Design and synthesis of novel 1,2,3-triazole-pyrimidine hybrids as potential anticancer agents

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · OCTOBER 2014

Impact Factor: 3.45 · DOI: 10.1016/j.ejmech.2014.08.010

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European Journal of Medicinal Chemistry 86 (2014) 368–380

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Design and synthesis of novel 1,2,3-triazole-pyrimidine hybrids as potential anticancer agents

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A R T I C L E I N F O

Article history:
Received 17 April 2014
Received in revised form
2 August 2014
Accepted 4 August 2014
Available online 5 August 2014

Keywords:
Triazole
Pyrimidine
Anticancer
Apoptosis
Cell cycle arrest

A B S T R A C T

A series of novel 1,2,3-triazole-pyrimidine hybrids were designed, synthesized and evaluated for their anticancer activity against four selected cancer cell lines (MGC-803, EC-109, MCF-7 and B16-F10). Most of the synthesized compounds exhibited moderate to good activity against all the cancer cell lines selected. Compound **17** showed the most excellent anticancer activity with single-digit micromolar IC_{50} values ranging from 1.42 to 6.52 μM. Further mechanism studies revealed that compound **17** could obviously inhibit the proliferation of EC-109 cancer cells by inducing apoptosis and arresting the cell cycle at G2/M phase.

1. Introduction

As one of the leading causes of death globally, cancer causes a great burden to both single human lives and the society as a whole. Although there have been progresses in the development of prevention and treatment of cancer, the successful treatment of cancer remains a challenge. Therefore, there is still an urgent need to search for some newer and safer anticancer agents that have broader spectrum of cytotoxicity to tumor cells [1,2]. Molecular hybridization which covalently combines two or more drug pharmacophores into a single molecule is an effective tool to design highly active novel entities [3,4]. In addition, the hybrids may also minimize the unwanted side effects and allow for synergic action [5].

The multi-functionalized pyrimidinones scaffold represents a class of heterocyclic compounds with significant pharmacological efficiency, including anti-viral [6,7], anti-HIV [8–10], anti-bacterial [11], especially anticancer [12–18]. For example, Compound (**1**), a benzimidazole–pyrimidine conjugates as potent antitumor agents, exhibited more potent cytotoxic activities than 5-fluorouracil against cervical carcinoma KB cells [19]. In addition, NSC23766 (**2**) is the first-generation small-molecule inhibitor of Rac GTPase targeting Rac activation by GEF with the ability to inhibit cell proliferation, anchorage-independent growth and invasion against human prostate cancer PC-3 cells [20]. Hoff et al. reported that thienopyrimidine (**3**) was identified as a novel and proprietary small molecule scaffold for potential antitumor agents as EGFR inhibitor [21] (Fig. 1).

On the other hand, 1,2,3-triazole has been a fruitful source of inspiration for medicinal chemists for many years. Due to their synthetic accessibility by click chemistry as well as their diverse inhibitory activities, including anti-fungal, anti-bacterial, anti-allergic, anti-inflammatory and others [22–29], we paid a lot of attention to that. Recent research on 1,2,3-triazoles became more appealing and promising for the design of anticancer agents. For example, M.J. Miller group reported that compound (**4**) exhibited an IC_{50} of 46 nM against MCF-7 cancer cell line [30]. Compound (**5**), a 1, 2, 3-triazol-dithiocarbamate-urea hybrid, showed IC_{50} values of 1.62 and 1.86 μM against MGC-803 and MCF-7 cell line, respectively [31]. Carboxyamidotriazole (**6**) [32], a 1,2,3-triazole-containing anticancer agent, is now available in the market (Fig. 2).

The study of new hybrid systems in which 1,2,3-triazole and pyrimidine are combined comprises an unexplored field of research. We have previously reported some 1,2,3-triazole-dithiocarbamate hybrids with good anticancer activity [33]. These findings have encouraged us to investigate the potential synergistic effect of 1,2,3-triazole and pyrimidine scaffolds. Herein, for the first time, we report the hybridization of these two pharmacophores

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http://dx.doi.org/10.1016/j.ejmech.2014.08.010
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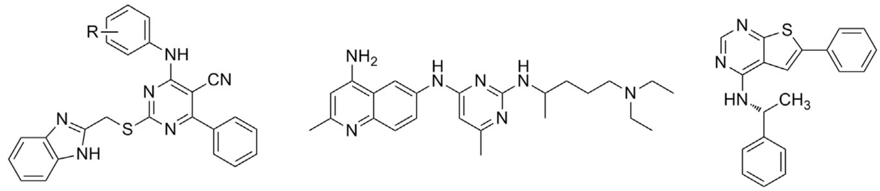

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Fig. 1. Pyrimidine derivatives with anticancer activity.

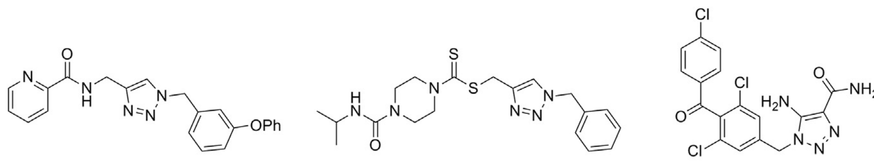

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Fig. 2. 1,2,3-triazole derivatives with anticancer activity.

and their anticancer ability against the four selected tumor cell lines.

2. Results and discussion

2.1. Chemistry

The general route for the synthesis of the target 1,2,3-triazole-pyrimidine hybrids was depicted in Scheme 1. The 6-aryl-5-cyano-2-thiouracils **10a**–**f** were prepared via prolonged heating of aldehydes**7a**–**f**, ethylcyanoacetate**8**, and thiourea**9** in ethanol, in the presence of potassium carbonate [34]. A mixture of the appropriate 2-mercapto-dihydroyrimidine derivatives **10a**–**f**, the propargyl bromide, and anhydrous potassium carbonate was refluxed in dry dioxane. Upon completion, phosphorous oxychloride was added to yield the target derivatives **11a**–**f**. These highly activated intermediates were then reacted with different aryl amines to obtain compounds **13a**–**e**. The compounds **12a**–**i** were prepared via click reaction of compound **11a**–**f** with appropriately substituted benzyl azides. The substituted benzyl azides were readily synthesized from the corresponding halides and sodium azide following literature procedures [35,36]. Target compounds **14**–**40** were synthesized in moderate to high yield using the same reaction condition as **13a**–**e**.

All the synthesized compounds were fully characterized by ^{1}H, ^{13}C NMR and high resolution mass spectra as described for compound **18** (Fig. 3). In the 1H NMR spectra of **18**, the NH proton resonated at δ 10.06 ppm as singlet. We have identified compound **18** from 1D NMR (^{1}H NMR, ^{13}C NMR and DEPT135) and 2D NMR (HSQC, COSY and HMBC). The numbers of the hydrogens and carbons corresponding to **18** were showed below (Fig. 3). The protons attached to S-CH_2, Ar-CH_2 and triazole-H occurred at δ 4.41 (s, 2H), 5.61 (s, 2H) and 7.73 (s, 1H), respectively. The carbons attached to S-CH_2, Ar-CH_2 and triazole-H occurred at δ 25.66, 51.04 and 124.17, respectively. In addition, some direct C–H correlations were observed, confirming that the signals of the aryl chain carbons appeared at 122.69–131.81 ppm and the aryl photons appeared at 7.12–7.89. The presence of a molecular ion peak at $m/z = 566.0699$ ($[M+Na]^+$) in the mass spectrum (calcd. 566.0697) further confirmed the structure of **18**. For all the spectra of compound **18**, please refer to the Supporting information.

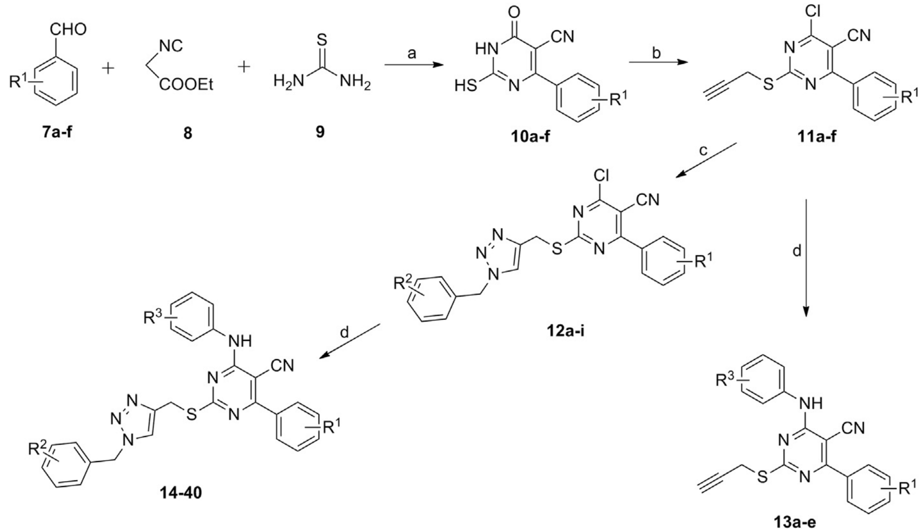
2.2. Evaluation of biological activity

2.2.1. Anticancer activity

All synthesized compounds were evaluated for their anticancer activity against four cancer cell lines, MGC-803 (human gastric cancer cell line), MCF-7 (human breast cancer cell line), B16-F10 (mouse melanoma cell line), and EC-109 (human esophageal cancer cell line) using MTT assay method and compared with the well-known anticancer drug 5-fluorouracil [37].

The anti-proliferative results of preliminary evaluation against the MGC-803, EC-109, B16-F10 and MCF-7 cancerous cell lines for the candidate compounds were shown in Table 1. The replacement of the alkyne substituent by the 1,2,3-triazole scaffolds resulted in a powerful improvement of activity for all the compounds (**14**–**18**), compared with the corresponding pyrimidine-analogs (**13a**–**e**). Especially, compound **17** showed excellent inhibitory effect against EC109 with an IC_{50} value of **1.42** μM (>90-fold and 7-fold more potent than 13**d** and 5-Fu, respectively). This result suggests that 1,2,3-triazole moiety may play an important role in determining activity. In order to complete an SAR study, a series of 1,2,3-triazole-pyrimidine hybrids were prepared and evaluated for their anti-proliferative activity (Table 2).

The SAR studies analysis, as listed on Table 2 showed that the majority of the synthesized compounds showed moderate to good cytotoxic activities against EC-109, MCF-7, and MGC-803. Among them, it was observed that compounds (**14**, **17**, **19**, and **24**) have an excellent anticancer activity with single-digit micromolar IC_{50} values against all the assayed cell lines. On the other hand, the electronic effect and the position of substituent on the aryl amine and benzyl groups had a remarkable effect on their cytotoxic activity. Compounds **14**–**24** were more cytotoxic than **25** against all the assayed cell lines, which means that the substitution on the aryl amine was important for the *in vitro* anticancer activity. Compounds **14**, **19** and **24** with electron-donating groups on the arylamine group have more potent inhibitory effect (7.96, 9.67 and 9.74 μM, respectively) against EC-109 than compounds (**15**, **16**, **18**, and **20**–**23**) (IC_{50} > 15 μM) with electron- withdrawing groups. Compared with compounds (**16**, **19**, and **22**) at the 2-substitution on the arylamine group, compounds (**17**, **14** and **23**) at the 3,4-substitution performed a relatively weak inhibitory effect against MCF-7 and MGC-803. The 4-substitution on the arylamine group (**17**) was more effective against MCF-7 and MGC-803 than those



Scheme 1. Reagents and conditions: **a**: absolute ethanol, absolute K_2CO_3, reflux, 10 h; **b**: (i) propargyl bromide, dioxane, reflux; (ii) phosphorous oxychloride, reflux, 1 h; **c**: $CuSO_4\cdot 5H_2O$, Sodium ascorbate, THF-H_2O (1:1), rt. **d**: appropriate aniline, absolute ethanol, reflux, 6 h.

