69.5% yield); mp 119-120^{o}C (Reported mp = 120-121^{o}C).

4-Allyl-3-(3,4-dimethoxyphenyl)-4*H*-1,2,4-triazole-3-thiol (4d) [36].

White solid (70.2% yield); mp 123-124^{o}C (Reported mp = 121-123^{o}C).

4-Allyl-3-(3,4,5-trimethoxyphenyl)-4*H*-1,2,4-triazole-3-thiol (4e) [70].

White solid (70.3% yield); mp 202-203^{o}C (Reported mp = 200-201^{o}C).

3,4-Diphenyl-4*H*-1,2,4-triazole-3-thiol (4f) [59].

White solid (67.9% yield); mp 281-283^{o}C (Reported mp = 280-283^{o}C).

3-(4-Chlorophenyl)-4-phenyl-4*H*-1,2,4-triazole-3-thiol (4g) [71].

White solid (77.0% yield); mp 239-241^{o}C (Reported mp = 243-245^{o}C).

3-(4-Methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole-3-thiol (4h) [59].

White solid (72.8% yield); mp 281-283^{o}C (Reported mp = 284-285^{o}C).

3-(3,4-Dimethoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole-3-thiol (4i) [59].

White solid (69.7% yield); mp 237-238^{o}C (Reported mp = 234-235^{o}C).

3-(3,4,5-Trimethoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole-3-thiol (4j) [59].

White solid (78.5% yield); mp 209-211^{o}C (Reported mp = 210-211^{o}C).

4.1.4. General procedure for the synthesis of 1-(4-aminophenyl)-3-arylprop-2-en-1-one (6a-d) [7].

An equimolar amount of *p*-aminoacetophenone (13.51 gm, 0.1 mol) and the appropriate aldehyde **5a-d** (0.1mol), were dissolved in a minimum amount of ethanol, aqueous NaOH (0.25 mol, 60%) was added dropwise. The reaction mixture was stirred in ice bath for 30 min then at rt until the precipitate was formed within 3 h. The precipitate was filtered off and washed thoroughly with cold distilled water and cold methanol (2x20 mL). The product was recrystallized from absolute ethanol. The structure of the product was confirmed by mp.

1-(4-Aminophenyl)-3-phenyl prop-2-en-1-one (6a) [46].

Buff solid (77.8% yield); mp 156-157^{o}C (Reported mp = 157-158^{o}C).

1-(4-Aminophenyl)-3-(4-chlorophenyl)prop-2-en-1-one (6b) [7].

Yellow solid (80.3% yield); mp 159-160^{o}C (Reported mp = 158-159^{o}C).

1-(4-Aminophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (6c) [7].

Yellow solid (67.3% yield); mp 115-116^{o}C (Reported mp = 114-115^{o}C).

1-(4-Aminophenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (6d) [7].

Yellow solid (70.4% yield); mp 166-168^{o}C as reported.

4.1.5. General procedure for synthesis of 2-bromo-*N*-(4-(3-arylacryloyl)phenyl)acetamides (7a-d) [46].

To a stirred mixture of the appropriate chalcone **6a-d** (6.30mmol) in dichloromethane (20 mL) and potassium carbonate (0.18gm, 1.302 mmol) in 100 mL water in an ice bath. bromoacetyl bromide (1.856g, 9.20 mmol) in 30 mL dichloromethane was added in a dropwise manner with stirring over 30 min. Stirring was continued for 2h at 0^{O}C and at RT overnight. The reaction mixture was extracted with dichloromethane (2 x 60 mL) and the organic layer was washed with distilled water (2x40 mL), dried over anhydrous sodium sulphate, filtered, evaporated under vacuum and the residue was recrystallized from ethanol.

2-Bromo-*N*-(4-((*E*)-3-phenylacryloyl)phenyl)acetamide (7a) [46].

Pale yellow powder (70.0% yield); mp 157-159^{o}C (Reported mp = 157-158^{o}C).

2-Bromo-*N*-(4-(*E*)-3-(4-chlorophenyl)acryloyl)phenyl)acetamide (7b) [7].

Pale yellow powder (71.5% yield); mp 191-192^{o}C (Reported mp = 190-192^{o}C).

2-Bromo-*N*-(4-((*E*)-3-(4-methoxyphenyl)acryloyl)phenyl)acetamide (7c) [46].

Pale orange crystal (69.70% yield); mp 155-156^{o}C (Reported mp = 160-162^{o}C).

2-Bromo-*N*-(4-((*E*)-3-(3,4,5-trimethoxyphenyl)acryloyl)phenyl)acetamide (7d) [46].

Yellow powder (78.0% yield); mp 166-167^{o}C (Reported mp= 160-162^{o}C).

4.1.6. General Procedure for synthesis of 2-(4-allyl/phenyl-5-phenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-phenylacryloyl)phenyl)acetamide derivatives (8-47).

An equimolar mixture of **4a-j** (0.09 mmol) and compound **7a-d** (0.09 mmol) in acetonitrile. TEA (0.18 mmol) was added as a base. The reaction mixture was stirred at room temperature until the precipitate formed. The formed precipitate was filtrated off then the precipitate was crystallized with acetonitrile to afford the target compounds [46].

4.1.6.1. 2-(4-Allyl-5-phenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-phenylacryloyl)-phenyl)acetamide (8).

Orange crystal (0.29g, 80% yield); mp 140-143^{O}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 4.08 (2H, s, SC*H*$_2$), 4.54 (2H, d, *J* = 4.00 Hz, NC*H*$_2$), 5.03 (1H, d, J_{trans}=17.22 Hz, N-CH_2CH=C*H*$_2$), 5.32 (1H, d, J_{cis} =11.04 Hz, N-CH_2CH=C*H*$_2$), 5.88-5.89 (1H, m, N-CH_2C*H*=CH_2), 7.35-7.36 (3H, m, Ar-*H*), 7.45-7.50 (4H, m, Ar-*H*), 7.57-7.58 (3H, m, Ar-*H* + C-*H*=CH), 7.71-7.76 (4H, m, Ar-*H* + CH=*CH*), 7.93 (2H, d, *J* = 8.22 Hz, Ar-*H*), 11.00 (1H, s, N*H*); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 37.18, 47.04, 97.32, 118.93, 119.13, 121.74, 126.00, 128.32, 128.43, 128.84, 129.04, 129.69, 130.01, 130.65, 133.60, 134.84, 142.64, 144.25, 152.60, 156.41, 166.94, 188.97; Anal. Calcd. For $C_{28}H_{24}N_4O_2S$ (480.58): C, 69.98; H, 5.03; N, 11.66, Found: C, 70.25; H, 5.11; N, 11.95.

4.1.6.2. 2-(4-Allyl-5-(4-chlorophenyl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-phenyl- acryloyl)phenyl)acetamide (9).

Off white powder (0.32g, 84% yield); mp:219-221^{O}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 4.03 (2H, s, SC*H*$_2$), 4.55 (2H, d, *J*=4.86 Hz, NC*H*$_2$), 5.07 (1H, d, J_{trans}=17.16 Hz, N-CH_2CH=C*H*$_2$), 5.37 (1H, d, J_{Cis}=10.32 Hz, N-CH_2 CH=C*H*$_2$), 5.89-5.93 (1H, m, N-CH_2C*H*=CH_2), 7.39-7.40 (3H, m, Ar-*H* + C-*H*=CH), 7.41-7.51 (3H, m, Ar-*H*), 7.56 (2H, d, *J*=8.94 Hz, Ar-*H*),7.60 (1H, d, *J* = 15.00 Hz, C-H=C*H*), 7.61-7.62 (1H, m, Ar-*H*), 7.73-7.78 (3H, m, Ar-*H*), 7.97 (2H, d, *J* = 8.94 Hz, Ar-*H*), 10.96 (1H, s, *NH*); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 36.90, 47.15, 119.18, 121.81, 124.55, 128.43, 128.93, 129.52, 129.78, 129.83, 130.00, 130.43, 130.48, 133.69, 134.98, 137.14, 142.58, 144.39, 153.27, 155.62, 166.94, 188.99; Anal. Calcd. For $C_{28}H_{23}ClN_4O_2S$ (515.03): C, 65.30; H, 4.50; N, 10.88. Found: C, 65.57; H, 4.33; N, 11.23.

4.1.6.3. 2-(4-Allyl-5-(4-methoxyphenyl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-phenyl- acryloyl)phenyl)acetamide (10)

Pale brown powder (0.34g, 84% yield); mp: 214-216^{O}C; ^{1}H NMR (600MHz, $CDCl_3$) δ (ppm): 3.84 (3H, s, OC*H*$_3$), 4.05 (2H, s, SC*H*$_2$), 4.54 (2H, d, *J* = 4.08 Hz, NC*H*$_2$), 5.06 (1H, d, J_{trans} = 17.16 Hz, NCH_2-CH=C*H*$_2$), 5.35 (1H, d, J_{Cis}=10.26 Hz, N-CH_2CH=C*H*$_2$), 5.89-5.92 (1H, m, N-CH_2C*H*=CH_2), 7.00 (2H, d, *J*=8.94 Hz, Ar-*H*),

7.38-7.39 (3H, m, Ar-*H*), 7.49 (1H, d, *J* = 15.78 Hz, C-*H*=CH), 7.54 (2H, d, *J* =8.94 Hz, Ar-*H*), 7.60-7.61 (2H, m, Ar-*H*), 7.74 (2H, d, *J* = 8.94 Hz, Ar-*H*),7.76 (1H, d, *J*=15.78 Hz, C-H=C*H*), 7.95 (2H, d, *J* =8.94 Hz, Ar-*H*),11.14 (1H, s, N*H*), ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 37.07, 47.06, 55.35, 97.39, 114.55, 118.23, 118.97, 119.19, 121.85, 128.40, 128.90, 129.80, 130.01, 130.78, 133.56, 134.98, 142.72, 144.29, 152.51, 156.48, 161.47, 167.12, 189.00; Anal. Calcd. For $C_{29}H_{26}N_4O_3S$ (510.61): C, 68.21; H, 5.13; N, 10.97. Found: C, 68.49; H, 5.06; N, 11.21.

4.1.6.4. 2-(4-Allyl-5-(3,4-dimethoxyphenyl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-phenyl acryloyl)phenyl)acetamide (11)

Brown Crystal (0.34g, 78.90% yield); mp: 117-120^{O}C; ^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 3.91 (3H, s, OC*H*$_3$), 3.95 (3H, s, OC*H*$_3$), 4.11 (2H, s, SC*H*$_2$), 4.61 (2H, d, *J*=4.07 Hz, NC*H*$_2$), 5.12 (1H, d, J_{trans} = 17.10 Hz, N-CH_2CH=C*H*$_2$), 5.41 (1H, d, J_{Cis} = 10.40 Hz, N-CH_2CH=C*H*$_2$), 5.92-6.02 (1H, m, N-CH_2C*H*=CH_2), 6.98 (1H, d, *J*=8.20 Hz, Ar-*H*), 7.17 (2H, d, *J* = 8.20 Hz, Ar-*H*), 7.21 (1H, s, Ar-*H*), 7.42-7.43 (2H, m, Ar-*H*), 7.53 (1H, d, *J* = 15.70 Hz, C-*H*=CH), 7.64-7.65 (2H, m, Ar-*H*), 7.78-7.84 (3H, m, Ar-*H* + C-H=C*H*), 8.00 (2H, d, *J* = 8.20 Hz, Ar-*H*), 11.12 (1H, s, N*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 37.25, 47.41, 56.05, 56.13, 111.25, 111.65, 112.71, 119.03, 119.26, 121.20, 121.85, 128.44, 128.96, 129.82, 130.46, 130.78, 134.97, 142.72, 144.41, 149.45, 151.22, 152.70, 155.96, 156.31, 166.93, 189.00; Anal. Calcd. For $C_{30}H_{28}N_4O_4S$ (540.36): C, 66.65; H, 5.22; N, 10.36. Found: C, 66.94; H, 5.36; N, 10.62.

4.1.6.5. 2-(4-Allyl-5-(3,4,5-trimethoxyphenyl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-phenylacryloyl)phenyl)acetamide (12)

Pale yellow Crystal (0.35g, 90.20% yield); mp =189-191^{O}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 3.82 (6H, s, 2OC*H*$_3$), 3.88 (3H, s, OCH_3), 4.05 (2H, s, SC*H*$_2$), 4.58 (2H, d, *J* = 4.04 Hz, NC*H*$_2$), 5.11 (1H, d, J_{trans} = 17.16 Hz,N-CH_2CH=C*H*$_2$), 5.39 (1H, d, J_{Cis} = 9.72 Hz, N-CH_2CH=C*H*$_2$), 5.94-5.99 (1H, m, N-CH_2C*H*=CH_2), 6.82 (2H, s, Ar-*H*), 7.36-7.37 (3H, m, Ar-*H*), 7.49 (1H, d, *J* = 15.84 Hz, C-H=C*H*), 7.59-7.60 (2H, m, Ar-*H*), 7.74-7.76 (3H, m, Ar-*H* + C-*H*=CH), 7.95 (2H, d, *J* = 7.56 Hz, Ar-*H*), 11.05 (1H, s, N*H*);^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 37.24, 47.46, 56.46, 61.15, 106.01, 119.02, 119.43, 121.10, 122.00, 128.64, 129.14, 130.01, 130.70, 131.26, 133.87, 135.19, 140.32, 142.86, 144.60, 153.12, 153.95, 156.84, 167.22,

189.18; Anal. Calcd. For $C_{31}H_{30}N_4O_5S$ (570.66): C, 65.25; H, 5.30; N, 9.82. Found: C, 65.57; H, 5.18; N, 10.06.