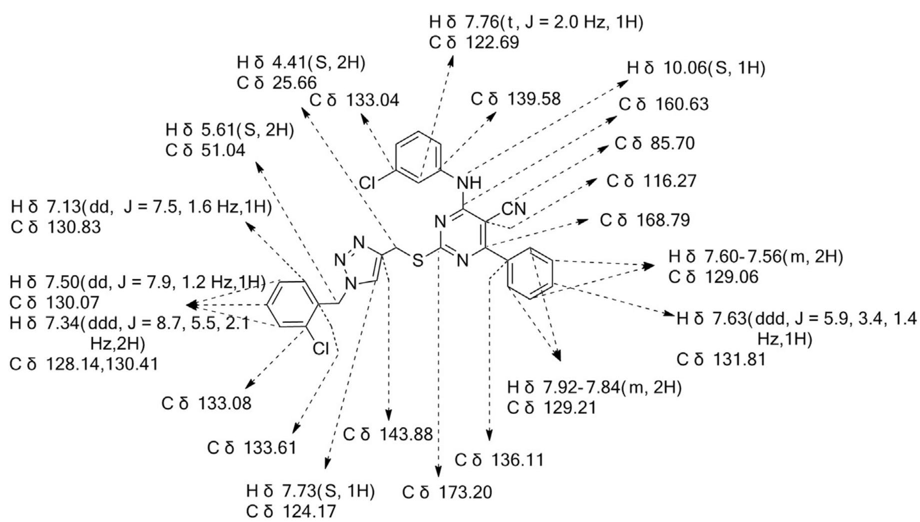


Fig. 3. Selected ^1H, ^{13}C NMR chemical shifts of compound **18**.

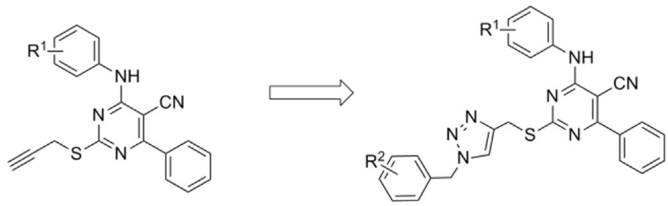
having 3-substitution as in **18**, **20**. Replacing the 2-substitution on the benzyl group (**22**, **24**) with 4-substitution (**30**, **31**, **26**, **27**) led to a loss of activity against EC-109, indicating the significance of the 2-substitution on the benzyl group in retaining activity. A similar trend was also observed to MCF-7 and MGC-803. In addition, we found that the substitution on the phenyl group (R_3) was also important for the *in vitro* anticancer activity showing the more potent activity against EC-109, when the unsubstitution on the phenyl group was replaced with 4-substitution (**34***vs***35**–**40**). An opposite trend was observed to MCF-7 and MGC-803. Furthermore, all the compounds were less potent than 5-Fu against B16-F10.

Compounds **14**, **17**, **19** and **24** were further examined for possible cytotoxicity against GES-1(normal human gastric epithelial cell line) and HET-1A (normal human esophageal epithelial cell line), respectively. As can be seen in Table 3, we found that compounds **14**, **17**, **19** and **24** exhibited no significant cytotoxicity against GES-1(37.12–64.69 μM) and HET-1A(16.17–22.95 μM), respectively. However, compounds **14**, **17**, **19** and **24** exhibited potent cytotoxicity against two selected cancer cell lines (MGC-803 and EC-109), as shown in Table 2. The results indicated that compounds **14**, **17**, **19** and **24** had good selectivity between cancer and normal cells.

2.2.2. Apoptosis assay

Apoptosis defects in cancer cells are the primary obstacle that limits the therapeutic efficacy of anticancer agents, hence the development of novel agents targeting programmed cell death has become an imperative mission for clinical application [38]. Due to the excellent cytotoxic activity against all tested cancer cell lines, compound **17** was chosen to be further investigated regarding its mechanism of action. To explore cytotoxicity of **17** in EC109 cells, cell apoptosis was investigated with Hoechst 33258 staining [39]. After 24 h incubation with **17** at indicated concentrations, characteristic apoptotic morphological changes were observed by fluorescence microscope, including cell rounding, chromatin shrinkage and formation of apoptotic bodies (Fig. 4A). In order to better characterize the mode of cell death induced by compound **17**, the apoptotic analysis was also performed with Annexin V-FITC/PI double staining and quantitated by flow cytometry [40]. Treatment

Table 1
Inhibitory results of preliminary evaluation against four cancer cell lines for the target compounds.

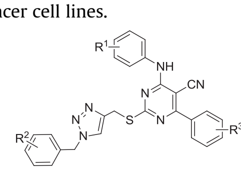


13a-e **14-18**

Comp.	R^1	R^2	IC_{50} (μM)[a]			
			EC-109	MCF-7	MGC-803	B16-F10
13a	*p*-OCH_3	*o*-Cl	23.22 ± 0.67	35.60 ± 1.76	28.40 ± 1.37	9.71 ± 2.11
14	*p*-OCH_3	*o*-Cl	**7.96 ± 0.55**	**8.05 ± 2.36**	**7.56 ± 1.58**	3.99 ± 0.58
13b	*m*-CF_3	*o*-Cl	>128	>128	>128	61.09 ± 0.87
15	*m*-CF_3	*o*-Cl	31.98 ± 0.11	12.56 ± 0.32	22.83 ± 0.25	3.71 ± 1.26
13c	*o*-Cl	*o*-Cl	>128	24.21 ± 0.22	52.80 ± 0.76	12.39 ± 1.12
16	*o*-Cl	*o*-Cl	28.45 ± 1.41	**1.95 ± 1.23**	**4.64 ± 0.45**	8.35 ± 0.34
13d	*p*-Cl	*o*-Cl	>128	>128	>128	19.79
17	*p*-Cl	*o*-Cl	**1.42 ± 1.25**	**6.52 ± 0.23**	**5.85 ± 0.15**	1.59 ± 0.56
13e	*m*-Cl	*o*-Cl	>128	>128	>128	10.52
18	*m*-Cl	*o*-Cl	24.39 ± 0.85	11.99 ± 0.75	15.82 ± 0.76	3.59 ± 0.32
5-Fu			10.81 ± 0.95	8.93 ± 1.26	7.69 ± 0.78	0.87 ± 1.21

[a] Inhibitory activity was assayed by exposure for 72 h to substances and expressed as concentration required to inhibit tumor cell proliferation by 50% (IC_{50}). Compounds with bold values showed more potent cytotoxic activities than 5-Fu against the selected cancer cell lines. Data are presented as the means ± SDs of three independent experiments.

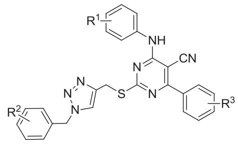
Table 2
Inhibitory results of 1,2,3-triazole-pyrimidine hybrids against four cancer cell lines.



Comp	R^1	R^2	R^3	IC_{50} (μM)[a]			
				EC-109	MCF-7	MGC-803	B16-F10
14	*p*-OCH_3	*o*-Cl	H	**7.96 ± 0.55**	**8.05 ± 2.36**	**7.56 ± 1.58**	3.99 ± 0.58
15	*m*-CF_3	*o*-Cl	H	31.98 ± 0.11	12.56 ± 0.32	22.83 ± 0.25	3.71 ± 1.26
16	*o*-Cl	*o*-Cl	H	28.45 ± 1.41	**1.95 ± 1.23**	**4.64 ± 0.45**	8.35 ± 0.34
17	*p*-Cl	*o*-Cl	H	**1.42 ± 1.25**	**6.52 ± 0.23**	**5.85 ± 0.15**	1.59 ± 0.56
18	*m*-Cl	*o*-Cl	H	24.39 ± 0.85	11.99 ± 0.75	15.82 ± 0.76	3.59 ± 0.32
19	*o*-OCH_3	*o*-Cl	H	**9.67 ± 0.11**	**6.17 ± 0.13**	**5.80 ± 0.07**	7.86 ± 1.41
20	*m*-CH_3	*o*-Cl	H	15.57 ± 0.43	19.62 ± 2.21	16.15 ± 2.38	13.50 ± 1.97
21	*m*-NO_2	*o*-Cl	H	>64	>64	43.07 ± 2.45	17.20 ± 1.39
22	*o*-F	*o*-Cl	H	23.04 ± 2.51	**8.06 ± 1.13**	**7.58 ± 0.80**	5.23 ± 1.92
23	*p*-F	*o*-Cl	H	25.76 ± 1.04	19.91 ± 2.38	8.74 ± 1.36	5.34 ± 1.08
24	*o*-CH_3	*o*-Cl	H	**9.74 ± 1.40**	**7.95 ± 0.78**	**7.28 ± 0.21**	2.76 ± 0.87
25	H	*o*-Cl	H	>64	>64	>64	28.25 ± 1.26
26	*o*-CH_3	*p*-Cl	H	>64	>64	>64	>64
27	*o*-CH_3	*p*-CH_3	H	>64	>64	>64	>64
28	*p*-CH_3	*p*-Cl	H	>64	>64	19.57 ± 1.59	15.10 ± 2.13
29	*p*-CH_3	*p*-F	H	42.73 ± 1.65	51.10 ± 2.41	>64	3.04
30	*o*-F	*p*-Cl	H	>64	>64	>64	>64
31	*o*-F	*p*-CH_3	H	>64	>64	22.86 ± 1.86	16.01 ± 0.65
32	*p*-CH_3	*p*-CH_3	H	55.73 ± 2.01	>64	>64	8.16 ± 1.76
33	*o*-Cl	*p*-CH_3	H	11.22 ± 0.65	34.43 ± 0.23	17.28 ± 2.34	7.08 ± 2.12
34	*p*-CH_3	*o*-Cl	H	>64	9.42 ± 0.45	7.19 ± 0.98	3.39 ± 1.34
35	*p*-CH_3	*o*-Cl	*p*-$CH(CH_3)_2$	**5.08 ± 0.21**	12.17 ± 1.01	>64	3.53 ± 1.23
36	*o*-OCH_3	*o*-Cl	*p*-$CH(CH_3)_2$	**3.58 ± 0.45**	10.25 ± 1.43	>64	2.69 ± 1.32
37	*p*-CH_3	*o*-Cl	*p*-CH_3	**4.65 ± 0.56**	30.94 ± 2.05	24.44 ± 2.15	2.63 ± 0.46
38	*p*-CH_3	*o*-Cl	*m,p,m*-triOCH_3	**5.85 ± 0.21**	20.58 ± 1.76	30.75 ± 1.58	20.34 ± 0.85
39	*p*-CH_3	*o*-Cl	*p*-Cl	**7.54 ± 0.89**	14.67 ± 0.58	18.54 ± 2.12	4.81 ± 2.76
40	*p*-CH_3	*o*-Cl	*p*-Br	**3.09 ± 1.21**	25.13 ± 0.47	15.63 ± 1.87	2.87 ± 0.32
5-Fu				10.81 ± 0.95	8.93 ± 1.26	7.69 ± 0.78	0.87 ± 1.21

[a] Inhibitory activity was assayed by exposure for 72 h to substances and expressed as concentration required to inhibit tumor cell proliferation by 50% (IC_{50}). Compounds with bold values showed more potent cytotoxic activities than 5-Fu against the selected cancer cell lines. Data are presented as the means ± SDs of three independent experiments.

Table 3
Inhibitory results of 1,2,3-triazole-pyrimidine hybrids against two normal cell lines.