4.1.6.6. 2-(4-Allyl-5-phenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(4-Chlorophenyl)- acryloyl)phenyl)acetamide (13)

Off white powder (0.30 g, 82% yield); mp 219-221^{O}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 4.05 (2H, s, SC$\underline{H_2}$), 4.56 (2H, d, *J* = 4.08 Hz, NC$\underline{H_2}$), 5.08 (1H, d, J_{trans} = 17.16 Hz, N-CH_2CH=C$\underline{H_2}$), 5.36 (1H, d, J_{Cis} = 10.32 Hz, N-CH_2CH=C$\underline{H_2}$), 5.89-5.92 (1H, m, N-CH_2C$\underline{H}$=CH_2), 7.35 (2H, d, *J* = 8.94 Hz, Ar-$\underline{H}$), 7.46 (1H, d , *J* = 15.78 Hz, C-$\underline{H}$=CH), 7.50-7.52 (3H, m, Ar-$\underline{H}$), 7.53 (2H, d, *J* = 8.22 Hz, Ar-$\underline{H}$), 7.61 (2H, d, *J* = 8.22 Hz, Ar-$\underline{H}$), 7.69 (1H, d, *J* = 15.78 Hz, C-H=C$\underline{H}$), 7.74 (2H, d, *J* = 8.94 Hz, Ar-$\underline{H}$), 7.94 (2H, d, *J* = 8.94 Hz, Ar-H), 11.11 (1H , s, N$\underline{H}$); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 36.74, 46.89, 118.97, 122.00, 125.84, 128.19, 128.29, 128.92, 129.31, 129.58, 129.65, 130.38, 131.01, 133.18, 133.38, 136.00, 142.44, 142.54, 152.71, 156.35, 166.84, 188.43; Anal. Calcd. For $C_{28}H_{23}ClN_4O_2S$ (515.03): C, 65.30; H, 4.50; N, 10.88. Found: C, 65.19; H, 4.57; N, 11.21.

4.1.6.7. 2-(4-Allyl-5-(4-chlorophenyl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(4-chloro- phenyl)acryloyl)phenyl)acetamide (14)

Off white powder (0.42 g, 85.01% yield); mp: 178-180^{O}C; ^{1}H NMR (500 MHZ, $CDCl_3$) δ (ppm): 4.17 (2H, s, SC$\underline{H_2}$), 4.60-4.62 (2H, m, NC$\underline{H_2}$), 5.12 (1H, d, J_{trans} = 17.16 Hz, N-CH_2CH=C$\underline{H_2}$), 5.43 (1H, d, *J*cis = 10.32 Hz, N-CH_2CH=C$\underline{H_2}$), 5.91-6.00 (1H, m, N-CH_2C$\underline{H}$=CH_2), 7.41 (2H, d, *J* = 7.10 Hz, Ar-$\underline{H}$), 7.49-7.53 (3H, m, Ar-$\underline{H}$ + C$\underline{H}$=CH), 7.58-7.62 (4H, m, Ar-$\underline{H}$), 7.75 (1H, d, *J* = 15.60 Hz, CH=C$\underline{H}$), 7.82 (2H, d, *J* = 7.80 Hz, Ar-$\underline{H}$), 8.00 (2H, d, *J* =7.80 Hz, Ar-$\underline{H}$), 11.06 (1H, s, N$\underline{H}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 37.16, 47.51, 118.46, 119.50, 122.32, 123.38, 124.05, 126.57, 128.30, 129.05, 129.33, 130.77, 133.30, 136.44, 137.47, 142.47, 143.51, 145.31, 152.69, 155.51, 166.78, 188.67; Anal. Calcd. For $C_{28}H_{22}Cl_2N_4O_2S$ (549.47): C, 61.20; H, 4.04; N, 10.20. Found: C, 61.48; H, 3.98; N, 10.47.

4.1.6.8. 2-(4-Allyl-5-(4-methoxyphenyl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(4-chloro- phenyl)acryloyl)phenyl)acetamide (15)

Off white powder (0.29 g, 79.5% yield); mp: 195-197^{O}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 3.84 (3H, s, OC$\underline{H_3}$), 4.05 (2H, s, SC$\underline{H_2}$), 4.54 (2H, d, *J* = 4.04 Hz ,

NC$\underline{H_2}$), 5.07 (1H, d, J_{trans} = 17.16 Hz, N-CH_2CH=C$\underline{H_2}$), 5.35 (1H, d, J_{Cis} = 10.26 Hz, N-CH_2CH=C$\underline{H_2}$), 5.89-5.92 (1H, m, N-CH_2C$\underline{H}$=CH_2), 6.99 (2H, d, *J* = 8.94 Hz, Ar-$\underline{H}$), 7.35 (2H, d, *J* = 8.28 Hz, Ar-$\underline{H}$), 7.46 (1H, d, *J* = 15.78 Hz, C-$\underline{H}$=CH), 7.52-7.54 (4H, m, Ar-$\underline{H}$), 7.68 (1H, d, *J* = 15.78 Hz, C-H-C$\underline{H}$), 7.73 (2H, d, *J* = 8.22 Hz, Ar-$\underline{H}$), 7.93 (2H, d, *J* = 8.22 Hz, Ar-$\underline{H}$), 11.10 (1H, s, N$\underline{H}$); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 37.08, 47.06, 55.35, 114.55, 118.22, 118.97, 119.20, 122.22, 129.16, 129.55, 129.80, 130.01, 130.78, 133.18, 133.48, 136.21, 142.72, 142.84, 152.49, 156.48, 161.47, 167.11, 188.64; Anal. Calcd. For $C_{29}H_{25}ClN_4O_3S$ (545.05): C, 63.90; H, 4.62; N, 10.28. Found: C, 64.23; H, 4.70; N, 10.45.

4.1.6.9. 2-(4-Allyl-5-(3,4-dimethoxyphenyl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(4-chlorophenyl)acryloyl)phenyl)acetamide (16)

Off white Crystal (0.31 g, 79.04% yield); mp: 138-139^{O}C; ^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 3.92 (3H, s, OC$\underline{H_3}$), 3.96 (3H, s, OC$\underline{H_3}$), 4.19 (2H, s, SC$\underline{H_2}$), 4.64 (2H, d, *J*=4.07 Hz, NC$\underline{H_2}$), 5.13 (1H, d, J_{trans} = 17.20 Hz, N-CH_2CH=C$\underline{H_2}$), 5.41 (1H, d, J_{Cis} = 10.40 Hz, N-CH_2CH=C$\underline{H_2}$), 5.92-6.01 (1H, m, N-CH_2C$\underline{H}$=CH_2), 6.99 (1H, d, *J* = 8.20 Hz, Ar-$\underline{H}$), 7.19 (1H, d, *J* = 8.20 Hz, Ar-$\underline{H}$), 7.23 (1H, s, Ar-$\underline{H}$), 7.40 (2H, d, *J* = 8.20 Hz, Ar-$\underline{H}$), 7.50 (1H, d, *J* = 15.60 Hz, C-$\underline{H}$=CH), 7.58 (2H, d, *J* = 8.20 Hz, Ar-$\underline{H}$), 7.75 (1H, d, *J* = 15.60 Hz, CH=C-$\underline{H}$), 8.85 (2H, d, *J* = 8.30 Hz, Ar-$\underline{H}$), 7.99 (2H, d, *J* = 8.30 Hz, Ar-$\underline{H}$), 11.22 (1H, s, N$\underline{H}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 37.31, 47.60, 56.08, 56.16, 111.28, 111.43, 111.69, 117.33, 119.28, 121.27, 122.24, 129.23, 129.60, 129.80, 133.46, 133.48, 136.30, 142.84, 142.87, 149.54, 151.17, 151.46, 152.95, 156.11, 166.75, 188.69; Anal. Calcd. For $C_{30}H_{27}N_4O_4S$ (575.08) C, 62.66; H, 4.73; N, 9.74. Found: C, 62.49; H, 4.81; N, 9.98.

4.1.6.10. 2-(4-Allyl-5-(3,4,5-trimethoxyphenyl)-4*H*-1,2,4triazol-3-ylthio)-*N*-(4-((*E*)-3-(4-chlorophenyl)acryloyl)phenyl)acetamide (17)

White powder (0.32 g, 85% yield); mp: 207-209^{O}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 3.83 (6H, s, 2OC$\underline{H_3}$), 3.86 (3H, s, OC$\underline{H_3}$), 4.06 (2H, s, SC$\underline{H_2}$), 4.58 (2H, d, *J* = 4.10 Hz, NC$\underline{H_2}$), 5.11 (1H, d, J_{trans} = 17.16 Hz, N-CH_2CH=C$\underline{H_2}$), 5.38 (1H, d, J_{Cis} = 10.26 Hz, CHC$\underline{H_2}$), 5.95-5.96 (1H, m, N-CH_2C$\underline{H}$=CH_2), 6.81 (2H, s, Ar-$\underline{H}$), 7.32 (2H, d, *J* = 8.22 Hz, Ar-$\underline{H}$), 7.43 (1H, d, *J* = 15.84 Hz, C-$\underline{H}$=CH), 7.51 (2H, d, *J* = 8.22 Hz, Ar-$\underline{H}$), 7.66 (1H, d, *J* = 15.84 Hz,CH =C-$\underline{H}$), 7.71 (2H, d, *J* = 8.28 Hz, Ar-$\underline{H}$), 7.91 (2H, d, *J* = 8.28 Hz, Ar-$\underline{H}$), 11.06 (1H, s, N$\underline{H}$); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm):

37.06, 47.17, 56.17, 60.85, 105.75, 118.73, 119.15, 121.10, 122.09, 129.10, 129.51, 129.70, 130.10, 131.01, 133.39, 136.17, 140.03, 142.72, 142.90, 152.76, 153.66, 156.53, 166.90, 188.50; Anal. Calcd. For $C_{31}H_{29}ClN_4O_5S$ (605.10): C, 61.53; H, 4.83; N, 9.26. Found: C, 61.76; H, 4.79; N, 9.44.

4.1.6.11. 2-(4-Allyl-5-phenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(4-methoxyphenyl)- acryloyl)phenyl)acetamide (18)

Pale yellow powder (0.35 g, 84% yield); mp: 199-200^{O}C; ^{1}H NMR (600MHz, $CDCl_3$) δ (ppm): 3.80 (3H, s, OC*H*$_3$), 4.07 (2H, s, SC*H*$_2$), 4.54 (2H, d, *J* = 4.14 Hz, NC*H*$_2$), 5.03 (1H, d , J_{trans} = 17.16 Hz, N-CH_2CH=C*H*$_2$), 5.31 (1H, d, J_{Cis} = 10.32 Hz, N-CH_2CH=C*H*$_2$), 5.87-5.89 (1H, m, N-CH_2C*H*=CH_2), 6.87 (2H, d, *J* = 8.94 Hz, Ar-*H*), 7.35 (1H, d, *J* = 15.78 Hz, C-*H*=CH), 7.43-7.47 (3H, m, Ar-*H*), 7.54 (2H, d, *J* = 8.94 Hz, Ar-*H*), 7.57 (2H, d, *J* = 7.56 Hz, Ar-*H*), 7.71 (1H, d, *J* = 15.78 Hz, CH=C-*H*), 7.75 (2H, d, *J* = 8.94 Hz, Ar-*H*), 7.92 (2H, d, *J* = 8.94 Hz, Ar-*H*), 10.98 (1H, s, N*H*); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 36.93, 45.71, 55.01, 114.03, 118.68, 118.83, 119.16, 125.74, 127.31, 128.19, 128.39, 128.80, 129.01, 129.85, 130.39, 133.51, 142.19, 143.87, 152.37, 156.15, 161.27, 166.66, 188.76; Anal. Calcd. For $C_{29}H_{26}N_4O_3S$ (510.61), C, 68.21; H, 5.13; N, 10.97. Found: C, 68.57; H, 5.03; N, 11.26.

4.1.6.12. 2-(4-Allyl-5-(4-chlorophenyl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(4-methoxy- phenyl)acryloyl)phenyl)acetamide (19)

Pale yellow powder (0.35 g, 81.10% yield); mp: 231-232^{O}C; ^{1}H NMR (600 MHZ, $CDCl_3$) δ (ppm): 3.84 (3H, s, OC*H*$_3$), 4.00 (2H, s, SC*H*$_2$), 4.54 (2H, d, *J* = 4.14 Hz, NC*H*$_2$), 5.08 (1H, d, J_{trans} = 17.16 Hz, N-CH_2CH=C*H*$_2$), 5.38 (1H, d, *J* = 10.32 Hz, N-CH_2CH=C*H*$_2$), 5.89-5.92 (1H, m, N-CH_2C*H*=CH_2), 6.92 (2H, d, *J* = 8.84 Hz, Ar-*H*), 7.38 (1H, d, *J* = 15.84 Hz, C-*H*=CH), 7.49 (2H, d, *J* = 8.84 Hz, Ar-*H*), 7.56-7.58 (4H, m, Ar-*H*), 7.74-7.77 (3H, m, Ar-*H* + CH=C-*H*), 7.98 (2H, d, *J* = 8.22 Hz, Ar-*H*), 10.92 (1H, s, N*H*); ^{13}C NMR (150 MHz, CDCl3) δ (ppm): 36.80, 47.17, 55.36, 114.40, 119.17, 119.28, 119.55, 125.55, 127.75, 129.55, 129.80, 130.21, 130.46, 133.90, 134.05, 137.18, 142.37, 144.28, 153.33, 155.65, 161.80, 166.96, 189.08; Anal. Calcd For $C_{29}H_{25}ClN_4O_3S$ (545.05) : C, 63.90; H, 4.62; N, 10.28 .Found: C, 64.16;H, 4.51; N,10.59.

4.1.6.13. 2-(4-Allyl-5-(4-methoxyphenyl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(4-methoxyphenyl)acryloyl)phenyl)acetamide (20)

Grayish yellow powder (0.30 g, 81%yield); mp: 170-173^{O}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 3.82 (3H, s, OC$\underline{H}_3$), 3.84 (3H, s, OC$\underline{H}_3$), 4.03 (2H, s, SC$\underline{H}_2$), 4.53 (2H, d, *J* = 4.10 Hz, NC$\underline{H}_2$), 5.06 (1H, d, J_{trans} = 17.16 Hz, N-CH_2CH=C$\underline{H}_2$), 5.34 (1H, d, J_{Cis} = 10.32 Hz, NH-CH_2CH=C$\underline{H}_2$), 5.88-5.91 (1H, m, N-CH_2C$\underline{H}$=CH_2), 6.90 (2H, d, *J* = 8.94 Hz, Ar-$\underline{H}$), 6.98 (2H, d, *J* = 8.94 Hz, Ar-$\underline{H}$), 7.38 (1H, d, *J* = 15.78 Hz, C-$\underline{H}$=CH), 7.53 (2H, d, *J* = 8.28 Hz, Ar-$\underline{H}$), 7.57 (2H, d, *J* = 8.28 Hz, Ar-$\underline{H}$), 7.72-7.75 (3H, m, Ar-$\underline{H}$ +CH=C-$\underline{H}$), 7.94 (2H, d, *J* = 8.28 Hz, Ar-$\underline{H}$), 11.07 (1H, s, N$\underline{H}$); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 37.04, 47.06, 55.35, 55.38, 114.36, 114.55, 118.22, 118.97, 119.15, 119.52, 127.70, 129.69, 130.02, 130.19, 130.79, 133.88, 142.50, 144.20, 152.48, 156.49, 161.47, 161.59, 167.11, 189.08; Anal. Calcd. For $C_{30}H_{28}N_4O_4S$ (540.63): C, 66.65; H, 5.22; N, 10.36. Found: C, 66.98; H, 5.08; N, 10.08.

4.1.6.14. 2-(4-Allyl-5-(3,4-dimethoxyphenyl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(4-methoxyphenyl)acryloyl)phenyl)acetamide (21)

Pale brown Crystal (0.51 g, 83.30% yield); mp: 181-184^{O}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 3.87 (3H, s, OC$\underline{H}_3$), 3.92 (3H, s, OC$\underline{H}_3$), 3.95 (3H, s, OC$\underline{H}_3$), 4.14 (2H, s, SC$\underline{H}_2$), 41 (2H, d, *J* = 4.07 Hz, NC$\underline{H}_2$), 5.13 (1H, d, J_{trans} = 17.20 Hz, N-CH_2CH=C$\underline{H}_2$), 5.42 (1H, d, J_{Cis} = 10.40 Hz, N-CH_2CH=C$\underline{H}_2$), 5.92-6.01 (1H, m, N-CH_2C$\underline{H}$=CH_2), 6.94 (2H, d, *J* = 8.20 Hz, Ar-$\underline{H}$), 6.98 (1H, d, *J* = 8.20 Hz, Ar-$\underline{H}$), 7.18 (1H, d, *J* = 8.10 Hz, Ar-$\underline{H}$), 7.22 (1H, s, Ar-$\underline{H}$), 7.41 (1H, d, *J* = 15.60 Hz, C-$\underline{H}$=CH),7.61 (2H, d, *J*=8.10 Hz, Ar-$\underline{H}$), 7.77 (1H, d, *J* = 15.60 Hz, CH=C-$\underline{H}$), 7.82 (2H, d, *J* = 8.20 Hz, Ar-$\underline{H}$), 7.99 (2H, d, *J* = 8.20 Hz, Ar-$\underline{H}$), 11.15 (1H, s, N$\underline{H}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 37.53, 47.47, 54.83, 56.06, 56.27, 110.63, 111.73, 114.57, 117.72, 121.29, 127.25, 129.34, 130.47, 130.73, 133.93, 142.52, 142.77, 144.19, 148.82, 150.23, 150.90, 151.30, 156.23, 160.45, 161.86, 166.80, 188.72; Anal. Calcd. For $C_{31}H_{30}N_4O_5S$ (570.66) C, 65.25; H, 5.30; N, 9.82. Found: C, 65.12; H, 5.47; N, 9.94.

4.1.6.15. 2-(4-Allyl-5-(3,4,5-trimethoxyphenyl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(4-methoxyphenyl)acryloyl)phenyl)acetamid (22)

Off white powder (0.36 g, 88% yield); mp: 174-176^{O}C; ^{1}H NMR (600 MHz, $CDCl_3$), δ (ppm): 3.81 (3H, s, OC*H*$_3$), 3.82 (6H, s, 2OC*H*$_3$), 3.87 (3H, s, OC*H*$_3$), 4.04 (2H, s, SC*H*$_2$), 4.58 (2H, d, J = 4.40 Hz, NC*H*$_2$), 5.09 (1H, d, J_{trans} = 17.16 Hz, N-CH_2CH=C*H*$_2$), 5.38 (1H, d, J_{Cis} = 10.32 Hz, N-CH_2CH=C*H*$_2$), 5.95-5.96 (1H, m, N-CH_2C*H*=CH_2), 6.81 (2H, s, Ar-*H*), 6.88 (2H, d, J = 8.28 Hz, Ar-*H*), 7.36 (1H, d, J = 15.84 Hz, C-*H*=CH), 7.55 (2H, d, J = 8.28 Hz, Ar-*H*), 7.70 (1H, d, J = 15.84 Hz, CH=C-*H*), 7.73 (2H, d, J = 8.22 Hz, Ar-*H*),7.93 (2H, d, J = 8.22 Hz, Ar-*H*), 10.99 (1H, s, N*H*); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 37.01, 47.19, 55.28, 56.19, 60.86, 105.73, 114.31, 118.70, 119.11, 119.39, 121.10, 127.61, 129.61, 130.13, 131.03, 133.87, 139.99, 142.40, 144.17, 152.79, 153.65, 156.54, 161.56, 166.93, 188.94; Anal. Calcd. For $C_{32}H_{32}N_4O_6S$ (600.68): C, 63.98; H, 5.37; N, 9.33. Found: C, 64.25; H, 5.28; N, 9.57.

4.1.6.16. 2-(4-Allyl-5-phenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(3,4,5-trimethoxy- phenyl)acryloyl)phenyl)acetamide (23)

Off white powder (0.35 g, 88% yield); mp165-168^{O}C, ^{1}H NMR (600 MHz, $CDCl_3$), δ (ppm): 3.87 (3H, s, OC*H*$_3$), 3.90 (6H, s, 2OC*H*$_3$), 4.04 (2H, s, SC*H*$_2$), 4.56 (2H, d, J = 4.20 Hz, NC*H*$_2$), 5.08 (1H, d, J_{trans} = 17.22 Hz, N-CH_2CH=C*H*$_2$), 5.35 (1H, d, J_{Cis} = 10.32 Hz, NCH2CH=C*H*$_2$), 5.89-5.92 (1H, m, N-CH_2C*H*=CH_2), 6.83 (2H, s, Ar-*H*), 7.35 (1H, d, J = 15.84 Hz, C-*H*=CH), 7.48-7.51 (3H, m, Ar-*H*), 7.61 (2H, d, J = 7.56 Hz, Ar-*H*), 7.66 (1H, d, J = 15.84 Hz,CH =C-*H*), 7.75 (2H, d, J = 8.22 Hz, Ar-*H*), 7.95 (2H, d, J = 8.22 Hz, Ar-*H*), 11.09 (1H, s, N*H*); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 36.99, 47.12, 56.14, 60.92, 105.56, 119.13, 119.19, 121.34, 126.09, 127.01, 128.52, 129.15, 129.80, 130.64, 130.77, 133.66, 140.29, 142.62, 144.52, 152.92, 153.46, 156.58, 167.07, 189.12; Anal. Calcd. For $C_{31}H_{30}N_4O_5S$ (570.66): C, 65.25; H, 5.30; N, 9.82. Found: C, 65.03; H, 5.42; N, 10.07.

4.1.6.17. 2-(4-Allyl-5-(4-chlorophenyl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(3,4,5-tri- methoxyphenyl)acryloyl)phenyl)acetamide (24)

White powder (0.37 g, 89% yield); mp: 194-196^{O}C; ^{1}H NMR (600 MHz, $CDCl_3$), δ (ppm): 3.86 (3H, s, OC*H*$_3$), 3.88 (6H, s, 2OC*H*$_3$), 4.04 (2H, s, SC*H*$_2$), 4.54 (2H, d, J

= 4.14 Hz, NC$\underline{H_2}$), 5.05 (1H, d, J_{trans} = 17.16 Hz, N-CH_2 CH=C$\underline{H_2}$), 5.36 (1H, d, J_{Cis} = 10.32 Hz, N-CH_2CH=C$\underline{H_2}$), 5.88-5.93 (1H, m, N-CH_2C$\underline{H}$=CH_2), 6.82 (2H, s, Ar-$\underline{H}$), 7.35 (1H, d, *J* = 15.78 Hz, C-$\underline{H}$=CH), 7.45 (2H, d, *J* = 8.88 Hz, Ar-$\underline{H}$), 7.54 (2H, d, *J*=8.88 Hz, Ar-$\underline{H}$), 7.64 (1H, d, *J* = 15.78 Hz, CH=C-$\underline{H}$), 7.71 (2H, d, *J* = 8.88 Hz, Ar-$\underline{H}$), 7.92 (2H, d, *J* = 8.88 Hz, Ar-$\underline{H}$), 10.94 (1H, s, N$\underline{H}$); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 36.94, 47.10, 56.11, 60.88, 105.55, 119.13, 119.30, 121.21, 124.51, 129.47, 129.57, 129.73, 130.18, 130.48, 133.66, 137.09, 140.26, 142.50, 144.53, 153.13, 153.41, 155.55, 166.86, 189.01; Anal. Calcd. For $C_{31}H_{29}ClN_4O_5S$ (605.1): C, 61.53; H, 4.83; N, 9.26. Found: C, 61.80; H, 4.70; N, 9.41.

4.1.6.18. 2-(4-Allyl-5-(4-methoxyphenyl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(3,4,5-tri- methoxyphenyl)acryloyl)phenyl)acetamide (25)

White powder (0.32 g , 85% yield); mp: 189-191^{O}C; ^{1}H NMR (600 MHZ, $CDCl_3$), δ (ppm): 3.83 (3H, s, OC$\underline{H_3}$), 3.86 (3H, s, OC$\underline{H_3}$), 3.89 (6H, s, 2OC$\underline{H_3}$), 4.04 (2H, s, SC$\underline{H_2}$), 4.54 (2H, d, *J* = 4.86 Hz, NC$\underline{H_2}$), 5.06 (1H, d, J_{trans} = 17.16 Hz, N-CH_2CH=C$\underline{H_2}$), 5.34 (1H, d, J_{Cis} = 10.98 Hz, N-CH_2CH=C$\underline{H_2}$), 5.88-5.93 (1H, m, N-CH_2C$\underline{H}$=CH_2), 6.82 (2H, s, Ar-$\underline{H}$), 6.98 (2H, d, *J* = 8.88 Hz, Ar-$\underline{H}$), 7.35 (1H, d, *J* = 15.84 Hz, C-$\underline{H}$=CH), 7.53 (2H, d, *J* = 8.88 Hz, Ar-$\underline{H}$), 7.64 (1H, d, *J* = 15.84 Hz, CH=C-$\underline{H}$), 7.73 (2H, d, *J* = 8.88 Hz, Ar-$\underline{H}$), 7.93 (2H, d, *J* = 8.88 Hz, Ar-$\underline{H}$), 11.13 (1H, s, N$\underline{H}$); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 37.04, 47.05, 55.34, 56.11, 60.90, 100.51, 105.52, 114.54, 118.21, 118.94, 119.16, 121.30, 129.75, 129.99, 130.50, 130.78, 133.58, 142.64, 144.47, 152.47, 153.43, 156.46, 161.46, 167.09, 189.08; Anal. Calcd. For $C_{32}H_{32}N_4O_6S$ (600.86): C, 63.98; H, 5.37; N, 9.33. Found: C, 63.87; H, 5.49; N, 9.57.