Comp	R^1	R^2	R^3	IC_{50} (μM)[a]	
				GES-1	HET-1A
14	*p*-OCH_3	*o*-Cl	H	39.49 ± 1.56	18.05 ± 1.23
17	*p*-Cl	*o*-Cl	H	39.88 ± 1.35	16.52 ± 1.34
19	*o*-OCH_3	*o*-Cl	H	37.12 ± 1.11	16.17 ± 1.25
24	*o*-CH_3	*o*-Cl	H	64.69 ± 1.42	22.95 ± 1.37

[a] Inhibitory activity was assayed by exposure for 72 h to substances and expressed as concentration required to inhibit tumor cell proliferation by 50% (IC_{50}). Data are presented as the means ± SDs of three independent experiments.

of E109 cells with compound **17** increased, in dose dependent manner, the percentage of the apoptotic population up to 12.2%, 29.3% and 52.8%, respectively, compared to control (6.4%) (Fig. 4B, C).

2.2.3. Cell cycle analysis

To have a better understanding of the mechanism of action of cytotoxic activity of compound **17**, a cell-cycle cytotoxicity assay was performed by treating EC-109 cells with different concentrations with compound **17** (0, 1, 2, 4 μM) [41]. After treatment EC-109 cells for 12 h, it was observed that the percentage of cells in G2/M phase at different concentrations were 19.97%, 24.25%, 29.22% and 36.91%, respectively (Fig. 5A), whereas treatment for 24 h, the percentage of cells in G2/M phase were17.13%, 25.45%, 36.62% and 50.72%, respectively (Fig. 5B). The results suggested that **17** caused an obvious G2/M arrest pattern in a concentration- and time-dependent manner with a concomitant decrease in terms of the number of cells in other phases of the cell cycle.

3. Conclusions

In summary, we have discovered a new class of 1,2,3-triazole-pyrimidine hybrids displaying high activities against the proliferation of different cancer cells *in vitro*. The promising compound **17** exhibited the potent and selective anticancer activity *in vitro* and was more potent than 5-fluorouracil against three human cancer cell lines. Further investigation indicated that compound **17** induced cell apoptosis and arrested cell cycle at G2/M phase in EC-109 cells. Further mechanism investigations are under way and will be reported in due course.

4. Experimental section

4.1. General

Reagents and solvents were purchased from commercial sources and were used without further purification. Melting points were determined on an X-5 micromelting apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 MHz and 100 MHz spectrometer respectively. High resolution mass spectra (HRMS) were recorded on a Waters Micromass Q-T of Micromass spectrometer by electrospray ionization (ESI).

4.2. General procedure for the synthesis of compounds *11a–f*

A mixture of the appropriate 2-mercapto-dihydroyrimidine derivatives **10a–f** (1 mmol), the propargyl bromide (1 mmol), and anhydrous potassium carbonate (1 mmol) was refluxed in dry dioxane. Upon completion, as judged by TLC, phosphorous oxychloride was added dropwise with stirring while maintaining the temperature of the reaction mixture. Stirring was continued for additional 1 h. The cooled reaction mixture was poured on crushed ice and the separated solid was filtered off, washed with water, dried and crystallized from aqueous ethanol to yield the pure product.

4.2.1. 4-Chloro-6-phenyl-2-(prop-2-yn-1-ylthio)pyrimidine-5-carbonitrile (*11a*)

Yield 70.5%. White solid. Mp: 131–132 °C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) δ 8.18–8.05 (m, 2H, Ar-H), 7.71–7.50 (m, 3H, Ar-H), 4.01 (d, *J* = 2.6 Hz, 2H, $-CH_2-$), 2.28 (t, *J* = 2.6 Hz, 1H, ≡C–H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm) δ 174.02, 168.73, 163.95, 134.07, 132.72, 129.35, 129.02, 114.43, 101.42, 78.17, 71.63, 20.36. HR-MS (ESI): Calcd. $C_{14}H_9ClN_3S$, $[M+H]^+$ *m/z*: 286.0206, found: 286.0202.

4.2.2. 4-Chloro-6-(4-isopropylphenyl)-2-(prop-2-yn-1-ylthio)pyrimidine-5-carbonitrile (*11b*)

Yield 72.8%. White solid. Mp: 109–110 °C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) δ 8.15–8.00 (m, 2H, Ar-H), 7.44 (m, 2H, Ar-H), 4.01 (d, *J* = 2.6 Hz, 2H, $-CH_2-$), 3.03 (hept, *J* = 6.9 Hz, 1H, CH), 2.28 (t, *J* = 2.6 Hz, 1H, ≡C–H), 1.33 (d, *J* = 6.9 Hz, 6H, $-CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm) δ 173.81, 168.55, 163.92, 131.62, 129.53, 127.21, 114.66, 100.97, 84.40, 78.06, 71.57, 34.29, 23.66, 20.31. HR-MS (ESI): Calcd. $C_{17}H_{15}ClN_3S$, $[M+H]^+$ *m/z*: 328.0675, found: 328.0677.

4.2.3. 4-Chloro-2-(prop-2-yn-1-ylthio)-6-(p-tolyl)pyrimidine-5-carbonitrile (*11c*)

Yield 65.5%. White solid. Mp: 111–112 °C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) δ 8.05 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.39 (d, *J* = 8.1 Hz, 2H, Ar-H), 4.01 (d, *J* = 2.6 Hz, 2H, $-CH_2-$), 2.28 (t, *J* = 2.6 Hz, 1H, ≡C–H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm) δ 173.79, 168.53, 163.91, 143.76, 131.30, 129.76, 114.64, 100.96, 84.11, 78.30, 71.57, 21.69, 20.32. HR-MS (ESI): Calcd. $C_{15}H_{11}ClN_3S$, $[M+H]^+$ *m/z*: 300.0362, found: 300.0363.

4.2.4. 4-Chloro-2-(prop-2-yn-1-ylthio)-6-(3,4,5-trimethoxyphenyl)pyrimidine-5-carbonitrile (*11d*)

Yield 68.3%. White solid. Mp: 104–105 °C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) δ 7.50 (s, 2H, Ar-H), 3.98 (d, *J* = 2.7 Hz, 2H, $-CH_2-$), 3.97 (s, 9H, $-CH_3$), 2.26 (t, *J* = 2.6 Hz, 1H, ≡C–H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm) δ 173.60, 167.69, 164.07, 153.33, 142.24, 128.70, 114.87, 106.98, 100.65, 78.72, 71.22, 61.08, 56.43, 20.35. HR-MS (ESI): Calcd. $C_{17}H_{14}ClN_3NaO_3S$, $[M+H]^+$ *m/z*: 398.0342, found: 398.0340.

4.2.5. 4-Chloro-6-(4-chlorophenyl)-2-(prop-2-yn-1-ylthio)pyrimidine-5-carbonitrile (*11e*)

Yield 77.2%. White solid. Mp: 121–122 °C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) δ 8.17–8.01 (m, 2H, Ar-H), 7.63–7.47 (m, 2H, Ar-H), 4.00 (d, *J* = 2.6 Hz, 2H, $-CH_2-$), 2.28 (t, *J* = 2.6 Hz, 1H, ≡C–H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm) δ 174.20, 167.41, 164.04, 139.39, 132.39, 130.69, 129.40, 114.27, 101.19, 78.07, 71.66, 20.40. HR-MS (ESI): Calcd. $C_{14}H_8Cl_2N_3S$, $[M+H]^+$ *m/z*: 319.9816, found: 319.9818.

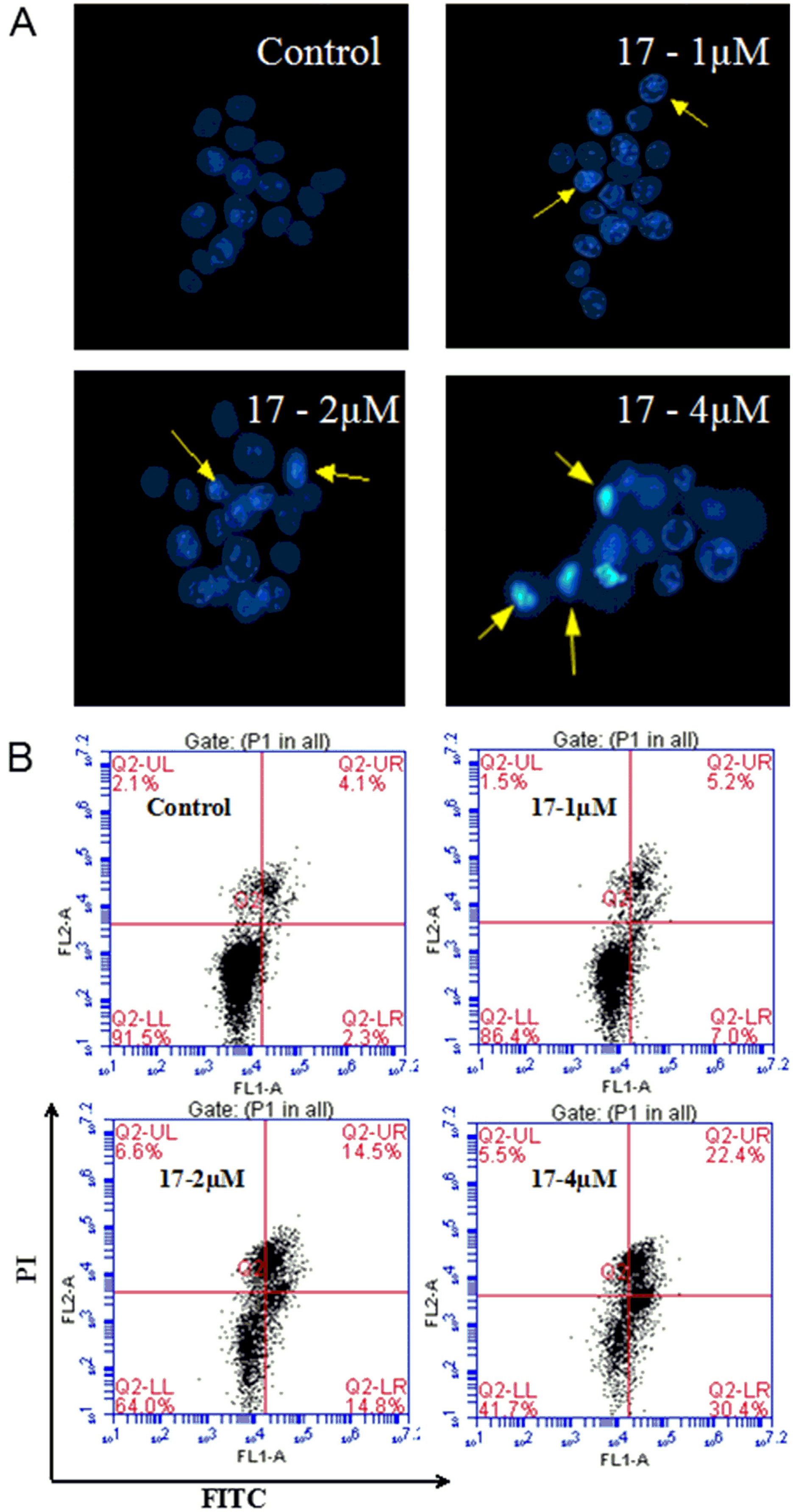


Fig. 4. Compound **17** induced apoptosis in EC109 cells. (A) Apoptosis analysis with Hoechst-33258 staining after 24 h of **17** in EC109 cells; (B and C) Quantitative analysis of apoptotic cells using Annexin V-FITC/PI double staining and flow-cytometry calculation. $^{**}P < 0.01$ was considered statistically highly significant. Dates are mean ± SD. All experiments were carried out at least three times.

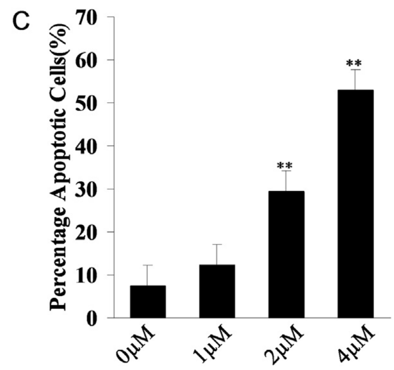


Fig. 4. (*continued*).

*4.2.6. 4-(4-Bromophenyl)-6-chloro-2-(prop-2-yn-1-ylthio)pyrimidine-5-carbonitrile (**11f**)*

Yield 80.5%. White solid. Mp: 137–138 °C. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) δ 8.08–7.96 (m, 2H, Ar-H), 7.84–7.60 (m, 2H, Ar-H), 4.00 (d, J = 2.6 Hz, 2H, −CH_2−), 2.27 (t, J = 2.6 Hz, 1H, ≡C−H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm) δ 174.24, 167.54, 164.06, 132.85, 132.39, 130.78, 127.98, 114.24, 101.19, 78.09, 71.65, 20.40. HR-MS (ESI): Calcd. $C_{14}H_8BrClN_3S$, $[M+H]^+$ m/z: 363.9311, found: 363.9314.

*4.3. General procedure for the synthesis of compounds **12a–i***

In a round-bottom flask equipped with a magnetic stirred bar, **11a–f** (5 mmol), azide derivatives (5 mmol), $CuSO_4 \cdot 5H_2O$ (62 mg, 0.25 mmol), sodium ascorbate (100 mg, 0.5 mmol), THF (20 mL) and H_2O (20 mL) were added. The resulting mixture was stirred at room temperature. The disappearance of compound **11a–f** was monitored by TLC. Upon completion, water was added and the reaction mixture was extracted with EtOAc. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum to afford the crude product. The crude product was recrystallized from acetone to yield the pure product.

*4.3.1. 4-Chloro-2-((1-(2-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-6-phenylpyrimidine-5-carbonitrile (**12a**)*

Yield 80.5%. Yellow solid. Mp: 168–169 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 8.05 (s, 1H), 7.99 (d, J = 7.4 Hz, 2H, ArH), 7.69 (t, J = 7.3 Hz, 1H, ArH), 7.61 (t, J = 7.5 Hz, 2H, ArH), 7.49 (d, J = 7.6 Hz, 1H, ArH), 7.36 (m, J = 14.7, 6.8 Hz, 2H, ArH), 7.18 (d, J = 7.3 Hz, 1H, ArH), 5.65 (s, 2H, Ar-CH_2), 4.60 (s, 2H, S-CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) δ 174.22, 168.88, 163.12, 134.65, 133.58, 133.07, 132.88, 130.97, 130.76, 130.10, 129.65, 129.34, 128.21, 124.68, 115.33, 102.24, 51.12, 26.37. HR-MS (ESI): Calcd. $C_{21}H_{15}Cl_2N_6S$, $[M+H]^+$ m/z: 453.0456, found: 453.0455.

*4.3.2. 4-Chloro-2-((1-(2-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-6-(p-tolyl)pyrimidine-5-carbonitrile (**12b**)*

Yield 90.1%. Yellow solid. Mp: 117–118 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 8.02 (d, J = 16.0 Hz, 1H), 7.90 (dd, J = 14.3, 8.2 Hz, 2H, ArH), 7.53–7.45 (m, 1H, ArH), 7.45–7.37 (m, 2H, ArH), 7.33 (dd, J = 14.6, 7.8 Hz, 2H, ArH), 7.17 (t, J = 6.9 Hz, 1H, ArH), 5.64 (d, J = 5.9 Hz, 2H, Ar-CH_2), 4.59 (s, 2H, S-CH_2), 2.41 (d, J = 7.4 Hz, 3H, Ar-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) δ 174.08, 168.60, 163.15, 143.44, 143.03, 133.57, 133.07, 131.81, 130.96, 130.73, 130.08, 129.94, 129.66, 129.27, 128.15, 124.58, 116.47, 115.45, 101.68, 51.10, 26.35, 25.63. HR-MS (ESI): Calcd. $C_{22}H_{17}Cl_2N_6S$, $[M+H]^+$ m/z: 467.0612, found: 467.0610.

*4.3.3. 4-(4-Bromophenyl)-6-chloro-2-((1-(2-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)pyrimidine-5-carbonitrile (**12c**)*

Yield 86.4%. Yellow solid. Mp: 110–111 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 8.06–7.87 (m,3H), 7.79 (dd, J = 28.8, 8.4 Hz, 2H, ArH), 7.52–7.43 (m, 1H, ArH), 7.37 (t, J = 7.5 Hz, 1H, ArH), 7.30 (t, J = 7.4 Hz, 1H, ArH), 7.16 (d, J = 7.1 Hz, 1H, ArH), 5.64 (d, J = 6.6 Hz, 2H, Ar-CH_2), 4.59 (s, 2H, S-CH_2). ^{13}C NMR (101 MHz, DMSO-d_6, δ, ppm) δ 167.77, 166.41, 163.08, 143.00, 134.63, 133.48, 133.08, 132.16, 131.59, 131.21, 130.72, 130.05, 128.12, 126.22, 124.60, 116.14, 115.16, 102.21, 93.70, 51.09, 25.68. HR-MS (ESI): Calcd. $C_{21}H_{14}BrCl_2N_6S$, $[M+H]^+$ m/z: 530.9561, found: 530.9559.

*4.3.4. 4-Chloro-2-((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-6-phenylpyrimidine-5-carbonitrile (**12d**)*

Yield 79.5%. Yellow solid. Mp: 145–146 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 8.06 (d, J = 6.1 Hz, 1H), 7.95 (dd, J = 15.2, 7.5 Hz, 2H, ArH), 7.63 (m, J = 14.5, 7.2 Hz, 2H, ArH), 7.53 (t, J = 7.3 Hz, 1H, ArH), 7.13 (t, J = 5.9 Hz, 4H, ArH), 5.48 (s, 2H, Ar-CH_2), 4.57 (s, 2H, S-CH_2), 2.25 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) δ 168.85, 167.63, 163.12, 137.96, 135.54, 134.63, 133.31, 132.87, 132.30, 129.75, 129.24, 129.07, 128.45, 128.42, 116.32, 115.32, 102.21, 93.60, 53.19, 26.43. HR-MS (ESI): Calcd. $C_{22}H_{18}ClN_6S$, $[M+H]^+$ m/z: 433.1002, found: 433.1001.

*4.3.5. 4-Chloro-2-((1-(2-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-6-(4-isopropylphenyl)pyrimidine-5-carbonitrile (**12e**)*

Yield 76.8%. Yellow solid. Mp: 143–144 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 8.06 (d, J = 8.3 Hz, 2H, ArH), 7.99 (s, 1H), 7.51 (d, J = 8.3 Hz, 2H, ArH), 7.47 (d, J = 7.8 Hz, 1H, ArH), 7.39 (t, J = 7.0 Hz, 1H, ArH), 7.32 (t, J = 7.2 Hz, 1H, ArH), 7.22 (d, J = 7.4 Hz, 1H, ArH), 5.69 (s, 2H, Ar-CH_2), 4.64 (s, 2H, S-CH_2), 3.06 (dt, J = 13.8, 6.9 Hz, 1H). 1.32 (d, J = 6.9 Hz, 6H). ^{13}C NMR (100 MHz, Acetone-d_6, δ, ppm) δ 174.51, 168.50, 163.16, 153.98, 143.12, 133.34, 133.14, 132.12, 130.47, 130.20, 129.71, 129.59, 127.66, 126.97, 123.55, 114.60, 101.18, 50.97, 34.04, 26.30, 23.13. HR-MS (ESI): Calcd. $C_{21}H_{15}ClFN_6S$, $[M+H]^+$ m/z: 437.0751, found: 437.0750.

*4.3.6. 4-Chloro-2-((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-6-phenylpyrimidine-5-carbonitrile (**12f**)*

Yield 91.5%. Yellow solid. Mp: 118–119 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 8.09 (s, 1H), 7.96 (dd, J = 15.2, 7.5 Hz, 2H, ArH), 7.72–7.52 (m, 3H, ArH), 7.34 (dd, J = 8.3, 5.7 Hz, 2H, ArH), 7.18 (dd, J = 11.2, 6.3 Hz, 2H, ArH), 5.55 (s, 2H, Ar-CH_2), 4.58 (s, 2H, S-CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) δ 167.62, 165.84, 163.54, 161.63, 161.11, 143.17, 135.56, 132.58, 132.26, 130.73, 130.64, 129.22, 129.03, 124.18, 116.14, 115.92, 93.58, 52.54, 25.70. HR-MS (ESI): Calcd. $C_{24}H_{21}Cl_2N_6S$, $[M+H]^+$ m/z: 495.0925, found: 495.0924.

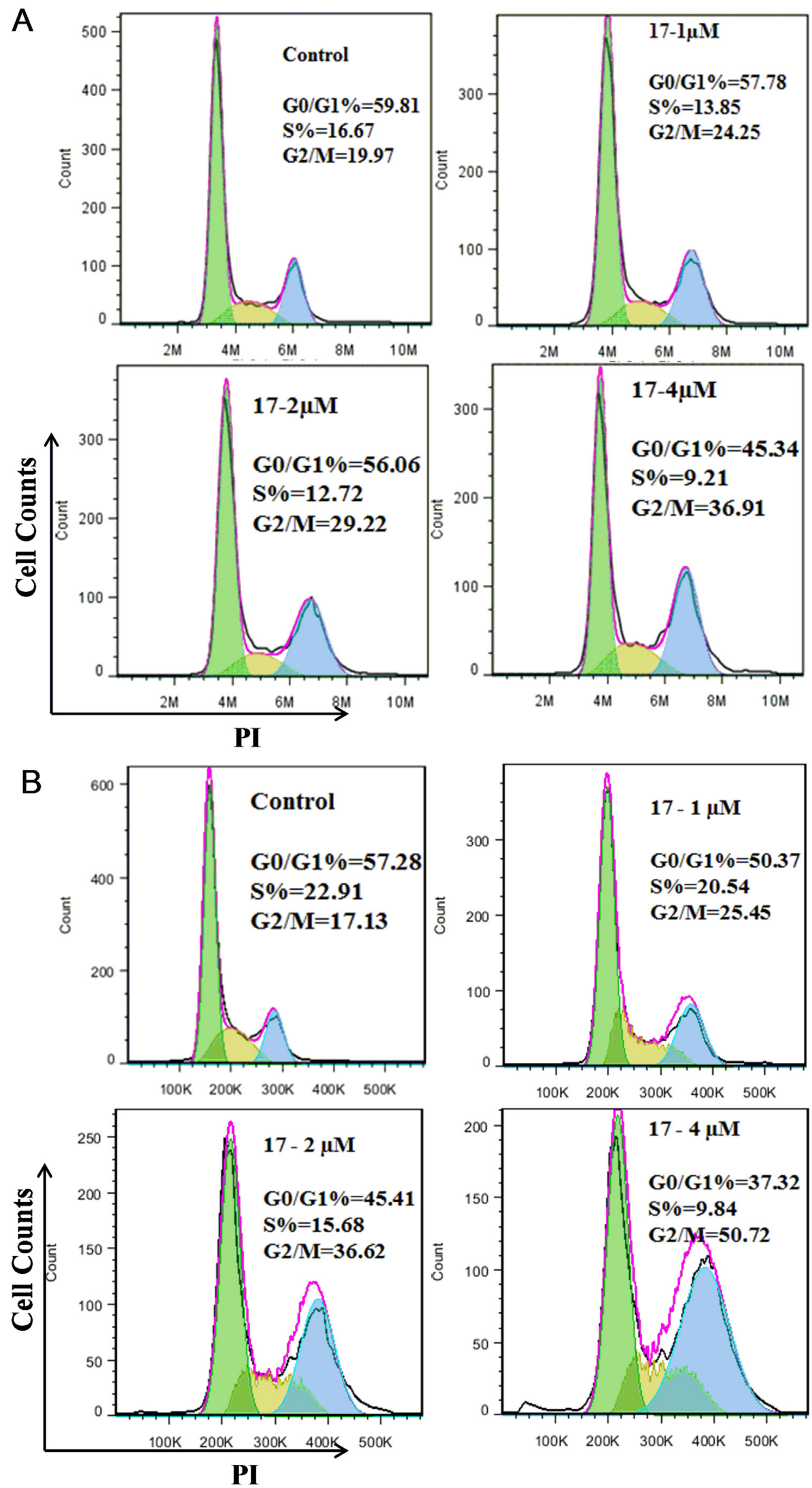


Fig. 5. Effect of compound **17** on the cell cycle distribution of EC-109 cells. Cells were treated with different concentrations (0, 1, 2, 4 μM) for 12 h 0r 24 h. Then the cells were fixed and stained with PI to analyze DNA content by flow cytometry. (A) incubated for 12 h; (B) incubated for 24 h. The experiments were performed three times, and a representative experiment is shown.