4.1.6.19. 2-(4-Allyl-5-(3,4-dimethoxyphenyl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(3,4,5-trimethoxyphenyl)acryloyl)phenyl)acetamide (26)

Pale yellow powder (0.29 g; 81.07%); mp: 173-175^{O}C; ^{1}H NMR (500MHz, $CDCl_3$), δ (ppm): 3.92 (3H, s, OC$\underline{H_3}$), 33.93 (3H, s, OC$\underline{H_3}$), 3.95 (6H, s, 2OC$\underline{H_3}$), 3.96 (3H, s, OC$\underline{H_3}$), 4.27 (2H, s, SC$\underline{H_2}$), 4.66 (2H, d, *J*=4.70, NC$\underline{H_2}$), 5.13 (1H, d, J_{trans} = 17.20 Hz, N-CH_2CH=C$\underline{H_2}$), 5.45 (1H, d, J_{Cis} = 10.40 Hz, N-CH_2CH=C$\underline{H_2}$), 5.92-6.01 (1H, m, N-CH_2C$\underline{H}$=CH_2), 6.88 (2H, s, Ar-$\underline{H}$), 6.99 (1H, d, *J* = 8.20 Hz, Ar-$\underline{H}$), 7.21 (1H, d, *J* = 7.90 Hz, Ar-$\underline{H}$), 7.26 (1H, s, Ar-$\underline{H}$), 7.40 (1H, d, *J* = 15.60 Hz, C-$\underline{H}$=CH), 7.70 (1H, d, *J* = 15.60 Hz, CH =C-$\underline{H}$), 7.87 (2H, d, *J*=8.30 Hz, Ar-$\underline{H}$), 7.98 (2H, d, *J*=8.30

Hz, Ar-*H*), 11.18 (1H, s, N*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 37.41, 45.90, 56.09, 56.22, 56.25, 61.03, 105.59, 111.31, 111.73, 116.53, 119.29, 119.50, 121.32, 121.40, 129.77, 130.28, 130.47, 133.73, 140.31, 142.61, 144.64, 149.61, 151.73, 153.08, 153.48, 155.80, 166.44, 189.17; Anal. Calcd. For $C_{33}H_{34}N_4O_7S$ (630.71) C, 62.84; H, 5.43; N, 8.88. Found: C, 63.12; H, 5.57; N, 9.04.

4.1.6.20. 2-(4-Allyl-5-(3,4,5-trimethoxyphenyl)-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(3,4,5-trimethoxyphenyl)acryloyl)phenyl)acetamide (27)

Off white powder (0.29 g, 79.6% yield); mp: 204-206^{O}C; ^{1}H NMR (600MHz, $CDCl_3$), δ (ppm): 3.82 (6H, s, 2OC*H*$_3$), 3.86 (3H, s, OC*H*$_3$), 3.87 (3H, s, OC*H*$_3$), 3.88 (6H, s, 2OC*H*$_3$), 4.04 (2H, s, SC*H*$_2$), 4.58 (2H, d, *J* = 4.14 Hz, NC*H*$_2$), 5.11 (1H, d, J_{trans} = 17.16 Hz, N-CH$_2$CH=C*H*$_2$), 5.39 (1H, d, J_{Cis} = 10.26 Hz, N-CH$_2$CH=C*H*$_2$), 5.94-5.98 (1H, m, N-CH$_2$C*H*=CH$_2$), 6.81 (2H, s, Ar-*H*), 6.83 (2H, s, Ar-*H*), 7.35 (1H, d, *J* = 15.78 Hz, C-*H*=CH), 7.64 (1H, d, *J* = 15.78 Hz, CH=C-*H*), 7.73 (2H, d, *J* = 8.94 Hz, Ar-*H*), 7.93 (2H, d, *J* = 8.94 Hz, Ar-*H*),11.04 (1H, s, N*H*), ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 37.21, 47.44, 56.35, 56.44, 61.13, 105.78, 105.99, 119.01, 119.40, 121.31, 121.45, 129.98, 130.80, 131.26, 133.88, 140.31, 140.50, 142.79, 144.78, 153.09, 153.66, 153.93, 156.83, 167.21, 189.25; Anal. Calcd. For $C_{34}H_{36}N_4O_8S$ (660.74): C, 61.80; H, 5.49; N, 8.48. Found: C, 62.13; H, 5.38; N, 8.76.

4.1.6.21. 2-(4,5-diphenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-phenylacryloyl)phenyl)- acetamide (28)

Brown powder (0.41 g, 79.80% yield); mp 157-159^{O}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 4.09 (2H, s, SC*H*$_2$), 7.19-7.21 (3H, m, Ar-*H*), 7.23-7.26 (2H, m, Ar-*H*), 7.32-7.35 (5H, m, Ar-*H*), 7.45-7.47 (2H, m, Ar-*H* + C-*H*=CH), 7.48-7.50 (2H, m, Ar-*H*), 7.56-7.59 (2H, m, Ar-*H*), 7.67 (1H, d, *J* = 15.60 Hz, CH=C-*H*), 7.78 (2H, d, *J* = 8.30 Hz, Ar-*H*), 7.94 (2H, d, *J* = 8.30 Hz, Ar-*H*), 10.98 (1H, s, N*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 36.58, 118.75, 119.22, 119.31, 121.89, 124.98, 127.03, 127.38, 128.32, 128.46, 128.87, 128.96, 132.17, 133.01, 133.73, 135.00, 139.64, 142.61, 144.42, 154.11, 155.14, 166.64, 189.06; Anal. Calcd. For $C_{31}H_{24}N_4O_2S$ (516.61): C, 72.07; H, 4.68; N, 10.85. Found: C, 72.41; H, 4.53; N, 11.08.

4.1.6.22. 2-(5-(4-Chlorophenyl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-phenyl- acryloyl)phenyl)acetamide (29)

Beige powder (0.30 g, 79.7% yield); mp: 223-225^{O}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 3.99 (2H, s, SC$\underline{H}_2$), 7.23-7.24 (2H, m, Ar-$\underline{H}$), 7.27 (2H, d, *J* = 8.94 Hz, Ar-$\underline{H}$), 7.34 (2H, d, *J* = 8.94 Hz, Ar-$\underline{H}$), 7.38-3.39 (3H, m, Ar-$\underline{H}$), 7.51 (1H, d, *J* = 15.12 Hz, C-$\underline{H}$=CH), 7.53-7.55 (3H, m, Ar-$\underline{H}$), 7.60-7.62 (2H, m, Ar-$\underline{H}$),7.77-7.79 (3H, m, Ar-$\underline{H}$ + CH=C-$\underline{H}$), 8.00 (2H, d, *J* = 8.22 Hz, Ar-$\underline{H}$), 10.83 (1H, s, N$\underline{H}$); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 36.27, 100.53, 119.21, 121.81, 124.30, 126.94, 128.43, 128.94, 129.10, 129.34, 129.87, 130.45, 130.68, 133.23, 133.72, 134.98, 136.57, 142.53, 144.41, 154.13, 155.10, 166.97, 189.00; Anal. Calcd. For $C_{31}H_{23}ClN_4O_2S$ (551.06): C, 67.57; H, 4.21; N, 10.17. Found: C, 67.28; H, 4.30; N, 10.41.

4.1.6.23. 2-(5-(4-Methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-phenyl- acryloyl)phenyl)acetamide (30)

Off white powder (0.35 g, 80.2% yield); mp: 245-247^{O}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 3.81 (3H, s, OC$\underline{H}_3$), 4.24 (2H, s, SC$\underline{H}_2$), 6.84 (2H, d, *J* = 6.80 Hz, Ar-$\underline{H}$), 7.25-7.30 (3H, m, Ar-$\underline{H}$), 7.38-7.42 (4H, m, Ar-$\underline{H}$), 7.53-7.59 (3H, m, Ar-$\underline{H}$ + C-$\underline{H}$=CH), 7.63-7.68 (3H, m, Ar-$\underline{H}$), 7.82 (1H, d, *J* = 15.60 Hz, CH=C-$\underline{H}$), 7.89 (2H, d, *J* = 6.60 Hz, Ar-$\underline{H}$), 8.02 (2H, d, *J* = 6.60 Hz, Ar-$\underline{H}$), 11.07 (1H, s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 36.83, 55.23, 114.14, 114.44, 119.39, 121.89, 127.20, 127.46, 128.32, 128.47, 128.96, 130.01, 130.11, 130.45, 130.54, 130.98, 132.88, 133.70, 135.01, 142.64, 144.40, 154.96, 166.48, 189.05; Anal. Calcd. For $C_{32}H_{26}N_4O_3S$ (546.64): C, 70.31; H, 4.79; N, 10.25. Found: C, 70.45; H, 4.88; N, 10.53.

4.1.6.24. 2-(5-(3,4-Dimethoxyphenyl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-phenylacryloyl)phenyl)acetamide (31)

Off white powder (0.41g, 70.08% yield); mp: 135-138^{O}C; ^{1}H NMR (500MHz, $CDCl_3$) δ (ppm): 3.71 (3H, s, OC$\underline{H}_3$), 3.88 (3H, s, OC$\underline{H}_3$), 4.04 (2H, s, SC$\underline{H}_2$), 6.77 (1H, d, *J* = 8.30 Hz, Ar-$\underline{H}$), 6.94 (1H, d, *J* = 8.30 Hz, Ar-$\underline{H}$), 7.03 (1H, s, Ar-$\underline{H}$), 7.31 (1H, d, *J* = 6.10 Hz, Ar-$\underline{H}$), 7.42-7.44 (4H, m, Ar-$\underline{H}$), 7.53-7.58 (4H, m, Ar-$\underline{H}$ + C-$\underline{H}$=CH), 7.65-7.67 (2H, m, Ar-$\underline{H}$), 7.82 (1H, d, *J* = 15.50 Hz, CH =C-$\underline{H}$), 7.84 (2H, d, *J* = 8.30 Hz, Ar-$\underline{H}$), 8.04 (2H, d, *J* = 8.30 Hz, Ar-$\underline{H}$), 11.00 (1H, s, N$\underline{H}$); ^{13}C NMR

(125 MHz, $CDCl_3$) δ (ppm): 36.48, 55.59, 56.62, 110.96, 118.11, 119.14, 121.28, 122.04, 125.71, 129.62, 129.87, 130.00, 130.24, 130.75, 133.55, 133.66, 134.92, 135.02, 142.63, 144.40, 148.93, 150.61, 150.85, 153.38, 155.26, 167.06, 189.02; Anal. Calcd. For $C_{33}H_{28}N_4O_4S$ (576.66): C, 68.73; H, 4.89; N, 9.72. Found: C, 69.08; H, 4.86; N, 9.96.

4.1.6.25. 2-(5-(3,4,5-Trimethoxyphenyl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-phenylacryloyl)phenyl)acetamide (32)

Pale beige Crystal (0.37 g; 78.80% yield); mp: 214-215^{O}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 3.63 (6H, s, 2OC*H*$_3$), 3.86 (3H, s, OC*H*$_3$), 4.13 (2H, s, SC*H*$_2$), 6.65 (2H, s, Ar-*H*), 7.32-7.33 (2H, m, Ar-*H*), 7.43-7.44 (3H, m, Ar-*H*), 7.54 (1H, d, *J* = 15.70 Hz, C-*H*=CH), 7.58-7.60 (3H, m, Ar-*H*), 7.66-7.68 (2H, m, Ar-*H*), 7.82 (1H, d, *J* = 15.70 Hz, CH=C-*H*), 7.86 (2H, d, *J* = 8.30 Hz, Ar-*H*), 8.04 (2H, d, *J* = 8.30 Hz, Ar-*H*), 11.01 (1H, s, N*H*); ^{13}C NMR (150 MHz, $CDCl_3$), δ (ppm): 35.81, 55.58, 60.96, 101.43, 118.46, 119.88, 120.18, 121.59, 126.88, 128.01, 128.97, 130.09, 130.48, 132.19, 134.98, 138.89, 139.27, 140.31, 143.16, 144.20, 153.06, 154.11, 155.15, 166.77, 188.71; Anal. Calcd. For $C_{34}H_{30}N_4O_5S$ (606.69): C, 67.31; H, 4.98; N, 9.23. Found: C, 67.68; H, 5.04; N, 9.61.

4.1.6.26. 2-(4,5-Diphenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(4-chlorophenyl)acryloyl)- phenyl)acetamide (33)

Off white powder (0.32 g, 79.40% yield); mp: 200-204^{O}C; ^{1}H NMR (600MHz, $CDCl_3$) δ (ppm): 4.22 (2H, s, SC*H*$_2$), 7.13 (1H, d, *J* = 8.50 Hz, Ar-*H*), 7.29-7.30 (2H, m, Ar-*H*), 7.35 (2H, d, *J* = 7.40 Hz, Ar-*H*), 7.40-7.44 (6H, m, Ar-*H*), 7.52 (1H, d, *J* = 15.50 Hz, C-*H*=CH), 7.55-7.60 (3H, m, Ar-*H*), 7.76 (1H, d, *J* = 15.50 Hz, CH=C-*H*), 7.88 (2H, d, *J* = 8.20 Hz, Ar-*H*), 8.02 (2H, d, *J* = 8.20 Hz, Ar-*H*), 11.09 (1H, s, NH); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 45.96, 118.17, 119.24, 119.35, 122.27, 124.73, 128.36, 128.90, 129.24, 129.61, 129.86, 129.95, 131.24, 132.88, 133.50, 133.54, 136.31, 142.73, 142.88, 154.20, 155.03, 166.53, 188.71; Anal. Calcd. For $C_{31}H_{23}ClN_4O_2S$ (551.06): C, 67.57; H, 4.21; N, 10.17. Found: C, 67.35; H, 4.37; N, 10.48.