4.3.7. 4-Chloro-2-((1-(2-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-6-(3,4,5-trimethoxyphenyl)pyrimidine-5-carbonitrile (***12g***)

Yield 80.6%. Yellow solid. Mp: 146–147 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm) δ 8.05 (s, 1H), 7.49 (d, J = 7.8 Hz, 1H, ArH), 7.44–7.34 (m, 3H, ArH), 7.31 (t, J = 7.5 Hz, 1H, ArH), 7.16 (d, J = 8.1 Hz, 1H, ArH), 5.65 (s, 2H, Ar-CH$_2$), 4.61 (s, 2H, S-CH$_2$), 3.81 (d, J = 18.2 Hz, 9H). ^{13}C NMR (100 MHz, DMSO-d$_6$, δ, ppm) δ 173.90, 167.99, 163.14, 153.31, 143.51, 141.58, 133.59, 133.05, 130.93, 130.73, 130.07, 129.43, 128.14, 124.50, 115.62, 107.50, 101.70, 60.75, 56.64, 51.09, 26.38. HR-MS (ESI): Calcd. $C_{24}H_{21}Cl_2N_6O_3S$, [M+H]$^+$ m/z: 543.0773, found: 543.0770.

4.3.8. 4-Chloro-2-((1-(2-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-6-(4-chlorophenyl)pyrimidine-5-carbonitrile (***12h***)

Yield 85.5%. Yellow solid. Mp: 115–116 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm) δ 8.00 (dd, J = 12.3, 8.4 Hz, 3H), 7.66 (dd, J = 28.7, 8.6 Hz, 2H, ArH), 7.54–7.42 (m, 1H, ArH), 7.37 (m, J = 7.7, 1.6 Hz, 1H, ArH), 7.30 (t, J = 7.5 Hz, 1H, ArH), 7.23–7.10 (m, 1H), 5.63 (s, 2H, Ar-CH$_2$), 4.59 (s, 2H, S-CH$_2$). ^{13}C NMR (100 MHz, DMSO-d$_6$, δ, ppm) δ 166.31, 163.09, 161.43, 143.02, 137.22, 134.29, 133.50, 133.09, 131.50, 131.09, 131.01, 130.74, 130.07, 129.51, 129.23, 128.13, 124.62, 116.16, 93.71, 51.10, 25.68. HR-MS (ESI): Calcd. $C_{21}H_{14}Cl_3N_6S$, [M+H]$^+$ m/z: 487.0066, found: 487.0065.

4.3.9. 4-Chloro-2-((1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-6-phenylpyrimidine-5-carbonitrile (***12i***)

Yield 74.68%. Yellow solid. Mp: 152–153 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm) δ 8.10 (d, J = 8.0 Hz, 1H), 7.95 (dd, J = 15.8, 7.6 Hz, 2H, ArH), 7.64 (m, J = 13.0, 6.9 Hz, 2H, ArH), 7.54 (t, J = 7.5 Hz, 1H, ArH), 7.41 (dd, J = 7.8, 5.1 Hz, 2H, ArH), 7.27 (t, J = 7.9 Hz, 2H, ArH), 5.56 (d, J = 4.0 Hz, 2H, Ar-CH$_2$), 4.58 (s, 2H, S-CH$_2$). ^{13}C NMR (100 MHz, DMSO-d$_6$, δ, ppm) δ 168.87, 167.63, 165.74, 163.13, 135.53, 135.33, 134.64, 133.33, 132.31, 130.31, 129.65, 129.33, 129.23, 129.06, 124.51, 116.31, 115.33, 102.26, 93.62, 52.53, 25.66. HR-MS (ESI): Calcd. $C_{21}H_{15}Cl_2N_6S$, [M+H]$^+$ m/z: 453.0456, found: 453.0454.

4.4. General procedure for the synthesis of compounds ***13a–e***

To a well stirred solution of the appropriate amine (5.30 mmol) in absolute ethanol (10 mL), equimolar amount of a solution of compounds **11a** (1.41 g, 5 mmol) in absolute ethanol (10 mL) was added. The reaction mixture was stirred for 1.5 h at room temperature then heated under reflux for additional 5 h. Upon completion, the precipitated product was filtered off, washed with ethanol to afford the crude product. The crude product was recrystallized from ethanol to yield the pure product.

4.4.1. 4-((4-Methoxyphenyl)amino)-6-phenyl-2-(prop-2-yn-1-ylthio)pyrimidine-5-carbonitrile (***13a***)

Yield 90.5%. Yellow solid. Mp: 202–203 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm) δ 9.83 (s, 1H, NH, D$_2$O exchangeable), 7.96–7.86 (m, 2H, Ar-H), 7.67–7.53 (m, 3H, Ar-H), 7.51 (d, J = 8.9 Hz, 2H, Ar-H), 6.95 (d, J = 8.9 Hz, 2H, Ar-H), 3.88 (d, J = 2.4 Hz, 2H, –CH$_2$–), 3.78 (s, 3H, –OCH$_3$), 3.20 (t, J = 2.4 Hz, 1H, ≡C–H). ^{13}C NMR (100 MHz, DMSO-d$_6$, δ, ppm) δ 172.26, 168.56, 160.65, 157.17, 136.27, 131.71, 130.69, 129.21, 129.01, 126.01, 116.39, 114.02, 85.07, 80.59, 73.82, 55.71, 19.40. HR-MS (ESI): Calcd. $C_{21}H_{17}N_4OS$, [M+H]$^+$ m/z: 373.1123, found: 373.1124.

4.4.2. 4-Phenyl-2-(prop-2-yn-1-ylthio)-6-((3-(trifluoromethyl)phenyl)amino)pyrimidine-5-carbonitrile (***13b***)

Yield 85.2%. Yellow solid. Mp: 153–154 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm) δ 10.19 (s, 1H, NH, D$_2$O exchangeable),8.09–7.88 (m, 4H, Ar-H), 7.68–7.50 (m, 5H, Ar-H), 3.92 (d, J = 2.3 Hz, 2H, –CH$_2$–), 3.18 (t, J = 2.3 Hz, 1H, ≡C–H). ^{13}C NMR (100 MHz, DMSO-d$_6$, δ, ppm) δ 172.47, 168.79, 160.65, 138.93, 136.05, 131.87, 129.97, 129.27, 129.06, 127.68, 125.91, 123.20, 121.58, 120.38, 116.13, 86.07, 80.16, 73.92, 19.44. HR-MS (ESI): Calcd. $C_{21}H_{14}F_3N_4S$, [M+H]$^+$ m/z: 411.0891, found: 411.0893.

4.4.3. 4-((2-Chlorophenyl)amino)-6-phenyl-2-(prop-2-yn-1-ylthio)pyrimidine-5-carbonitrile (***13c***)

Yield 75.5%. Yellow solid. Mp: 158–159 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm) δ 9.96 (s, 1H, NH, D$_2$O exchangeable), 7.94 (m, 2H, Ar-H), 7.69–7.51 (m, 5H, Ar-H), 7.40 (m, 2H, Ar-H), 3.79 (d, J = 2.5 Hz, 2H, –CH$_2$–), 3.11 (t, J = 2.5 Hz, 1H, ≡C–H). ^{13}C NMR (100 MHz, DMSO-d$_6$, δ, ppm) δ 172.37, 168.44, 161.32, 136.07, 135.09, 131.87, 131.04, 130.11, 129.62, 129.25, 129.09, 128.81, 128.11, 116.14, 84.98, 80.20, 73.76, 19.33. HR-MS (ESI): Calcd. $C_{20}H_{14}ClN_4S$, [M+H]$^+$ m/z: 377.0628, found: 377.0631.

4.4.4. 4-((4-Chlorophenyl)amino)-6-phenyl-2-(prop-2-yn-1-ylthio)pyrimidine-5-carbonitrile (***13d***)

Yield 77.3%. Yellow solid. Mp: 231–232 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm) δ 10.03 (s, 1H, NH, D$_2$O exchangeable), 7.97–7.81 (m, 2H, Ar-H), 7.74–7.50 (m, 5H, Ar-H), 7.43 (m, 2H, Ar-H), 3.90 (d, J = 2.5 Hz, 2H, –CH$_2$–), 3.21 (t, J = 2.4 Hz, 1H, ≡C–H). ^{13}C NMR (100 MHz, DMSO-d$_6$, δ, ppm) δ 172.43, 168.74, 160.56, 136.99, 136.11, 131.82, 129.25, 129.04, 128.74, 125.76, 116.25, 100.11, 85.81, 80.54, 73.85, 19.50. HR-MS (ESI): Calcd. $C_{20}H_{14}ClN_4S$, [M+H]$^+$ m/z: 377.0628, found: 377.0628.

4.4.5. 4-((3-Chlorophenyl)amino)-6-phenyl-2-(prop-2-yn-1-ylthio)pyrimidine-5-carbonitrile (***13e***)

Yield 73.8%. Yellow solid. Mp: 189–190 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm) δ 10.05 (s, 1H, NH, D$_2$O exchangeable), 8.05–7.31 (m, 9H, Ar-H), 3.93 (s, 2H, –CH$_2$–), 3.20 (s, 1H, ≡C–H). ^{13}C NMR (100 MHz, DMSO-d$_6$, δ, ppm) δ 172.43, 168.79, 160.57, 139.58, 136.08, 133.16, 131.86, 130.41, 129.28, 129.06, 125.08, 123.54, 122.47, 116.17, 86.01, 80.38, 73.97, 19.49. HR-MS (ESI): Calcd. $C_{20}H_{14}ClN_4S$, [M+H]$^+$ m/z: 377.0628, found: 377.0630.

4.5. General procedure for the synthesis of compounds ***14–40***

To a well stirred solution of the appropriate amine (5.30 mmol) in absolute ethanol (10 mL), equimolar amount of a solution of compounds **12a–i** (5 mmol) in absolute ethanol (10 mL) was added. The reaction mixture was stirred for 1.5 h at room temperature then heated under reflux for additional 5 h. Upon completion, the precipitated product was filtered off, washed with ethanol to afford the crude product. The crude product was recrystallized from ethanol to yield the pure product.

4.5.1. 2-((1-(2-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-4-((4-methoxyphenyl)amino)-6-phenylpyrimidine-5-carbonitrile(***14***)

Yield 80.9%. Yellow solid. Mp: 286–287 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$, δ, ppm) δ 9.82 (s, 1H, NH, D$_2$O exchangeable), 7.86 (d, J = 7.0 Hz, 2H, ArH), 7.64–7.53 (m, 4H, ArH), 7.50 (s, 1H), 7.37 (m, 4H, ArH), 7.14 (d, J = 7.4 Hz, 1H, ArH), 6.88 (d, J = 8.8 Hz, 2H, ArH), 5.59 (s, 2H, Ar-CH$_2$), 4.32 (s, 2H, S-CH$_2$), 3.71 (s, 3H, OCH$_3$) ^{13}C NMR (100 MHz, DMSO-d$_6$, δ, ppm): δ 173.01, 168.57, 160.87, 157.31, 136.30, 133.61, 133.05, 131.64, 130.89, 130.72, 130.09, 129.13, 129.00, 128.16, 126.50, 124.21, 116.47, 114.04, 84.71, 56.49, 55.67, 51.02, 25.58, 19.03. HR-MS (ESI): Calcd. $C_{28}H_{22}ClN_7NaOS$, [M+Na]$^+$ m/z: 562.1193, found: 562.1194.

4.5.2. 2-((1-(2-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-4-phenyl-6-((3-(trifluoromethyl)phenyl)amino)pyrimidine-5-carbonitrile (**15**)

Yield 80.9%. Yellow solid. Mp: 185–186 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 10.20 (s, 1H, NH, D_2O exchangeable), 7.89 (t, $J = 61.6$ Hz, 5H, ArH), 7.69–7.42 (m, 6H, ArH), 7.35 (d, $J = 24.0$ Hz, 2H, ArH), 7.11 (s, 1H), 5.59 (s, 2H, Ar-CH_2), 4.38 (s, 2H, S-CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): δ 172.98, 168.76, 160.66, 141.33, 136.16, 134.97, 133.59, 133.00, 131.80, 130.79, 130.70, 130.23, 130.07, 129.19, 129.08, 128.14, 127.88, 124.09, 122.04, 120.60, 116.27, 115.35, 115.22, 73.49, 50.86, 25.59. HR-MS (ESI): Calcd. $C_{28}H_{19}ClF_3N_7NaS$, $[M+Na]^+$ m/z: 600.0961, found: 600.0959.

4.5.3. 2-((1-(2-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-4-((2-chlorophenyl)amino)-6-phenylpyrimidine-5-carbonitrile(**16**)

Yield 74.8%. Yellow solid. Mp: 190–191 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 10.03 (s, 1H, NH, D_2O exchangeable), 7.89 (d, $J = 7.0$ Hz, 2H, ArH), 7.68–7.56 (m, 3H, ArH), 7.54 (m, 3H, ArH), 7.44 (s, 1H), 7.42–7.23 (m, 4H, ArH), 7.16–7.10 (m, 1H, ArH), 5.60 (s, 2H, Ar-CH_2), 4.23 (s, 2H, S-CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): δ 173.15, 168.51, 161.31, 143.92, 136.05, 135.23, 133.60, 133.04, 131.81, 131.34, 130.89, 130.73, 130.12, 130.10, 129.79, 129.17, 129.08, 128.90, 128.19, 128.07, 123.89, 116.22, 84.66, 51.00, 25.46. HR-MS (ESI): Calcd. $C_{27}H_{20}Cl_2N_7S$, $[M+H]^+$ m/z: 544.0878, found: 544.0876.

4.5.4. 2-((1-(2-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-4-((4-chlorophenyl)amino)-6-phenylpyrimidine-5-carbonitrile(**17**)

Yield 76.5%. Yellow solid. Mp: 195–196 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 10.03 (s, 1H, NH, D_2O exchangeable), 7.87 (d, $J = 6.0$ Hz, 2H, ArH), 7.70 (s, 1H), 7.58 (d, $J = 6.4$ Hz, 5H, ArH), 7.50 (d, $J = 7.5$ Hz, 1H, ArH), 7.38 (m, 4H, ArH), 7.16 (d, $J = 6.8$ Hz, 1H, ArH), 5.61 (s, 2H, Ar-CH_2), 4.37 (s, 2H, S-CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): δ 173.11, 168.72, 160.62, 139.42, 136.15, 133.56, 133.07, 131.76, 130.95, 130.75, 130.10, 130.05,129.18, 129.05, 128.74, 128.18, 126.06, 119.59, 118.19, 116.35, 85.49, 51.07, 25.66. HR-MS (ESI): Calcd. $C_{27}H_{19}Cl_2N_7NaS$, $[M+Na]^+$ m/z: 566.0697, found: 566.0696.

4.5.5. 2-((1-(2-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-4-((3-chlorophenyl)amino)-6-phenylpyrimidine-5-carbonitrile(**18**)

Yield 79.6%. Yellow solid. Mp: 189–190 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 10.06 (s, 1H, NH, D_2O exchangeable), 7.92–7.84 (m, 2H, ArH), 7.76 (t, $J = 2.0$ Hz, 1H, ArH), 7.73 (s, 1H), 7.63 (ddd, $J = 5.9, 3.4, 1.4$ Hz, 1H, ArH), 7.60–7.56 (m, 2H, ArH), 7.56–7.53 (m, 1H, ArH), 7.50 (dd, $J = 7.9, 1.2$ Hz, 1H, ArH), 7.39 (td, $J = 7.7, 1.7$ Hz, 1H, ArH), 7.34 (ddd, $J = 8.7, 5.5, 2.1$ Hz, 2H, ArH), 7.19 (dd, $J = 8.0, 1.2$ Hz, 1H, ArH), 7.13 (dd, $J = 7.5, 1.6$ Hz, 1H, ArH), 5.61 (s, 2H, Ar-CH_2), 4.41 (s, 2H, S-CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): δ173.20, 168.79, 160.63, 143.88, 139.58, 136.11, 133.61, 133.08, 133.04, 131.81, 130.83, 130.70, 130.41, 130.07, 129.21,129.06, 128.14, 125.12, 124.17, 123.92, 122.69, 116.27, 85.70, 51.04, 25.66. HR-MS (ESI): Calcd. $C_{27}H_{19}Cl_2N_7NaS$, $[M+Na]^+$ m/z: 566.0697, found: 566.0699.

4.5.6. 2-((1-(2-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-4-((2-methoxyphenyl)amino)-6-phenylpyrimidine-5-carbonitrile(**19**)

Yield 75.8%. Yellow solid. Mp: 191–192 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 9.34 (s, 1H, NH, D_2O exchangeable), 7.87 (d, $J = 6.7$ Hz, 2H, ArH), 7.58 (d, $J = 6.8$ Hz, 4H, ArH), 7.52 (s, 1H, ArH), 7.50 (s, 1H), 7.40 (t, $J = 7.3$ Hz, 1H, ArH), 7.34 (t, $J = 7.1$ Hz, 1H, ArH), 7.18 (d, $J = 7.0$ Hz, 1H, ArH), 7.11 (d, $J = 7.2$ Hz, 2H, ArH), 6.91 (t, $J = 6.9$ Hz, 1H, ArH), 5.59 (s, 2H, Ar-CH_2), 4.30 (s, 2H, S-CH_2), 3.83 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): δ 173.09, 168.08, 160.76, 153.26, 136.14, 133.60, 133.00, 131.75, 130.82, 130.70, 130.07, 129.12, 129.07, 128.15, 127.58, 126.39, 126.31, 120.71, 116.39, 112.18, 84.88, 56.27, 51.04, 25.58. HR-MS (ESI): Calcd. $C_{28}H_{22}ClN_7NaOS$, $[M+Na]^+$ m/z: 562.1193, found: 562.1194.

4.5.7. 2-((1-(2-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-4-phenyl-6-(m-tolylamino)pyrimidine-5-carbonitrile(**20**)

Yield 90.8%. Yellow solid. Mp: 163–164 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 9.90 (s, 1H, NH, D_2O exchangeable), 7.87 (d, $J = 7.0$ Hz, 2H, ArH), 7.66–7.53 (m, 4H, ArH), 7.50 (d, $J = 7.7$ Hz, 1H, ArH), 7.42 (s, 1H), 7.35 (m, 3H, ArH), 7.21 (t, $J = 7.8$ Hz, 1H, ArH), 7.09 (d, $J = 7.3$ Hz, 1H, ArH), 6.94 (d, $J = 7.4$ Hz, 1H, ArH), 5.59 (s, 2H, Ar-CH_2), 4.37 (s, 2H, S-CH_2), 2.22 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): δ 173.11, 168.71, 160.71, 138.14, 137.86, 136.23, 133.64, 132.98, 131.70, 130.73, 130.67, 130.06, 129.17, 129.02, 128.69, 128.13, 126.30, 125.15, 124.24, 121.67, 116.40, 85.15, 51.01, 25.56, 21.33. HR-MS (ESI): Calcd. $C_{28}H_{23}ClN_7S$, $[M+H]^+$ m/z: 524.1424, found: 524.1425.

4.5.8. 2-((1-(2-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-4-((3-nitrophenyl)amino)-6-phenylpyrimidine-5-carbonitrile(**21**)

Yield 85.6%. Yellow solid. Mp: 208–209 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 10.31 (s, 1H, NH, D_2O exchangeable), 8.63 (t, $J = 1.9$ Hz, 1H, ArH), 8.08 (m, 1H, ArH), 7.97 (m, 1H, ArH), 7.90 (d, $J = 6.9$ Hz, 2H, ArH), 7.78 (s, 1H), 7.68–7.54 (m, 4H, ArH), 7.48 (d, $J = 7.9$ Hz, 1H, ArH), 7.38 (m, 1H, ArH), 7.32 (t, $J = 7.4$ Hz, 1H, ArH), 7.11 (m, 1H, ArH), 5.60 (s, 2H, Ar-CH_2), 4.44 (s, 2H, S-CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): δ 173.30, 168.82, 160.61, 148.11, 143.70, 139.42, 136.03, 133.60, 133.00, 131.87, 130.80, 130.68, 130.08, 130.05, 129.95, 129.23, 129.08, 128.12, 124.15, 119.59, 118.19, 116.16, 86.07, 51.01, 25.80. HR-MS (ESI): Calcd. $C_{27}H_{19}ClN_8NaO_2S$, $[M+Na]^+$ m/z: 577.0938, found: 577.0942.

4.5.9. 2-((1-(2-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-4-((2-fluorophenyl)amino)-6-phenylpyrimidine-5-carbonitrile(**22**)

Yield 81.5%. Yellow solid. Mp: 157–158 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 10.01 (s, 1H, NH, D_2O exchangeable), 7.89 (d, $J = 7.0$ Hz, 2H, ArH), 7.65–7.54 (m, 3H, ArH), 7.52 (m, 1H, ArH), 7.47 (d, $J = 7.8$ Hz, 1H, ArH), 7.43 (s, 1H), 7.42–7.33 (m, 2H, ArH), 7.28 (m, 2H, ArH), 7.15 (m, 2H, ArH), 5.59 (s, 2H, Ar-CH_2), 4.25 (s, 2H, S-CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): δ 173.17, 168.52, 161.22, 158.57, 156.11, 136.08, 133.57, 133.06, 131.78, 130.93, 130.74, 130.09, 129.19, 129.05, 128.93, 128.80, 128.18, 125.50, 124.89, 123.99, 116.46, 116.27, 84.89, 51.01, 25.49. HR-MS (ESI): Calcd. $C_{27}H_{19}ClFN_7NaS$, $[M+Na]^+$ m/z: 550.0993, found: 550.0996.