4.1.6.27. 2-(5-(4-Chlorophenyl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(4-chloro- phenyl)acryloyl)phenyl)acetamide (34)

Off white powder (0.39 g, 89% yield); mp: 200-203^{O}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 3.98 (2H, s, SC$\underline{H}_2$), 7.23 (2H, d, *J* = 7.56 Hz, Ar-$\underline{H}$), 7.27 (2H, d, *J* = 8.22 Hz, Ar-$\underline{H}$), 7.33 (2H, d, *J* = 8.22 Hz, Ar-$\underline{H}$), 7.36 (2H, d, *J* = 7.56 Hz, Ar-$\underline{H}$), 7.48 (1H, d, *J* = 15.84 Hz, C-$\underline{H}$=CH), 7.51-7.55 (5H, m, Ar-$\underline{H}$), 7.71 (1H, d, *J* = 15.84 Hz, CH=C-$\underline{H}$), 7.77 (2H, d, *J* = 8.28 Hz, Ar-$\underline{H}$), 7.98 (2H, d, *J* = 8.28 Hz, Ar-$\underline{H}$), 10.85 (1H, s, N$\underline{H}$); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 36.24, 119.25, 122.23, 124.31, 126.94, 127.10, 129.13, 129.34, 129.59, 130.01, 130.48, 130.71, 133.10, 133.50, 136.19, 137.02, 142.67, 142.89, 154.01, 154.16, 154.60, 167.02, 188.69; Anal. Calcd. For $C_{31}H_{22}Cl_2N_4O_2S$ (585.5): C, 63.59; H, 3.79; N, 9.57. Found: C, 63.81; H, 3.85; N, 9.72.

4.1.6.28. 2-(5-Methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(4-chloro- phenyl)acryloyl)phenyl)acetamide (35)

Off white powder (0.29 g, 79.50% yield); mp: 218-219^{O}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 3.78 (3H, s, OC$\underline{H}_3$), 3.96 (2H, s, SC$\underline{H}_2$), 6.81 (2H, d, *J* = 8.94 Hz, Ar-$\underline{H}$),7.23 (2H, d, *J* = 6.90 Hz, Ar-$\underline{H}$), 7.33 (2H, d, *J* = 8.28 Hz, Ar-$\underline{H}$), 7.37 (2H, d, 2H, *J* = 8.28 Hz, Ar-$\underline{H}$), 7.48-7.52 (4H, m, Ar-$\underline{H}$ + C-$\underline{H}$=CH),7.56 (2H, d, *J* = 8.28 Hz, Ar-$\underline{H}$), 7.73 (1H, d, *J* = 15.84 Hz, CH=C-$\underline{H}$), 7.79 (2H, d, *J* = 8.94 Hz, Ar-$\underline{H}$), 7.99 (2H, d, *J* = 8.94 Hz, Ar-$\underline{H}$), 11.02 (1H, s, N$\underline{H}$); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 36.29, 55.28, 114.21, 118.13, 119.29, 122.31, 127.07, 127.17, 129.24, 129.60, 130.03, 130.31, 130.44, 133.55, 133.65, 136.10, 136.20, 142.80, 142.86, 155.51, 155.61, 161.11, 167.27, 188.74; Anal. Calcd. For $C_{32}H_{25}ClN_4O_3S$ (581.08): C, 66.14; H, 4.34; N, 9.64. Found: C, 66.03; H, 4.08; N, 9.38.

4.1.6.29. 2-(5-(3,4-Dimethoxyphenyl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(4-chlorophenyl)acryloyl)phenyl)acetamide (36)

Pale brown powder (0.21 g, 68.93% yield); mp: 155-157^{O}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 3.73 (3H, s, OC$\underline{H}_3$), 3.87 (3H, s, OC$\underline{H}_3$), 4.32 (2H, s, SC$\underline{H}_2$), 6.76 (1H, d, *J* = 8.20 Hz, Ar-$\underline{H}$), 6.96 (1H, d, *J* = 8.20 Hz, Ar-$\underline{H}$), 7.12 (1H, s, Ar-$\underline{H}$), 7.36-7.37 (1H, m, Ar-$\underline{H}$), 7.41 (2H, d, *J* = 7.50 Hz, Ar-$\underline{H}$), 7.51 (1H, d, *J* = 7.10 Hz, Ar-$\underline{H}$), 7.60-7.64 (4H, m, Ar-$\underline{H}$ & C-$\underline{H}$=CH), 7.75 (1H, d, *J* = 15.80 Hz, CH =C-$\underline{H}$), 7.91 (2H,

d, *J* = 8.10 Hz, Ar-*H*), 8.00-8.02 (3H, m, Ar-*H*), 8.07 (1H, d, *J* = 8.10 Hz, Ar-*H*), 11.03 (1H, s, N*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 37.00, 54.94, 55.79, 110.94, 118.74, 119.90, 120.51, 121.62, 121.89, 126.85, 129.20, 129.41, 131.18, 134.07, 138.08, 140.40, 141.26, 142.69, 143.02, 143.57, 146.74, 149.06, 151.07, 153.70, 160.90, 166.15, 188.65; Anal. Calcd. For $C_{33}H_{27}ClN_4O_4S$ (611.11): C, 64.86; H, 4.45; N, 9.17. Found: C, 65.09; H, 4.62; N, 9.45.

4.1.6.30. 2-(5-(3,4,5-Trimethoxyphenyl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(4-chlorophenyl)acryloyl)phenyl)acetamide (37)

Off white powder (0.38 g, 78.60% yield); mp: 205-207^{O}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 3.63 (6H, s, 2OC*H*$_3$), 3.86 (3H, s, OC*H*$_3$), 4.19 (2H, s, SC*H*$_2$), 6.66 (2H, s, Ar-*H*), 7.34-7.36 (2H, m, Ar-*H*), 7.41 (2H, d, *J* = 7.70 Hz, Ar-*H*), 7.52 (1H, d, *J* =15.60 Hz, C-*H*=CH), 7.59-7.61 (5H, m, Ar-*H*), 7.76 (1H, d, *J* = 15.60 Hz, CH=C-*H*), 7.87 (2H, d, *J* = 7.80 Hz, Ar -*H*), 8.02 (2H, d, *J* = 7.80 Hz, Ar-*H*), 11.03 (1H, s, N*H*); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 36.63, 55.97, 60.96, 105.52, 111.00, 112.79, 117.34, 119.33, 119.56, 122.21, 123.38, 126.20, 127.28, 128.30, 133.60, 135.76, 138.23, 140.31, 141.07, 144.93, 147.76, 153.36, 154.77, 166.60, 188.68; Anal. Calcd. For $C_{34}H_{29}ClN_4O_5S$ (641.14): C, 63.69; H, 4.56; N, 8.74. Found: C, 64.01; H, 4.63; N, 8.98.

4.1.6.31. 2-(4,5-Diphenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(4-methoxyphenyl)- acryloyl)phenyl)acetamide (38)

Off white powder (0.29 g, 70.65% yield); mp:205-207^{O}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 3.87 (3H, s, OCH_3), 4.14 (2H, s, SC*H*$_2$), 6.95 (2H, d, *J* = 8.50 Hz, Ar-*H*), 7.25-7.27 (2H, m, Ar-*H*), 7.34 (2H, d, *J* = 7.60 Hz, Ar-*H*), 7.39 (2H, *J* = 7.60 Hz, Ar-*H*), 7.43 (1H, d, *J* = 15.00 Hz, C-*H*=CH), 7.54-7.58 (3H, m, Ar-*H*), 7.62 (2H, d, *J* = 8.50 Hz, Ar-*H*), 7.76-7.81 (2H, m, Ar-*H* + CH=C-*H*), 7.85 (2H, d, *J* = 8.20 Hz, Ar-*H*), 8.02 (2H, d , *J* = 8.20 Hz, Ar-*H*), 11.03 (1H, s, N*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 36.50, 55.44, 111.01, 114.21, 119.52, 123.75, 126.89, 127.26, 130.46, 132.18, 132.55, 132.93, 133.60, 133.99, 138.23, 138.59, 142.10, 143.51, 143.88, 152.31, 157.62, 161.87, 166.48, 189.08; Anal. Calcd. For $C_{32}H_{26}N_4O_3S$ (546.64): C, 70.31; H, 4.79; N, 10.25. Found: C, 70.67; H, 4.85; N, 10.61.

4.1.6.32. 2-(5-(4-Chlorophenyl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(4-methoxyphenyl)acryloyl)phenyl)acetamide (39)

Pale brown powder (0.24 g, 72.34% yield); mp: 175-177^{O}C; ^{1}H NMR (500 MHz, $CDCL_3$) δ (ppm): 3.87 (3H, s, OC$\underline{H}_3$), 4.11 (2H, s, SC$\underline{H}_2$), 6.96 (2H, d, *J* = 8.30 Hz, Ar-$\underline{H}$), 7.31 (2H, d, *J* = 8.20 Hz, Ar-$\underline{H}$), 7.37 (2H, d , *J* = 8.20 Hz, Ar-$\underline{H}$), 7.43 (1H, d, *J* = 15.50 Hz, C-$\underline{H}$=CH), 7.53-7.57 (5H, m, Ar-$\underline{H}$), 7.62 (2H, d, *J* = 7.30 Hz, Ar-$\underline{H}$), 7.67 (1H, d, *J* = 15.50 Hz, CH=C -$\underline{H}$), 7.83 (2H, d, *J* = 8.30 Hz, Ar-$\underline{H}$), 8.02 (2H, d, *J* = 8.30 Hz, Ar-$\underline{H}$), 10.88 (1H, s, N$\underline{H}$); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 36.49, 55.22, 118.46, 118.90, 119.55, 119.92, 121.58, 123.89, 124.82, 129.93, 130.27, 130.44, 130.76, 130.81, 131.33, 132.99, 134.15, 136.82, 144.24, 154.22, 154.33, 161.53, 166.65, 189.04; Anal. Calcd. For $C_{32}H_{25}ClN_4O_3S$ (581.08): C, 66.14; H, 4.34; N, 9.64. Found: C, 66.42; H, 4.21; N, 9.89.

4.1.6.33. 2-(5-(4-Methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(4-methoxyphenyl)acryloyl)phenyl)acetamide (40)

Off white powder (0.35 g, 78% yield); mp: 215-218^{O}C; ^{1}H NMR (600MHz, $CDCl_3$) δ (ppm): 3.78 (3H, s, OC$\underline{H}_3$), 3.85 (3H, s, OC$\underline{H}_3$), 3.96 (2H, s, SC$\underline{H}_2$), 6.81 (2H, d, *J* = 8.94 Hz, Ar-$\underline{H}$), 6.92 (2H, d, *J* = 8.94 Hz, Ar-$\underline{H}$), 7.23-7.24 (2H, m, Ar-$\underline{H}$), 7.34 (2H, d, *J* = 8.94 Hz, Ar-$\underline{H}$), 7.40 (1H, d, *J* = 15.12 Hz, C-$\underline{H}$=CH), 7.49-7.52 (3H, m, Ar-$\underline{H}$), 7.59 (2H, d, *J* = 8.94 Hz, Ar-$\underline{H}$), 7.75-7.79 (3H, m, Ar-$\underline{H}$ + CH=C-$\underline{H}$), 8.00 (2H, d, *J* = 8.94 Hz, Ar-$\underline{H}$), 10.96 (1H, s, N$\underline{H}$); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 36.28, 55.28, 55.38, 114.21, 114.41, 117.30, 119.24, 120.10, 127.09, 128.20, 129.71, 129.91, 130.23, 130.30, 130.35, 133.67, 133.90, 142.30, 145.30, 151.70, 154.10, 156.20, 161.10, 167.23, 188.70; Anal. Calcd. For $C_{33}H_{28}N_4O_4S$ (576.66): C, 68.73; H, 4.89; N, 9.72. Found: C, 69.07; H, 5.03; N, 9.48.

4.1.6.34. 2-(5-(3,4-Dimethoxyphenyl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(4-methoxyphenyl)acryloyl)phenyl)acetamide (41)

Pale brown powder (0.30 g, 66.98% yield); mp: 150-153^{O}C; ^{1}H NMR (500MHz, $CDCl_3$) δ (ppm): 3.70 (3H, s, OC$\underline{H}_3$), 3.87(6H, s, 2OC$\underline{H}_3$), 4.09 (2H, s, SC$\underline{H}_2$), 6.67 (1H, d, *J* = 7.60 Hz, Ar-$\underline{H}$), 6.92- 6.95 (3H, m, Ar-$\underline{H}$), 7.02 (1H, s, Ar-$\underline{H}$), 7.29-7.30 (2H, m, Ar-$\underline{H}$), 7.42 (1H, d, *J* = 15.60 Hz, C-$\underline{H}$=CH), 7.55-7.57 (3H, m, Ar-$\underline{H}$), 7.61 (2H, d, *J* = 7.60 Hz, Ar-$\underline{H}$), 7.78 (1H, d , *J* = 15.60 Hz, CH=C -$\underline{H}$), 7.84 (2H, d, *J* =

7.30 Hz, Ar-*H*), 8.01 (2H, d, *J* = 7.30 Hz, Ar-*H*), 10.99 (1H, s, N*H*); ^{13}CNMR (125 MHz, $CDCl_3$) δ (ppm): 36.61, 55.43, 55.78, 55.92, 110.98, 111.05, 114.42, 117.67, 119.25, 119.38, 119.57, 121.33, 127.24, 127.75, 129.74, 130.21, 130.38, 130.59, 133.98, 142.46, 144.24, 144.66, 148.94, 150.83, 153.58, 155.09, 166.88, 189.04.; Anal. Calcd. For $C_{34}H_{30}N_4O_5S$ (606.69): C, 67.31; H, 4.98; N, 9.23. Found: C, 67.64; H, 4.87; N, 9.50.