4.5.10. 2-((1-(2-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-4-((4-fluorophenyl)amino)-6-phenylpyrimidine-5-carbonitrile(**23**)

Yield 74.6%. Yellow solid. Mp: 218–219 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 9.98 (s, 1H, NH, D_2O exchangeable), 7.87 (d, $J = 4.3$ Hz, 2H, ArH), 7.56 (t, $J = 25.8$ Hz, 7H, ArH), 7.40 (s, 1H), 7.35 (d, $J = 6.6$ Hz, 1H, ArH), 7.15 (s, 3H, ArH), 5.61 (s, 2H, Ar-CH_2), 4.34 (s, 2H, S-CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): δ 173.10, 168.65, 160.84, 136.19, 134.20, 133.60, 133.06, 131.73, 130.94, 130.73, 130.09, 129.16, 129.03, 128.16, 126.85, 126.77, 124.08, 116.40, 115.63, 115.41, 85.08, 51.04, 25.61. HR-MS (ESI): Calcd. $C_{27}H_{20}ClFN_7S$, $[M+H]^+$ m/z: 528.1173, found: 528.1172.

4.5.11. 2-((1-(2-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-4-phenyl-6-(o-tolylamino)pyrimidine-5-carbonitrile(**24**)

Yield 77.5%. Yellow solid. Mp: 159–160 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 9.77 (s, 1H, NH, D_2O exchangeable), 7.92–7.84 (m, 2H, ArH), 7.64–7.57 (m, 2H, ArH), 7.56 (s, 1H), 7.55–7.51 (m, 1H, ArH), 7.41 (m, 1H, ArH), 7.39–7.29 (m, 2H, ArH), 7.25 (d, $J = 6.9$ Hz, 2H, ArH), 7.15 (m, 2H, ArH), 7.08 (m, 1H, ArH), 5.58 (s, 2H, Ar-CH_2), 4.18 (s, 2H, S-CH_2), 2.19 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): δ 173.06, 168.47, 161.38, 144.04, 136.52, 136.24, 135.44, 133.64, 132.97, 131.67, 130.85, 130.73, 130.70, 130.08, 129.15, 129.01, 128.26, 128.18, 127.49, 126.60, 123.95, 116.43, 84.34, 50.96, 25.37, 18.31. HR-MS (ESI): Calcd. $C_{28}H_{23}ClN_7S$, $[M+H]^+$ m/z: 524.1424, found: 524.1425.

*4.5.12. 2-((1-(2-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-4-phenyl-6-(phenylamino)pyrimidine-5-carbonitrile(**25**)*

Yield 76.8%. Yellow solid. Mp: 202–203 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 9.99 (s, 1H, NH, D_2O exchangeable), 7.86 (s, 2H, ArH), 7.53 (s, 7H, ArH), 7.40 (s, 1H), 7.33 (d, J = 5.8 Hz, 2H, ArH), 7.13 (s, 2H, ArH), 5.59 (s, 2H, Ar-CH_2), 4.34 (s, 2H, S-CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): δ 173.07, 168.71, 160.75, 137.95, 136.21, 133.60, 133.02, 131.72, 131.50, 130.87, 130.72, 130.07, 129.17, 129.03, 128.86, 128.16, 128.06, 125.64, 124.71, 116.46, 85.19, 51.01, 25.58. HR-MS (ESI): Calcd. $C_{27}H_{21}ClN_7S$, $[M+H]^+$ m/z: 510.1268, found: 510.1269.

*4.5.13. 2-((1-(4-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-4-phenyl-6-(o-tolylamino)pyrimidine-5-carbonitrile(**26**)*

Yield 71.4%. Yellow solid. Mp: 162–163 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 9.79 (s, 1H, NH, D_2O exchangeable), 7.88 (d, J = 6.8 Hz, 2H, ArH), 7.63–7.52 (m, 3H, ArH), 7.46 (s, 1H), 7.44 (s, 1H, ArH), 7.33 (d, J = 6.9 Hz, 1H, ArH), 7.30–7.21 (m, 4H, ArH), 7.21–7.12 (m, 2H, ArH), 5.48 (s, 2H, Ar-CH_2), 4.17 (s, 2H, S-CH_2), 2.20 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_{66}, δ, ppm): δ 173.13, 168.47, 161.41, 144.26, 136.53, 136.25, 135.48, 135.37, 133.34, 131.64, 130.88, 130.15, 129.22, 129.15, 129.00, 128.30, 127.51, 126.65, 123.59, 116.45, 84.36, 52.41, 25.41, 18.31. HR-MS (ESI): Calcd. $C_{28}H_{22}ClN_7NaS$, $[M+Na]^+$ m/z: 546.1244, found: 546.1249.

*4.5.14. 2-((1-(4-Methylbenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-4-phenyl-6-(o-tolylamino)pyrimidine-5-carbonitrile(**27**)*

Yield 80.3%. Yellow solid. Mp: 143–144 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 9.77 (s, 1H, NH, D_2O exchangeable), 7.86 (d, J = 6.9 Hz, 2H, ArH), 7.66–7.57 (m, 2H, ArH), 7.56 (s, 1H), 7.32 (d, J = 7.0 Hz, 1H, ArH), 7.30–7.25 (m, 1H, ArH), 7.24–7.09 (m, 7H, ArH), 5.41 (s, 2H, Ar-CH_2), 4.15 (s, 2H, S-CH_2), 2.29 (s, 3H, CH_3), 2.20 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): δ 173.10, 168.49, 161.39, 144.10, 137.94, 136.52, 136.25, 135.47, 133.38, 131.64, 130.88, 130.15, 129.74, 129.15, 129.00, 128.27, 127.53, 126.66, 123.38, 116.43, 84.33, 53.01, 25.39, 21.17, 18.31. HR-MS (ESI): Calcd. $C_{29}H_{25}N_7NaS$, $[M+Na]^+$ m/z: 526.1790, found: 526.1792.

*4.5.15. 2-((1-(4-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-4-phenyl-6-(p-tolylamino)pyrimidine-5-carbonitrile(**28**)*

Yield 80.7%. Yellow solid. Mp: 208–209 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 9.91 (s, 1H, NH, D_2O exchangeable), 7.90–7.80 (m, 2H, ArH), 7.65–7.53 (m, 3H, ArH), 7.45 (s, 1H), 7.42 (m, 4H, ArH), 7.25 (d, J = 8.3 Hz, 2H, ArH), 7.10 (d, J = 8.2 Hz, 2H, ArH), 5.49 (s, 2H, Ar-CH_2), 4.30 (s, 2H, S-CH_2), 2.21 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): δ 173.07, 168.67, 160.77, 144.29, 136.25, 135.40, 135.31, 134.93, 133.32, 131.66, 130.19, 129.31, 129.23, 129.14, 129.01, 124.81, 123.77, 116.46, 84.95, 52.42, 25.53, 20.94. HR-MS (ESI): Calcd. $C_{28}H_{23}ClN_7S$, $[M+H]^+$ m/z: 524.1424, found: 524.1425.

*4.5.16. 2-((1-(4-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-4-phenyl-6-(p-tolylamino)pyrimidine-5-carbonitrile(**29**)*

Yield 87.6%. Yellow solid. Mp: 213–214 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 9.90 (s, 1H, NH, D_2O exchangeable), 7.88–7.81 (m, 2H, ArH), 7.62–7.54 (m, 3H, ArH), 7.46 (s, 1H), 7.41 (d, J = 8.3 Hz, 2H, ArH), 7.34–7.27 (m, 2H, ArH), 7.20 (t, J = 8.8 Hz, 2H, ArH), 7.11 (d, J = 8.3 Hz, 2H, ArH), 5.48 (s, 2H, Ar-CH_2), 4.31 (s, 2H, S-CH_2), 2.22 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): δ 173.08, 168.67, 160.76, 136.25, 135.32, 134.92, 132.68, 131.66, 130.62, 130.53, 129.31, 129.14, 129.01, 124.80, 123.65, 116.46, 116.18, 115.96, 84.95, 52.43, 25.55, 20.95. HR-MS (ESI): Calcd. $C_{28}H_{23}FN_7S$, $[M+H]^+$ m/z: 508.1720, found: 508.1720.

*4.5.17. 2-((1-(4-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-4-((2-fluorophenyl)amino)-6-phenylpyrimidine-5-carbonitrile(**30**)*

Yield 70.5%. Yellow solid. Mp: 160–161 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 10.03 (s, 1H, NH, D_2O exchangeable), 7.92–7.85 (m, 2H, ArH), 7.64–7.54 (m, 3H, ArH), 7.49 (d, J = 7.9 Hz, 1H, ArH), 7.46 (s, 1H), 7.45–7.40 (m, 2H, ArH), 7.31–7.24 (m, 4H, ArH), 5.49 (s, 2H, Ar-CH_2), 4.23 (s, 2H, S-CH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): δ 173.23, 168.52, 161.24, 158.60, 156.13, 144.08, 136.07, 135.34, 133.35, 131.76, 130.27, 129.22, 129.19, 129.04, 125.61, 125.49, 124.87, 123.59, 116.46, 116.26, 84.89, 52.42, 25.51. HR-MS (ESI): Calcd. $C_{27}H_{19}ClFN_7NaS$, $[M+H]^+$ m/z: 550.0993, found: 550.0996.

*4.5.18. 4-((2-Fluorophenyl)amino)-2-((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-6-phenylpyrimidine-5-carbonitrile(**31**)*

Yield 69.6%. Yellow solid. Mp: 172–173 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 10.01 (s, 1H, NH, D_2O exchangeable), 7.90–7.85 (m, 2H, ArH), 7.60 (m, 3H, ArH), 7.47 (t, J = 7.8 Hz, 1H, ArH), 7.37 (s, 1H), 7.28 (m, 2H, ArH), 7.16 (m, 5H, ArH), 5.42 (s, 2H, Ar-CH_2), 4.21 (s, 2H, S-CH_2), 2.29 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): δ 173.23, 168.53, 161.23, 158.60, 156.14, 143.93, 137.96, 136.08, 133.34, 131.76, 129.74, 129.19, 129.04, 128.38, 125.60, 125.48, 124.93, 123.37, 116.47, 116.26, 84.87, 53.04, 25.51, 21.17. HR-MS (ESI): Calcd. $C_{28}H_{23}FN_7S$, $[M+H]^+$ m/z: 508.1720, found: 508.1722.

*4.5.19. 2-((1-(4-Methylbenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-4-phenyl-6-(p-tolylamino)pyrimidine-5-carbonitrile(**32**)*

Yield 76.7%. Yellow solid. Mp: 226–227 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 9.90 (s, 1H, NH, D_2O exchangeable), 7.85 (d, J = 7.0 Hz, 2H, ArH), 7.64–7.53 (m, 3H, ArH), 7.44–7.38 (m, 3H, ArH), 7.18 (s, 1H, ArH), 7.16 (s, 1H, ArH), 7.12 (m, 4H, ArH), 5.42 (s, 2H, Ar-CH_2), 4.29 (s, 2H, S-CH_2), 2.28 (s, 3H, CH_3), 2.22 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): δ 173.09, 168.67, 160.76, 144.13, 137.92, 136.26, 135.32, 134.95, 133.40, 131.65, 129.74, 129.33, 129.14, 129.00, 128.30, 124.83, 123.59, 116.46, 84.94, 53.04, 25.55, 21.17, 20.94. HR-MS (ESI): Calcd. $C_{29}H_{26}N_7S$, $[M+H]^+$ m/z: 504.1970, found: 504.1971.

*4.5.20. 4-((2-Fluorophenyl)amino)-2-((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-6-phenylpyrimidine-5-carbonitrile(**33**)*

Yield 72.7%. Yellow solid. Mp: 182–183 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 10.01 (s, 1H, NH, D_2O exchangeable), 7.90–7.85 (m, 2H, ArH), 7.60 (m, 3H, ArH), 7.47 (t, J = 7.8 Hz, 1H, ArH), 7.37 (s, 1H), 7.28 (m, 2H, ArH), 7.16 (m, 5H, ArH), 5.42 (s, 2H, Ar-CH_2), 4.21 (s, 2H, S-CH_2), 2.29 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): δ 173.23, 168.53, 161.23, 158.60, 156.14, 143.93, 137.96, 136.08, 133.34, 131.76, 129.74, 129.19, 129.04, 128.38, 125.60, 125.48, 124.93, 123.37, 116.47, 116.26, 84.87, 53.04, 25.51, 21.17. HR-MS (ESI): Calcd. $C_{28}H_{22}ClN_7NaS$, $[M+Na]^+$ m/z: 546.1244, found: 546.1246.

*4.5.21. 2-((1-(2-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-4-phenyl-6-(p-tolylamino)pyrimidine-5-carbonitrile(**34**)*

Yield 73.6%. Yellow solid. Mp: 200–201 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 9.89 (s, 1H, NH, D_2O exchangeable), 7.90–7.83 (m, 2H, ArH), 7.58 (m, 3H, ArH), 7.52 (m, 1H, ArH), 7.45 (s, 1H), 7.43–7.32 (m, 4H, ArH), 7.12 (d, J = 8.6 Hz, 3H), 5.59 (s, 2H, Ar-CH_2), 4.33 (s, 2H, S–CH_2), 2.22 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): δ 173.05, 168.66, 160.75, 144.04, 136.25, 135.32, 134.93, 133.63, 133.00, 131.68, 130.79, 130.71, 130.09, 129.31, 129.15, 129.02, 128.18, 124.77, 124.11, 116.47, 84.96, 50.99, 25.54, 20.98. HR-MS (ESI): Calcd. $C_{28}H_{23}ClN_7S$, $[M+H]^+$ m/z: 524.1424, found: 524.1423.

*4.5.22. 2-((1-(2-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-4-(4-isopropylphenyl)-6-(p-tolylamino)pyrimidine-5-carbonitrile(**35**)*

Yield 67.8%. Yellow solid. Mp: 189–190 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 9.86 (s, 1H, NH, D_2O exchangeable), 7.81 (d, J = 8.2 Hz, 2H, ArH), 7.50 (t, J = 7.9 Hz, 1H, ArH), 7.48 (s, 1H), 7.45–7.30 (m, 6H, ArH), 7.11 (d, J = 8.0 Hz, 3H, ArH), 5.59 (s, 2H, Ar-CH_2), 4.33 (s, 2H, S-CH_2), 2.98 (m, 1H, Ar-CH), 2.22 (s, 3H, CH_3), 1.25 (d, J = 6.9 Hz, 6H, C-$(CH_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): δ 172.98, 168.38, 160.83, 152.46, 144.10, 135.38, 134.86, 133.79, 133.64, 132.99, 130.77, 130.69, 130.08, 129.30, 128.17, 126.99, 124.72, 124.12, 116.66, 84.55, 50.98, 33.90, 29.49, 25.54, 24.08, 20.97. HR-MS (ESI): Calcd. $C_{31}H_{29}ClN_7S$, $[M+H]^+$ m/z: 566.1894, found:566.1896.

*4.5.23. 2-((1-(2-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-4-(4-isopropylphenyl)-6-(p-tolylamino)pyrimidine-5-carbonitrile(**36**)*

Yield 62.5%. Yellow solid. Mp: 128–129 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) ^{1}H NMR (400 MHz, DMSO) δ 9.22 (s, 1H), 7.94–7.78 (m, 2H, ArH), 7.65 (d, J = 7.7 Hz, 1H, ArH), 7.54 (s, 1H, ArH), 7.49 (d, J = 7.8 Hz, 1H, ArH), 7.43 (d, J = 8.2 Hz, 2H, ArH), 7.40–7.29 (m, 2H, ArH), 7.19 (dd, J = 11.2, 4.2 Hz, 1H, ArH), 7.11 (d, J = 7.9 Hz, 2H, ArH), 6.92 (t, J = 7.5 Hz, 1H, ArH), 5.59 (s, 2H, Ar-CH_2), 4.28 (d, J = 39.5 Hz, 2H, S-CH_2), 3.91 (m, 3H), 3.12 (m, 1H, Ar-CH), 1.37 (d, J = 6.9 Hz, 6H, C-$(CH_3)_2$). ^{13}C NMR (101 MHz, DMSO, δ, ppm) δ 173.10, 167.70, 160.75, 152.88, 152.57, 133.69, 133.63, 133.01, 130.78, 130.65, 130.04, 129.24, 128.11, 127.29, 127.04, 126.53, 125.79, 124.13, 120.73, 116.57, 112.11, 84.58, 56.31, 51.02, 33.90, 25.63, 24.05.HR-MS (ESI): Calcd. $C_{31}H_{28}ClN_7NaOS$, $[M+Na]^+$ m/z: 604.1662, found: 604.1664.

*4.5.24. 2-((1-(2-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-4-(p-tolyl)-6-(p-tolylamino)pyrimidine-5-carbonitrile(**37**)*

Yield 80.7%. Yellow solid. Mp: 208–209 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 9.85 (s, 1H, NH, D_2O exchangeable), 7.78 (d, J = 7.9 Hz, 2H, ArH), 7.51 (d, J = 7.8 Hz, 1H, ArH), 7.45 (s, 1H), 7.36 (m, 6H, ArH), 7.11 (d, J = 7.8 Hz, 3H, ArH), 5.58 (s, 2H, Ar-CH_2), 4.33 (s, 2H, S-CH_2), 2.41 (s, 3H, CH_3), 2.22 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): δ 172.96, 168.42, 160.83, 144.09, 141.84, 135.37, 134.86, 133.62, 133.40, 133.00, 130.78, 130.69, 130.08, 129.58, 129.29, 129.14, 128.17, 124.73, 124.10, 116.61, 84.57, 50.99, 25.54, 21.52, 20.98. HR-MS (ESI): Calcd. $C_{29}H_{25}ClN_7S$, $[M+H]^+$ m/z: 538.1581, found: 538.1581.

*4.5.25. 2-((1-(2-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-4-(p-tolylamino)-6-(3,4,5-trimethoxyphenyl)pyrimidine-5-carbonitrile(**38**)*

Yield 91.7%. Yellow solid. Mp: 140–141 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 9.84 (s, 1H, NH, D_2O exchangeable), 7.51 (d, J = 6.8 Hz, 2H, ArH), 7.40 (s, 1H), 7.39 (s, 2H, ArH), 7.33 (t, J = 7.3 Hz, 1H, ArH), 7.23 (s, 2H, ArH), 7.12 (t, J = 7.8 Hz, 3H, ArH), 5.59 (s, 2H, Ar-CH_2), 4.35 (s, 2H, S-CH_2), 3.84 (s, 6H, OCH_3), 3.77 (s, 3H, OCH_3), 2.24 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): δ 172.79, 167.96, 160.83, 153.12, 144.35, 140.50, 135.36, 134.90, 133.64, 133.00, 131.22, 130.76, 130.69, 130.08, 129.31, 128.14, 124.73, 124.07, 116.74, 106.92, 84.72, 60.66, 56.53, 50.99, 25.60, 20.98. HR-MS (ESI): Calcd. $C_{31}H_{28}ClN_7NaO_3S$, $[M+Na]^+$ m/z: 636.1561, found: 636.1558.

*4.5.26. 2-((1-(2-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-4-(4-chlorophenyl)-6-(p-tolylamino)pyrimidine-5-carbonitrile(**39**)*

Yield 70.5%. Yellow solid. Mp: 218–219 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 9.93 (s, 1H, NH, D_2O exchangeable), 7.89 (d, J = 7.3 Hz, 2H, ArH), 7.62 (t, J = 16.7 Hz, 2H, ArH), 7.51 (d, J = 7.1 Hz, 1H, ArH), 7.44 (s, 1H), 7.38 (m, 4H ArH), 7.12 (d, J = 6.4 Hz, 3H, ArH), 5.58 (s, 2H, Ar-CH_2), 4.33 (s, 2H, S-CH_2), 2.23 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): δ 173.13, 167.41, 160.65, 160.75, 143.99, 136.59, 135.26, 135.00, 133.60, 133.01, 131.04, 130.81, 130.71, 130.08, 129.32, 129.16, 128.17, 124.79, 124.10, 116.31, 84.98, 50.99, 25.57, 20.98. HR-MS (ESI): Calcd. $C_{28}H_{22}Cl_2N_7S$, $[M+H]^+$ m/z: 558.1034, found: 558.1035.

*4.5.27. 4-(4-Bromophenyl)-2-((1-(2-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methylthio)-6-(p-tolylamino)pyrimidine-5-carbonitrile(**40**)*

Yield 76.9%. Yellow solid. Mp: 225–226 °C. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) δ 9.92 (s, 1H, NH, D_2O exchangeable), 7.80 (q, J = 8.3 Hz, 4H, ArH), 7.50 (t, J = 10.6 Hz, 1H, ArH), 7.44 (s, 1H), 7.42–7.22 (m, 4H, ArH), 7.12 (d, J = 7.6 Hz, 3H, ArH), 5.58 (s, 2H, Ar-CH_2), 4.33 (s, 2H, S-CH_2), 2.23 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): δ 173.15, 167.51, 160.65, 143.98, 135.37, 135.26, 135.00, 133.60, 133.02, 132.09, 131.20, 130.82, 130.71, 130.08, 129.32, 128.17, 125.49, 124.78, 124.09, 116.29, 84.96, 50.99, 25.57, 20.98. HR-MS (ESI): Calcd. $C_{28}H_{22}BrClN_7S$, $[M+H]^+$ m/z: 602.0529, found: 602.0523.

4.6. Effect of compounds on cell viability

Exponentially growing cells were seeded at 5×10^3 cells per well into 96-well plates. After 24 h incubation at 37 °C, the culture medium was removed and replaced with fresh medium containing the candidate compounds in different concentrations. The cells were incubated for another 72 h. Then, 20 μl of MTT (3-(4, 5-dimethylthiazol- 2-yl)-2, 5-diphenyltetrazolium bromide) solution (5 mg/mL) was added to each well and incubated for 4 h at 37 °C. The medium containing MTT was discarded, then 150 mL of dimethyl sulfoxide (DMSO) was added to each well and the plates agitated until the dark blue crystals (formazan) had completely dissolved; the absorbance was measured using a microplate reader at a wavelength of 570 nm. Each concentration was analyzed in triplicate and the experiment was repeated three times. The average 50% inhibitory concentration (IC_{50}) was determined from the concentration–response curves according to the inhibition ratio for each concentration.

4.7. Analysis of cellular apoptosis

EC-109 cells were plated in 6-well plates (1.0×10^6 cells/well) and incubated at 37 °C for 24 h. Exponentially growing cells were then incubated for 24 h with complete medium (blank) or with the compound **17.** Cells were then harvested and the Annexin-V-FITC/PI apoptosis kit (Biovision) was used according to the manufacturer's instructions to detect apoptotic cells. Ten thousand events were collected for each sample and analyzed by Accuri C6 flow cytometer.

4.8. Flow cytometric analysis of cell cycle distribution

For flow cytometric analysis of DNA content, 5×10^5 EC-109 cells in exponential growth were treated with different concentrations of the test compounds for 24 h. After an incubation period, the cells were collected, centrifuged and fixed with ice–cold ethanol (70%). The cells were then treated with buffer containing RNAse A and 0.1% Triton X-100 and then stained with PI. Samples were analyzed on Accuri C6 flow cytometer (Becton, Dickinson). Data obtained from the flow cytometer was analyzed using the FlowJo software (Tree Star, Inc., Ashland, OR, USA).

Acknowledgments

This work was supported by National Natural Science Foundation of China (Project No. 81172937 by Dr. Hong-Min Liu). We are grateful to Dr. Shaomin Wang, Analysis and Testing Center, Zhengzhou University, for performing the HR-ESI-MS date; Bing Zhao, School of Pharmaceutical Sciences, Zhengzhou University, for the NMR analysis.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.08.010.

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