4.1.6.35. 2-(5-(3,4,5-Trimethoxyphenyl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(4-methoxyphenyl)acryloyl)phenyl)acetamide (42)

Yellow powder (0.43 g, 83% yield); mp 186-187^{O}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 3.63 (6H, s, 2OC*H*$_3$), 3.85 (3H, s, OC*H*$_3$), 3.87 (3H, s, OC*H*$_3$), 4.15 (2H, s, SC*H*$_2$), 6.65 (2H, s, Ar-*H*) , 6.95 (2H, d, *J* = 8.50 Hz, Ar-*H*), 7.33-7.34 (2H, m, Ar-*H*), 7.43 (1H, d , *J*=15.60 Hz, C-*H*=CH), 7.59-7.60 (3H, m, Ar-*H*), 7.62 (2H, d, *J* = 8.50 Hz, Ar-*H*), 7.79 (1H, d, *J* = 15.60 Hz, CH=C-*H*), 7.85 (2H, d, *J* = 8.20 Hz, Ar-*H*), 8.02 (2H, d, *J* = 8.20 Hz, Ar-*H*), 10.99 (1H, s, N*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 36.57, 55.44, 55.95, 60.95, 105.48, 114.42, 119.25, 119.52, 119.93, 127.29, 127.72, 129.74, 130.23, 130.48, 130.77, 133.42, 134.02, 139.92, 142.42, 144.30, 153.25, 153.96, 154.84, 161.62, 166.69, 189.04; Anal. Calcd. For $C_{35}H_{32}N_4O_6S$ (636.72): C, 66.02; H, 5.07; N, 8.80. Found: C, 66.31; H, 5.13; N, 8.97.

4.1.6.36. 2-(4,5-Diphenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(3,4,5-trimethoxyphenyl)- acryloyl)phenyl)acetamide (43)

Off white powder (0.43 g, 81.54% yield); mp: 218-220^{O}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 3.92 (3H, s, OC*H*$_3$), 3.94 (6H, s, 2OC*H*$_3$), 4.11 (2H, s, SC*H*$_2$), 6.88 (2H, s, Ar-*H*), 7.24-7.27 (2H, m, Ar-*H*), 7.34 (2H, d, *J* = 7.50 Hz, Ar-*H*), 7.36-7.40 (1H m, Ar-*H*), 7.42 (2H, d, *J* = 7.50 Hz, Ar-*H*), 7.52-7.557 (3H, m, Ar-*H* + C-*H*=CH), 7.64-7.73 (2H, m, Ar-*H* + CH =C-*H*), 7.85 (2H, d, *J* = 8.60 Hz, Ar-*H*), 8.02 (2H, d, *J* = 8.60 Hz, Ar-*H*), 11.04 (1H, s, N*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 36.56, 56.02, 60.96, 105.61, 118.68, 119.54, 121.58, 124.77, 126.56, 128.23, 129.78, 130.37, 130.64, 131.51, 132.65, 133.24, 139.67, 140.22, 142.24, 144.29, 157.70, 158.87, 160.90, 166.43, 188.90; Anal. Calcd. For $C_{34}H_{30}N_4O_5S$ (606.69): C, 67.31; H, 4.98; N, 9.23. Found: C, 67.63; H, 5.04; N, 9.36.

4.1.6.37. 2-(5-(4-Chlorophenyl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(3,4,5-tri- methoxyphenyl)acryloyl)phenyl)acetamide (44)

Off white powder (0.40 g, 88.7% yield) ; mp 230-233^{O}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 3.87 (3H, s, OC*H*$_3$), 3.89 (6H, s, 2OC*H*$_3$), 3.99 (2H, s, SC*H*$_2$), 6.83 (2H, s, Ar-*H*), 7.22 (2H, d, *J* = 8.22 Hz, Ar-*H*),7.24 (2H, d, *J* = 8.94 Hz, Ar-*H*), 7.32 (2H, d, *J* = 8.94 Hz, Ar-*H*), 7.37 (1H, d, *J* = 15.84 Hz, C-*H*=CH), 7.51-7.54 (3H, m, Ar-*H*), 7.67 (1H, d, *J* = 15.84 Hz, CH =C-*H*), 7.76 (2H, d, *J* = 8.22 Hz, Ar-*H*), 7.97 (2H, d, *J* = 8.22 Hz, Ar-*H*), 10.83 (1H, s, N*H*); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 36.25, 56.14, 60.92, 105.57, 119.20, 121.25, 124.30, 126.93, 129.08, 129.31, 129.90, 130.43, 130.67, 133.22, 133.80, 136.56, 140.30, 142.47, 142.67, 144.59, 153.46, 153.90, 154.55, 166.95, 189.06; Anal. Calcd. For $C_{34}H_{29}ClN_4O_5S$ (641.14): C, 63.69; H, 4.56; N, 8.74. Found: C, 64.02; H, 4.68; N, 8.98.

4.1.6.38. 2-(5-(4-Methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(3,4,5-trimethoxyphenyl)acryloyl)phenyl)acetamide (45)

Off white powder (0.31g, 80% yield); mp 243-245^{O}C; ^{1}H NMR (600MHz, $CDCl_3$), δ (ppm): 3.77 (3H, s, OC*H*$_3$), 3.88 (3H, s, OC*H*$_3$), 3.90 (6H, s, 2OC*H*$_3$), 3.97 (2H, s, SC*H*$_2$), 6.80 (2H, d, *J* = 8.22 Hz, Ar-*H*), 6.85 (2H, s, Ar-*H*), 7.23 (2H, d, *J* = 8.22 Hz, Ar-*H*), 7.32 (2H, d, *J* = 6.90 Hz, Ar-*H*), 7.38 (1H, d, *J* = 15.12 Hz, C-*H*=CH), 7.50-7.53 (3H, m, Ar-*H*), 7.68 (1H, d, *J* = 15.12 Hz, CH=C-*H*), 7.79 (2H, d, *J* = 8.94 Hz, Ar-*H*), 7.99 (2H, d, *J* = 8.94 Hz, Ar-*H*), 11.00 (1H, s, N*H*); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 36.29, 55.26, 56.18, 60.96, 105.57, 114.19, 118.11, 119.26, 121.38, 127.07, 129.67, 129.77, 129.85, 130.29, 130.43, 133.72, 137.80, 140.27, 144.10, 146.09, 153.29, 153.39, 155.05, 161.20, 168.15, 189.18; Anal. Calcd. For $C_{35}H_{32}N_4O_6S$ (636.72): C, 66.02; H, 5.07; N, 8.80. Found: C, 65.78; H, 4.96; N, 8.97.

4.1.6.39. 2-(5-(3,4-Dimethoxyphenyl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(3,4,5-trimethoxyphenyl)acryloyl)phenyl)acetamide (46)

Olive green powder (0.42 g, 80.03% yield); mp;176-178^{O}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 3.70 (3H, s, OC*H*$_3$), 3.86 (3H, s, OC*H*$_3$), 3.91 (3H, s, OC*H*$_3$), 3.94 (6H, s, 2OC*H*$_3$), 4.17 (2H, s, SC*H*$_2$), 6.76 (1H, d, *J*=8.30 Hz, Ar-*H*), 6.87 (2H, s, Ar-*H*), 6.94 (1H, d, *J* = 8.30 Hz, Ar-*H*), 7.05 (1H, s, Ar-*H*), 7.38 (1H, d, *J* = 15.60 Hz, C-*H*=CH), 7.40-7.42 (2H, m, Ar-*H*), 7.57 (2H, d, *J* = 7.60 Hz, Ar-*H*), 7.67-7.72 (2H, m,

Ar-*H* + CH=C-*H*), 7.88 (2H, d, *J* = 7.90 Hz, Ar-*H*), 7.99-8.02 (2H, m, Ar-*H*), 10.97 (1H, s, N*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 36.49, 54.69, 54.91, 56.63, 57.48, 105.94, 110.16, 118.90, 119.50, 120.26, 121.18, 121.46, 127.37, 128.72, 131.28, 133.77, 134.38, 140.56, 141.68, 142.53, 143.95, 144.79, 149.32, 151.27, 153.54, 154.71, 161.68, 166.76, 189.03; Anal. Calcd. For $C_{36}H_{34}N_4O_7S$ (666.74): C, 64.85; H, 5.14; N, 8.40. Found: C, 65.02; H, 5.01; N, 8.35.

4.1.6.40. 2-(5-(3,4,5-Trimethoxyphenyl)-4-phenyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(4-((*E*)-3-(3,4,5-trimethoxyphenyl)acryloyl)phenyl)acetamide (47)

Off white powder (0.25 g, 76.89% yield); mp: 222-225^{O}C; ^{1}H NMR (500MHz, $CDCl_3$) δ (ppm): 3.63 (6H, s, 2OC*H*$_3$), 3.86 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 3.94 (6H, s, 2OC*H*$_3$), 4.15 (2H, s, SC*H*$_2$), 6.65 (2H, s, Ar-*H*), 6.88 (2H, s, Ar-*H*), 7.33-7.34 (2H, m, Ar-*H*), 7.41 (1H, d, *J* = 15.60 Hz, C-*H*=CH), 7.59-7.60 (3H, m, Ar-*H*), 7.72 (1H, d, *J* = 15.60 Hz, CH=C-*H*), 7.86 (2H, d, *J* = 8.40 Hz, Ar-*H*), 8.02 (2H, d, *J* = 8.40 Hz, Ar-*H*), 11.02 (1H, s, N*H*); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 36.56, 55.95, 56.25, 60.95, 61.03, 105.47, 105.61, 119.30, 119.83, 121.29, 127.27, 129.84, 130.48, 130.51, 130.82, 133.38, 133.78, 139.97, 140.34, 142.55, 144.66, 153.27, 153.48, 153.98, 154.82, 166.68, 189.10; Anal. Calcd. For $C_{37}H_{36}N_4O_8S$ (696.77): C, 63.78; H, 5.21; N, 8.04. Found: C, 64.07; H, 5.37; N, 8.35.

4.2. Biology section:

Cell culture and reagents

Human lung adenocarcinoma (A549) cell line was obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA) and was cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich Co., LLC., St. Louis, MO, USA), containing 10% fetal bovine serum (FBS; Sigma-Aldrich), 100 U/mL penicillin, and 100 μg/mL streptomycin (Life Technologies) in a humidified atmosphere with 5% CO2 at 37°C. Triazole-chalcone hybrids were chemically synthesized (**8**-**47**). Cisplatin was purchased from Sigma-Aldrich. Pan-caspase inhibitor (z-VAD-fmk) was purchased from Enzo Life Sciences, PA, USA. All chemicals used in this study were of the analytical or cell-culture grade.

4.2.1. Proliferation assay

The methodology of the NCI anticancer screening has been described in detail elsewhere (http://www.dtp.nci.nih.gov). Briefly, the primary anticancer assay was performed at approximately 60 human tumor cell lines panel derived from nine neoplastic diseases, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda. Tested compounds were added to the culture at a single concentration (10^{-5} M) and the cultures were incubated for 48 h. Endpoint determinations were made with a protein binding dye, SRB. Results for each tested compound were reported as the percent of the growth of the treated cells when compared to the untreated control cells. The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents.

Compounds **24, 25, 27, 41, 47** which Showed significant cell growth inhibition in the One-Dose Screen were evaluated against the 60 cell panel at five different concentrations by a solubilizing drug in dimethyl sulfoxide. After drug addition, cells were incubated at 37° C, 5 % CO_2, 95 % air and 100 % relative humidity for 48h then Staining by SRB and the absorbance was evaluated spectrophotometrically by using an automated plate. The growth percentage was calculated at different drug concentrations levels and at a different time.

4.2.2. Anti-proliferative assay [72].

On the other hand, proliferation assay for A549 cells was quantified as BrdU incorporation using a Cell Proliferation ELISA, BrdU (colorimetric) Kit (Roche Diagnostics, Indianapolis, IN, USA) following the manufacturer's prescribed instructions. A549 cells were seeded as 5×10^3 cells/well and cultured overnight in a 96-well plate. The cells were treated with 1, 5, 10, 25, 50 or 100 μM triazole-chalcone hybrids **8-47,** cisplatin as a positive control, or DMSO as a negative control for 24 h. BrdU was added to be a final concentration of 10 μM and cultured for further two h. BrdU incorporation by the cells was quantified according to the manufacturer's instructions. At least three independent experiments were performed. Percentage of growth inhibition was determined as [1− (OD of treated cells/OD of control cells)] ×100.

4.2.3. Apoptosis analysis [73].

Apoptosis of A549 cells induced by triazole-chalcone hybrids, compounds **24**, **25**, **27**, **41** and **47**, was analyzed by FACS Calibur flow cytometer (BD Biosciences, San Jose, CA, USA) using the Annexin V/propidium iodide (PI) staining kit (BioLegend Inc., San Diego, CA, USA), according to the manufacturer's instructions. Briefly, A549 cells were treated with 1, 5 or 10 µM triazole-chalcone hybrids or DMSO for 24 h. After harvesting, the cells were stained with FITC-labeled annexin V antibody and PI for apoptosis assay in annexin V binding buffer (BioLegend). Quantitative analysis of the FACS data was done by using FlowJo software (Flowjo, Ashland, OR, USA).

4.2.4. Immunoblotting analysis [74].

A549 cells were harvested, washed twice with chilled PBS, and lysed with ice-cold lysis buffer containing 0.1% SDS, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 2 µg/mL aprotinin, 5 µg/mL pefabloc SC (4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride), a protease inhibitor cocktail, 1 % phosphatase inhibitor cocktail and 50 mM Tris–HCl (pH 7.4) after 24 h incubation with **24, 25, 27, 41, 47** or DMSO (control). The cell lysates were kept on ice for 30 min after gentle vortex and then centrifuged at 14,000g for 15 min at 4°C. The supernatants were loaded onto SDS-PAGE gel immediately after protein extraction, or otherwise, the supernatants were stored at −80°C until use. The protein concentration was determined by Bradford assay (Bio-Rad Laboratories, CA, USA) according to the instructions of the manufacturer. For Western blotting analysis, a weight of 30 µg protein cell lysates was loaded onto a 15% SDS-PAGE gel. Proteins separated on an SDS-PAGE gel were transferred onto a PVDF membrane. The PVDF membrane was incubated in blocking buffer containing 3% non-fat milk powder, 1% bovine serum albumin (Sigma-Aldrich), and 0.5% Tween-20 in PBS for 1 h. Subsequently, the PVDF membrane was incubated with anti-Bax monoclonal antibody (mAb, clone no. D2E11), anti-cytochrome c polyclonal antibody (pAb), anti-cleaved caspase 3 (mAb, 5A1E), anti-cleaved caspase 8 (mAb, 11G10), anti-cleaved caspase-9 polyclonal antibody (pAb), or anti β Actin mAb (clone no. 8H10D10) (Cell Signaling Technologies, Danvers, MA, USA) for overnight, and followed by HRP-conjugated anti-rabbit IgG (Cell Signaling Technologies) or HRP-conjugated anti-mouse IgG (Becton Dickinson Co, Durham, NC, USA) for 1 h with agitation at room

temperature. Finally, the specific bindings to each primary antibody were detected on an X-ray film (Konica Minolta Medical Imaging, Wayne, NJ, USA) with ECL prime immunodetection reagent (GE Healthcare, Little Chalfont, UK).

4.2.5. Pan-caspase inhibitor (z-VAD-fmk) experiments [75].

Pan-caspase inhibitor **(**z-VAD-fmk**;** 20 µM) was added to the cells with ~50% confluency. After 24 h the cells were incubated with 10 µM of compounds **24**, **25**, **27**, **41** or **47** and/or 10 µM z-VAD-fmk (total z-VAD-fmk concentration were therefore 30 µM) for 24h. DMSO was used as a control. In order to confirm the inhibition of cleaved caspase-3, whole cell lysates were prepared and western blotting analyses were performed for cleaved caspase-3 expression as described above. Apoptosis by flow cytometry was performed to analyze the effect of z-VAD-fmk on triazole-chalcone hybrids induced apoptosis as described above under apoptosis analysis protocol.

4.2.6. Statistical analysis

Data are represented as means ± SD. Student's t-test was performed to determine the statistical significance of triazole-chalcone hybrids and control or between triazole-chalcone hybrids and triazole chalcone hybrids with z-VAD-fmk. Statistical significance was defined as *P < 0.05 or **P<0.005. The IC_{50} of compounds were calculated by Graph Pad Prism 5 (Version 5.01, GraphPad Software, San Diego, CA, USA). The data shown in the figures are representative data for three independent experimental results.

5. Acknowledgment

Authors thank the Development Therapeutics Program of the National Cancer Institute, Bethesda, MD, USA, for *in vitro* evaluation of the anticancer activity. Grateful thanks are expressed to **Dr. Mohammed El Amir Fathi Higazy,** National Research Centre, Egypt and **Dr. Mohammed Abdel Wahaab**, Um Al-Qura University, Saudi Arabia for helping in measuring the NMR spectral analysis.

Conflicts of Interest

The authors declare no conflicts of interest

6. References

[1] M.J. Thun, J.O. DeLancey, M.M. Center, A. Jemal, E.M. Ward, The global burden of cancer: priorities for prevention, Carcinogenesis. 31 (**2010**) 100–110.

[2] W.M. Eldehna, H. Almahli, G.H. Al-Ansary, H.A. Ghabbour, M.H. Aly, O.E. Ismael, A. Al-Dhfyan, H.A. AbdelAziz, Synthesis and in vitro anti-proliferative activity of some novel isatins conjugated with quinazoline/phthalazine hydrazines against triple-negative breast cancer MDA-MB-231 cells as apoptosis inducing agents, J Enzyme Inhib Med Chem. 32 (**2017)** 600-613.

[3] R.SY. Wong, Apoptosis in cancer: From pathogenesis to treatment, J. Exp. Clin. Cancer Res. 30 (**2011**) 87-100.

[4] S. Elmore, Apoptosis: A review of programmed cell death, Toxicol. Pathol. 35 (**2007**) 495–516.

[5] B. Conradt, Genetic control of programmed cell death during animal development, Annu. Rev. Genet. 43 (**2009**) 493–523.

[6] A. Modzelewska, C. Pettit, G. Achanta, N.E. Davidson, P. Huang, S.R. Khan, Anticancer activities of novel chalcone and bis-chalcone derivatives, Bioorg. Med. Chem. 14 (**2006**) 3491–3495.

[7] M.A.E. Mourad, M. Abdel-Aziz, G.E.-D.A. A.Abuo-Rahma, H.H. Farag, Design, Synthesis and Anticancer Activity of Nitric Oxide Donating/Chalcone Hybrids, *Eur. J. Med. Chem.* 54 (**2012**) 907–913.

[8] S. Syam, S.I. Abdelwahab, M.A. Al-Mamary, S. Mohan, Synthesis of Chalcones with Anticancer Activities, Molecules 17 (**2012**) 6179–6195.

[9] C. Karthikeyan, N.S.H.N. Moorthy, S. Ramasamy, U. Vanam, E. Manivannan, D. Karunagaran, P. Trivedi, Advances in chalcones with anticancer activities, Recent Pat. Anticancer Drug Discov. 10 (**2015**) 97–115.

[10] R.N. Gacche, N.A. Dhole, S.G. Kamble, B. P. Bandgar, *In-vitro* Evaluation of Selected Chalcones for Antioxidant Activity, J. Enzyme Inhib. Med. Chem. 23 (**2008**) 28–31.

[11] G. Cioffi, L.M. Escobar, A. Braca, N. De Tommasi, Antioxidant Chalcone Glycosides and Flavanones from *Maclura* (*Chlorophora*) *tinctoria*. J. Nat. Prod. 66 (**2003**) 1061–1064.

[12] A.A. Bekhit, N.S. Habib, A. el-Din, A. Bekhit, A. Synthesis and Antimicrobial Evaluation of Chalcone and Syndrome Derivatives of 4(3*H*)-Quinazolinone, Boll. Chim. Farm. 140 (**2001**) 297–301.

[13] A. Husain, M. Rashid, R. Mishra, D. Kumar, Bis-Chalcones and Flavones: Synthesis and Antimicrobial Activity, Acta Pol. Pharm. 70 (**2013**) 443–449.

[14] R.L. Macdonald, M.J. McLean, Anticonvulsant Drugs: Mechanisms of Action, Adv. Neurol. 44 (**1986**) 713–736.

[15] S. Kaushik, N. Kumar, S. Drabu, Synthesis and Anticonvulsant Activities of PhenoxyChalcones, The Pharma.Research. 1 (**2010**) 257-262.

[16] H.K. Hsieh, L.T. Tsao, J.P. Wang, C.N. Lin, Synthesis and Anti-inflammatory Effect of Chalcones, J. Pharm. Pharmacol. 52 (**2000**) 163–171.

[17] Y.H. Kim, J. Kim, H. Park, H.P. Kim, Anti-inflammatory Activity of The Synthetic Chalcone Derivatives: Inhibition of Inducible Nitric Oxide Synthase-Catalyzed Nitric Oxide Production from Lipopolysaccharide-Treated RAW 264.7 Cells, Biol. Pharm. Bull. 30 (**2007**) 1450–1455.

[18] Z. Wan, D. Hu, P. Li, D. Xie, X. Gan, Synthesis, Antiviral Bioactivity of Novel 4-Thioquinazoline Derivatives Containing Chalcone Moiety, Mol. Basel Switz. 20 (**2015**) 11861–11874.

[19] T.T. Dao, P.H. Nguyen, H.S. Lee, E. Kim, J. Park, S.I. Lim, W.K. Oh, Chalcones as Novel Influenza A (H1N1) Neuraminidase Inhibitors from *Glycyrrhiza inflate*, Bioorg. Med. Chem. Lett. 21 (**2011**) 294–298.

[20] N. Yadav, S.K. Dixit, A. Bhattacharya, L.C. Mishra, M. Sharma, S.K. Awasthi, V.K. Bhasin, Antimalarial Activity of Newly Synthesized Chalcone Derivatives, In Vitro. Chem. Biol. Drug Des. 80 (**2012**) 340–347.

[21] J.N. Domínguez, C. León, J. Rodrigues, N. Gamboa de Domínguez, J. Gut, P.J. Rosenthal, Synthesis and Antimalarial Activity of Sulfonamide Chalcone Derivatives, Farmaco. 60 (**2005**) 307–311.

[22] D.D. Jandial, C.A. Blair, S. Zhang, L.S. Krill, Y.-B. Zhang, X. Zi, Molecular Targeted Approaches to Cancer Therapy and Prevention Using Chalcones, Curr. Cancer Drug Targets 14 (**2014**) 181–200.

[23] A. Boumendjel, X. Ronot, J. Boutonnat, Chalcones Derivatives Acting as Cell Cycle Blockers: Potential AntiCancer Drugs?, Curr. Drug Targets 10 (**2009**) 363–371.

[24] S. Ducki, The Development of Chalcones as Promising Anticancer agents, I.Drugs. 10 (**2007**) 42–46.

[25] V. Martel-Frachet, M. Keramidas, A. Nurisso, S. DeBonis, C. Rome, J.-L Coll, A. Boumendjel, D.A. Skoufias, X. Ronot, IPP51, a Chalcone Acting as A Microtubule Inhibitor with *In vivo* Antitumor Activity Against Bladder Carcinoma, Oncotarget. 6 (**2015**) 14669–14686.

[26] M. Das, K. Manna, Chalcone Scaffold in Anticancer Armamentarium: A Molecular Insight, J. Toxicol. 2016 (**2016**) 1-14.

[27] C.W. Mai, M. Yaeghoobi, N. Abd-Rahman, Y.B. Kang, M.R. Pichika, Chalcones with electron-withdrawing and electron-donating substituents: Anticancer activity against TRAIL resistant cancer cells, structure-activity relationship analysis and regulation of apoptotic proteins, Eur. J. Med. Chem. 77(**2014**) 378–387.

[28] E. Winter, L.D. Chiaradia, A.H. Silva, R.J. Nunes, R.A. Yunes, T.B. Creczynski-Pasa, Involvement of extrinsic and intrinsic apoptotic pathways together with endoplasmic reticulum stress in cell death induced by naphthylchalcones in a leukemic cell line: advantages of multi-target action, Toxicol. In Vitro. J. 28 (**2014**) 769–777.

[29] A.M. Vijesh, A.M. Isloor, P. Shetty, S. Sundershan, H.K. Fun, New pyrazole derivatives containing 1,2,4-triazoles and benzoxazoles as potent antimicrobial and analgesic agents. Eur. J. Med. Chem. 62 (**2013**) 410–415.

[30] X. Collin, A. Sauleau, J. Coulon, 1,2,4-Triazolo mercapto and aminonitriles as potent antifungal agents, Bioorg. Med. Chem. Lett. 13 (**2003**) 2601–2605.

[31] U. Salgın-Gökşen, N. Gökhan-Kelekçi, Ö. Göktaş, Y. Köysal, E. Kılıç, Ş. Işık, G. Aktay, M. Özalp, 1-Acylthiosemicarbazides, 1,2,4-triazole-5(4*H*)-thiones, 1,3,4-thiadiazoles and hydrazones containing 5-methyl-2-benzoxazolinones: Synthesis, analgesic-anti-inflammatory and antimicrobial activities, Bioorg. Med. Chem. 15 (**2007**) 5738–5751.

[32] I. Khan, S. Ali, S. Hameed, N.H. Rama, M.T. Hussain, A. Wadood, R. Uddin, Z. Ul-Haq, A. Khan, S. Ali, M. Choudhary, Synthesis, antioxidant activities and urease inhibition of some new 1,2,4-triazole and 1,3,4-thiadiazole derivatives, Eur. J. Med. Chem. 45 (**2010**) 5200–5207.

[33] B. Tozkoparan, E. Küpeli, E. Yeşilada, M. Ertan, Preparation of 5-aryl-3-alkylthio-l,2,4-triazoles and corresponding sulfones with antiinflammatory–analgesic activity, Bioorg. Med. Chem. 15 (**2007**) 1808–1814.

[34] B.S. Holla, B. Veerendra, M.K. Shivananda, B. Poojary, Synthesis characterization and anticancer activity studies on some Mannich bases derived from 1,2,4-triazoles, Eur. J. Med. Chem. 38 (**2003**) 759–767.

[35] R. Lin, P.J. Connolly, S. Huang, S.K. Wetter, Y. Lu, W.V. Murray, S.L. Emanuel, R.H. Gruninger, A.R. Fuentes-Pesquera, C.A. Rugg, S.A. Middleton, L.K. Jolliffe, 1-Acyl-1*H*-[1,2,4]triazole-3,5-diamine Analogues as Novel and Potent Anticancer Cyclin-Dependent Kinase Inhibitors: Synthesis and Evaluation of Biological Activities, J. Med. Chem. 48 (**2005**) 4208–4211.

[36] G.El.-D.A.A. Abuo-Rahma, M. Abdel-Aziz, M. E.A.M. Beshr, T.F.S. Ali, 1,2,4-Triazole/oxime hybrids as new strategy for nitric oxide donors: Synthesis, anti-inflammatory, ulceroginicity and antiproliferative activities, Eur. J. Med. Chem. 71 (**2014**) 185–198.

[37] İ. Küçükgüzel, Ş. Güniz Küçükgüzel, S. Rollas, G. Ötük-Sanış, O. Özdemir, İ. Bayrak, T. Altuğ, J.P. Stables, Synthesis of some 3-(Arylalkylthio)-4-alkyl/aryl-5-(4-aminophenyl)-4*H*-1,2,4-triazole derivatives and their anticonvulsant activity, *Farmaco.* 59 (**2004**) 893–901.

[38] R.W. Sidwell, J.H. Huffman, G.P. Khare, L.B. Allen, J.T. Witkowski, R.K. Robins, Broad-Spectrum Antiviral Activity of Virazole: 1-beta-D-Ribofuranosyl-1,2,4-triazole- 3-carboxamide, Science 177 (**1972**) 705–706.

[39] E. Palaska, G. Şahin, P. Kelicen, N.T. Durlu, G. Altinok, Synthesis and anti-inflammatory activity of 1-acylthiosemicarbazides, 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazole-3-thiones. Farmaco. 57 (**2002**) 101–107.

[40] B. Tozkoparan, N. Gökhan, G. Aktay, E. Yeşilada, M. Ertan, 6-Benzylidenethiazolo[3,2-b]-1,2,4-triazole-5(6*H*)-onessubstituted with ibuprofen: synthesis, characterization and evaluation of anti-inflammatory activity, Eur. J. Med. Chem. 35 (**2000**) 743–750.

[41] A. Varvaresou, A. Tsantili-Kakoulidou, T. Siatra-Papastaikoudi, E. Tiligada, Synthesis and Biological Evaluation of Indole Containing Derivatives of Thiosemicarbazide and Their Cyclic 1,2,4-Triazole and 1,3,4-Thiadiazole Analogs, Arzneimittel-forschung. 50 (**2000**) 48-54.

[42] M. Asif, Analgesic and Anti-Inflammatory Activities of Different Triazole Analogues, PharmaTutor Magazine 2 (**2014**) 62–71.

[43] R. Romagnoli, P.G. Baraldi, O. Cruz-Lopez, C.L. Cara, M.D. Carrion, A. Brancale, E. Hamel, L. Chen, R. Bortolozzi, G. Basso, G. Viola, Synthesis and Antitumor Activity of 1,5-Disubstituted 1,2,4-Triazoles as Cis-Restricted Combretastatin Analogues, J. Med. Chem. 53 (**2010**) 4248–4258.

[44] Z. Mao, X. Zheng, Y. Qi, M. Zhang, Y. Huang, C. Wan, G. Rao, Synthesis and biological evaluation of novel hybrid compounds between chalcone and piperazine as potential antitumor agents, RSC Adv. 6 (**2016**) 7723–7727.

[45] R. Ferrer, G. Lobo, N. Gamboa, J. Rodrigues, C. Abramjuk, K. Jung, M. Lein, J.E. Charris, Synthesis of [(7-Chloroquinolin-4-yl)amino]chalcones: Potential Antimalarial and Anticancer Agents, Sci. Pharm. 77 (**2009**) 725–741.

[46] M. Abdel-Aziz, S.-E. Park, G.E.-D.A.A. Abuo-Rahma, M.A. Sayed, Y. Kwon, Novel *N*-4-piperazinyl-ciprofloxacin-chalcone hybrids: synthesis, physicochemical properties, anticancer and topoisomerase I and II inhibitory activity, Eur. J. Med. Chem. 69 (**2013**) 427–438.

[47] K.V. Sashidhara, A. Kumar, M. Kumar, J. Sarkar, S. Sinha, Synthesis and *in vitro* evaluation of novel coumarin-chalcone hybrids as potential anticancer agents, Bioorg. Med. Chem. Lett. 20 (**2010**) 7205–7211.

[48] H. Wei, J. Ruan, X. Zhang, Coumarin–chalcone hybrids: promising agents with diverse pharmacological properties, RSC Adv. 6 (**2016**) 10846–10860.

[49] Z.A. Wani, A.S. Pathania, G. Mahajan, A. Behl, M.J. Mintoo, S.K. Guru, A. Viswanath, F. Malik, A. Kamal, D.M. Mondhe, Anticancer activity of a novel quinazolinone-chalcone derivative through cell cycle arrest in pancreatic cancer cell line, J. Solid Tumors 5 (**2015**) 73-85.

[50] R. Pingaew, A. Saekee, P. Mandi, C. Nantasenamat, S. Prachayasittikul, S. Ruchirawat, V. Prachayasittikul, Synthesis, biological evaluation and molecular docking of novel chalcone-coumarin hybrids as anticancer and antimalarial agents, Eur. J. Med. Chem. 85 (**2014**) 65–76.

[51] G.D. da Silva, M.G. da Silva, E.M.P.V.E. Souza, A. Barison, S.C. Simões, F.P. Varotti, L.A. Barbosa, G.H.R. Viana, J.A.F.P. Villar, Design and Synthesis of New Chacones Substituted with Azide/Triazole Groups and Analysis of Their Cytotoxicity Towards HeLa Cells, Molecules**.** 17 (**2012**) 10331–10343.

[52] S.-Y. Zhang, D.-J. Fu, X.-X. Yue, Y.-C. Liu, J. Song, H.-H. Sun, H.-M. Liu, Y.-B. Zhang, Design, Synthesis and Structure-Activity Relationships of Novel Chalcone-1,2,3-triazole-azole Derivates as Antiproliferative Agents, Molecules 21 (**2016**) 653-657.

[53] A.H. Banday, V.V. Kulkarni, V.J. Hruby, Design, synthesis, and biological and docking studies of novel epipodophyllotoxin–chalcone hybrids as potential anticancer agents, Med. Chem. Comm. 6 (**2015**) 94–104.

[54] P. Yadav, K. Lal, A. Kumar, S.K. Guru, S. Jaglan, S. Bhushan, Green synthesis and anticancer potential of chalcone linked-1,2,3-triazoles. Eur. J. Med. Chem. 126 (**2017**) 944-953.

[55] R. Kaur, A.R. Dwivedi, B. Kumar, V. Kumar, Recent developments on 1,2,4-triazole nucleus in anticancer compounds: a review. Anticancer Agents Med Chem. 16 (**2016**) 465-489.

[56] H.A. El-Sherief, B.G. Youssif, S.N. Bukhari, M. Abdel-Aziz, H.M. Abdel-Rahman, Novel 1, 2, 4-triazole derivatives as potential anticancer agents: Design, synthesis, molecular docking and mechanistic studies. Bioorg. Chem. 76 (**2018**) 314-325.

[57] H. Shi, Y. Wang, R. Hua, Acid-catalyzed carboxylic acid esterification and ester hydrolysis mechanism: acylium ion as a sharing active intermediate via a spontaneous trimolecular reaction based on density functional theory calculation and supported by electrospray ionization-mass spectrometry, Phys. Chem. Chem. Phys. 17 (**2015**) 30279–30291.

[58] X. Zhang, M. Breslav, J. Grimm, K. Guan, A. Huang, F. Liu, C.A. Maryanoff, D. Palmer, M. Patel, Y. Qian, C. Shaw, K. Sorgi, S. Stefanick, D. Xu, A new procedure for preparation of carboxylic acid hydrazides, J. Org. Chem. 67 (**2002**) 9471–9474.

[59] J.R. Maxwell, D.A. Wasdahl, A.C. Wolfson, V.I. Stenberg, Synthesis of 5-aryl-2*H*-tetrazoles, 5-aryl-2*H*-tetrazole-2-acetic acids, and [(4-phenyl-5-aryl-4*H*-1,2,4-triazol-3-yl)thio]acetic acids as possible superoxide scavengers and antiinflammatory agents, J. Med. Chem. 27 (**1984**) 1565–1570.

[60] L. Popiołek, U. Kosikowska, L. Mazur, M. Dobosz, A. Malm, Synthesis and antimicrobial evaluation of some novel 1,2,4-triazole and 1,3,4-thiadiazole derivatives, Med. Chem. Res. 22 (**2013**) 3134–3147.

[61] D.W. Nicholson, N.A. Thornberry, Caspases: killer proteases, Trends Biochem. Sci. 22 (**1997**) 299-306.

[62] Y.-C. Chen, S.-Y. Lin-Shiau, J.K. Lin, Involvement of Reactive Oxygen Species and Caspase 3 Activation in Arsenite-Induced Apoptosis, Cell. Physiol. 177 (**1998**) 324-333.

[63] G. M. Cohen, Caspases: the executioners of apoptosis, Biochem. J. 326 (**1997**) 1-16.

[64] D. Wallach, E.E. Varfolomeev, N.L. Malinin, Y.V. Goltsev, A.V. Kovalenko, M.P. Boldin, Tumor necrosis factor receptor and Fas signalingmechanisms, Annu. Rev. Immunol. 17 (**1999**) 331–367.

[65] S.W. Tait, D.R. Green, Mitochondria and cell death: outer membrane permeabilization and beyond, Nat. Rev. Mo1.Cell. Biol. 11(**2010**) 621-632.

[66] E. Eldering, W.J. Mackus, I.A. Derks, L.M. Evers, E. Beuling, P. Teeling, S.M. Lens, M.H. van Oers, R.A. van Lier, Apoptosis via the B cell antigen receptor requires Bax translocation and involves mitochondrial depolarization, cytochrome C release, and caspase-9 activation, Eur. J. lmmunol. 34 (**2004**) 1950-1960.

[67] D.-J. Fu, S.-Y. Zhang, Y.-C. Liu, X.-X. Yue, J.-J. Liu, J. Song, R.-H. Zhao, F. Li, H.-H. Sun, Y.-B. Zhang, H. M. Liu, Design, synthesis and antiproliferative activity studies of 1, 2, 3-triazole–chalcones, Med. Chem. Comm. 7 (**2016**) 1664-1671.

[68] H. He, W. Wang, Y. Zhou, Q. Xia, Y. Ren, J. Feng, H. Peng, H. He, L Feng, Rational design, synthesis and biological evaluation of 1,3,4-oxadiazole pyrimidine derivatives as novel pyruvate dehydrogenase complex E1 inhibitors, Bioorg. Med. Chem. 24 (**2016**) 1879–1888.

[69] H. Rajak, B.S. Thakur, P. Parmar, P. Kumar, A.K. Gupta, N. Agrawal, P.C. Sharma, Antimicrobial activity of some novel triazole-3-thione containing substituted piperazine moiety, Der Pharma Chemica. 3 (**2011**) 422–462.

[70] U.S. Pathak, M.B. Devani, C.J. Shishoo, S.A. Shah, Synthesis of 5-Aryl-3-Mercapto-4-Substituted-Triazoles, Indian J. Chem. Sect. B-Org. Chem. Med. Chem. 28 (**1989**) 83–86.

[71] K. Sung, A.-R. Lee, Synthesis of [(4,5-disubstituted-4*H*-1,2,4-triazol-3-yl) thio] alkanoic acids and their analogues as possible anti-inflammatory agents, J. Heterocycl. Chem. 29 (**1992**) 1101–1109.

[72] J.-P. Magaud, I. Sargent, D. Y. Mason, Detection of human white cell proliferative responses by immunoenzymatic measurement of bromodeoxyuridine uptake, J. Immunol. Methods 106 (**1988**) 95-100.

[73] I. Vermes, C. Haanen, H. Steffens-Nakken, C. Reutelingsperger, A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V, J. Immunol. Methods. 184 (**1995**) 39-51.

[74] http://www.abcam.com/protocols/general-western-blot-protocol.

[75] E.A. Slee, H. Zhu, S.C. Chow, M. Macfarlane, D.W. Nicholson, G.M. Cohen, Benzyloxycarbonyl-Val-Ala-Asp (OMe) fluoromethylketone (Z-VAD. FMK) inhibits apoptosis by blocking the processing of CPP32. Biochem J. 315 (**1996**) 21-24.

- New 1,2,4-Triazole/Chalcone hybrids **8-47** were synthesized.
- Compounds **24**, **25**, **27**, **41** and **47** had shown the highest cytotoxicity against A549 cells with IC_{50} ranging from 4.4 to 16.04 µM.
- The apoptotic cells number increased in a dose-dependent manner.
- The tested hybrids induced caspase-3 dependent apoptosis through both extrinsic and intrinsic pathways.